Toxoplasma gondii-specific IgG avidity testing in pregnant women

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Abstract

Background

The parasite *Toxoplasma gondii* (Tg) can cause congenital toxoplasmosis following primary infection in a pregnant woman. It is therefore important to distinguish between recent and past infection when both Tg-specific IgM and IgG are detected in a single serum in pregnant women. Tg-specific IgG avidity testing is an essential tool to help to date the infection. However, interpretation of its results can be complex.

Objectives:

To review Tg-specific avidity testing benefits and limitations in pregnant women, in order to help practitioners to interpret the results and adapt the patient management.

Sources:

PubMed search with the keywords avidity, toxoplasmosis and *Toxoplasma gondii* for articles published from 1989 to 2019.

Content:

Tg-specific IgG avidity testing remains a key tool for dating a Tg infection in immunocompetent pregnant women. Several commercial assays are available and display comparable performances. A high avidity result obtained on a first-trimester serum sample is indicative of a past infection, which occurred before pregnancy. To date, a low avidity result must still be considered as non-informative to date the infection, although some authors suggested that very low avidity results are highly suggestive of recent infections depending on the assay. Interpretation of low or grey zone avidity results on a first-trimester serum sample, as well as any avidity result on a second or third-trimester serum sample, is more complex and requires recourse to expert toxoplasmosis laboratories.

Implications:

Although used for about 30 years, Tg-specific avidity testing has scarcely evolved. The same difficulties in interpretation have persisted over the years. Some authors proposed additional

thresholds to exclude an infection of less than 9 months, or in contrast to confirm a recent infection. Such thresholds would be of great interest to adapt management of pregnant women and avoid unnecessary treatment: however, they need confirmation and further studies.

Introduction

Toxoplasma gondii (Tg) is a cosmopolitan intracellular parasite. Congenital toxoplasmosis can occur following primary infection in a pregnant woman. The risk of transplacental transmission and severity of foetal disease depend on the gestational age at the time of maternal infection [1,2]. As the infection is usually asymptomatic in pregnant women, toxoplasmosis detection and screening during pregnancy rely on serological techniques. While detection of both Tg-specific IgM and IgG in a single serum sample must suggest an acute infection, a past infection cannot be excluded either since Tg-specific IgM antibodies can persist for months or years after infection (Figure 1, Table 1). Tg-specific IgG-avidity testing can be helpful in discriminating between these two possibilities, but its interpretation can be complex and sometimes confusing for non-experts. This review is designed to support interpretation of complex Tg serological results, and focuses on the benefits and limitations of Tg-specific IgG-avidity testing in pregnant women. It is based on a literature research in Pubmed using the keywords avidity, toxoplasmosis and *Toxoplasma gondii* from 1989 to 2019.

What is avidity?

Avidity, or functional affinity, is a measure of the binding strength of IgG antibodies to an antigen. Avidity increases with time, as, following prolonged or repeated exposure to the antigen, the IgG hypervariable regions successively adapt through antigen-driven B cell selection, to bind to it more tightly [3]. Measuring the avidity of specific IgG-antibodies directed against a given pathogen, e.g.*Toxoplasma gondii*, can therefore help to discriminate between a recent and a past infection.

Avidity testing is of particular interest in helping to date a *T. gondii* (Tg) infection in pregnant women after both specific IgM and IgG have been detected, and guide the patient management (see below).

How to measure avidity?

Several approaches exist for the measurement of Tg-specific IgG avidity. Most of the techniques rely on the use of a protein denaturing agent, e.g. hypermolar urea (4-8M), as a diluent or a washing

agent (Table 1). This denaturing agent either prevents the formation (diluent) or allows the dissociation of antigen/low-avidity antibodies complex by disruption of hydrogen bonds (washing agent). Basically, Tg-specific IgG testing is performed twice in parallel, with and without denaturing agent, on a single dilution or serial dilutions of serum. Avidity is then calculated as the ratio of IgG testing (optical density, luminescence or IgG titre) with denaturing agent (corresponding to high-avidity specific IgG antibodies) to IgG testing without denaturing agent. The result is expressed as an index (Avidity Index (AI)) or a percentage. As the relationship between OD and IgG titre (UI/mL) is not linear, the result can vary according to the mode of calculation [4,5].

Other techniques, i.e. Architect or Alinity Toxo IgG avidity (Abbott Diagnostics) and Elecsys Toxo IgG Avidity (Roche Diagnostics) assays, use recombinant antigens as blocking agents. These soluble blocking agents bind to and neutralize high-avidity specific IgG antibodies. As the previous techniques, two reactions are performed in parallel, with or without blocking agent, on a single-dilution of serum, but by contrast, low-avidity specific IgG antibodies are detected. Avidity (%) is therefore calculated according to the following formula: [1 – (IgG testing with blocking agent/IgG testing without blocking agent)] x 100.

Avidity testing can be performed either on serial dilutions (end-titre method) or on a single dilution of serum. The automated commercial assays, detailed in Table 1, measure avidity using the single dilution serum method. As AI can vary according to the total concentration of IgG in the sample [6], some manufacturers recommend adjusting each serum concentration within a standard range of IgG titres before measuring avidity. In addition, all the assays require a minimal IgG titre to be performed. The end-titre method is considered the gold-standard as it does not depend on IgG concentration, but it is not adapted for routine diagnosis [6,7].

Interpretation of Tg-specific IgG avidity results

The aim of avidity testing is to discriminate between recently-acquired and past infections. Briefly, a high Tg-specific IgG avidity result strongly indicates a past infection, while a low or intermediate avidity result is not informative in dating the infection. However, rules of interpretation vary according to the assay, notably the definition of time elapsed for infection to be considered acute or past, the degree of certainty in ruling out a recent infection with a high avidity result, and the interpretation of a low avidity result (Table 1). Most of the manufacturers consider a past infection as an infection older than 4 months, whereas some others set a time frame of more than 3 months (Chorus or Enzywell Toxoplasma IgG avidity, Diesse Diagnostica Senese) or 20 weeks (Platelia™ TOXO IgG Avidity, Biorad). Some assays interpret a high avidity result as ruling out a recent infection, while others do not exclude a recent infection with complete certainty. Similarly, some manufacturers interpret a low avidity result as suggesting a possible but not confirmed recent infection (Biorad, Diasorin, Vircell, Technogenetics), while others offer no interpretation of low avidity results (bioMerieux, Abbott, Roche).

Despite this general principle of interpretation and the specifications of the different manufacturers, many questions persist about avidity. One of them is: can a recent infection be ruled out reliably with a high avidity result? The answer is "yes". The ability of high avidity results to exclude a recent infection has been shown for all commercial assays currently available, with Positive Predictive Values (PPV) very close or equal to 100% [8–12]. According to some authors, a very high avidity result (>90%) with the Elecsys (Roche Diagnosis) and Architect (Abbott) assays could even rule out an infection of <9 months [8,12]. High avidity results have been only exceptionally reported in cases of recent infections and have not been explained to date [10,13].

Another frequent but complex question about avidity is: can a low avidity result confirm a recent infection? The answer is "no, but". To date, a low avidity result must still be considered as non-informative in dating the infection. Indeed, persistent low avidity results have been commonly

reported in serum from patients with past infections of months or even years, whatever the technique used. One study estimated the PPVs of a low avidity result at 61.1%, 73.5%, 74.3% and 77.5% for the Platelia, Architect, Liaison and VIDAS assays, respectively, and a similar PPV was found for the Elecsys assay: 69.9% [8,11,12,14–16]. The reasons for delayed (or absence of) Tg-specific IgG maturation in some patients have not yet been fully elucidated.[17] Considerable inter-individuals variations have been observed [5,18]. Tg-specific IgG maturation is delayed in pregnant women and in neonates with congenital toxoplasmosis.[15,19,20] Although controversial, it has been suggested that anti-*Toxoplasma* treatment, e.g. with spiramycin, may lead to delayed Tg-specific IgG maturation in pregnant women by reducing the parasite load [5,15,16,20,21]. In a study in mice comparing different antiparasitic treatment regimen, only atovaquone was observed to impact avidity maturation, whereas spiramycin or pyrimethamine-sulfadiazine were not [22]. In addition, some authors did not find any difference in avidity between treated or untreated pregnant women [16]. Finally, the kinetics of IgG avidity increase over time varies between assays [8,11,23]. The frequency of persistent low avidity results in past infection is difficult to estimate accurately, as most of the studies on avidity are retrospective and performed on selected samples from bank sera.

While a low avidity result cannot confirm a recent infection, some authors suggested that a very low avidity result could be highly suggestive of this [8,12,24,25]. Among them, Fricker-Hidalgo *et al* and Murat *et al* concluded that Tg-specific IgG avidity results ≤15% with the Elecsys assay or <17% with the Architect assay could reliably confirm recent infections of <3 or <2 months, respectively [8,12]. Similarly, Boquel *et al* reported that a very low avidity result <0.05 with the Laison XL Toxo IgG avidity assay was indicative of a probable recent infection of <3 months, with a PPV of 97% [25]. To date, and until additional studies are performed, the hypothesis of a very recent infection based on a very low avidity result should be confirmed by repeat Tg-specific IgM and IgG testing on a serum sample taken 2-3 weeks later.

As stated above, there are some differences in interpretation of an avidity result according to the assay, but are the overall performances of the different commercial assays comparable? The answer appears to be "yes", since many studies comparing 2 to 4 Tg-specific avidity assays in parallel found that avidity values were highly correlated whatever the technique (correlation coefficients ≥0.795), with similar kinetics of avidity maturation [8,11,26–29]. General agreement between assays was good, around 80% [8,27,30]. Although the oldest (developed in 1998) and the cheapest, the VIDAS assay performed best for the diagnosis of past infections [11,27,31,32]. In a study, the accuracy compared to the date of infection was 93.4% vs 86.8% for the VIDAS and the Architect assays respectively [31]. Several studies also suggested that AI in the grey zone with the VIDAS assay could rule out a recent infection of <4 months [16,31]. A similar observation was made with the Architect assay [31]. This last assay was the most dynamic one in recent infections, probably due to the use of early and late recombinant antigens [11]. The Elecsys assay, also based on the use of recombinant antigens, appeared correlate well (83%) with the Architect assay [8]. Due to these inter-assay variations, the use of two different techniques for avidity testing could be helpful in some particular situations (see below and Figure 1) [30,33].

Tg-specific IgG avidity testing in pregnant women

Tg-specific IgG avidity testing is mainly performed in pregnant women. It is recommended in an immunocompetent pregnant woman displaying both IgM and IgG on first serological testing, in addition to follow-up serological testing 2-3 weeks later, in order to distinguish between acute and latent infection and therefore adapt the patient management.

Interpretation of high, grey-zone or low avidity results depends on the term of pregnancy at the time of sampling (Figure 1). First, what about high avidity results? In several European countries including France, some regions of Italy and Austria, a toxoplasmosis serological screening is recommended during the first trimester for any pregnant women [34,35]. Avidity testing is performed when both

Tg-specific IgG and IgM are detected in this screening sample. In this situation, interpretation of a high avidity result is straightforward, as the serum is taken early in pregnancy: it allows identification of a past infection, which occurred before the beginning of pregnancy, with residual IgM [24]. Monthly serological screening can therefore be stopped (provided stable antibody titres are found in a second serum taken 3 weeks later). Even if rare, it must be kept in mind that foetal transmission of *T. gondii* can occur following anteconceptional infection (usually in the 2 months prior to conception): consequently, a high avidity result in a serum sampled at the end of the first trimester must be interpreted with caution [36]. Avidity testing can also be performed later in pregnancy in countries in which no systematic toxoplasmosis screening is implemented or in pregnant women with no previous follow-up. To date, a high avidity result obtained in a serum sampled >4 months after the beginning of pregnancy does not exclude an infection during pregnancy. Study of the Tg-specific IgG and IgM kinetics +/- additional techniques (e.g. Tg-specific IgA testing) are therefore required. Antiparasitic treatment, ultrasound follow-up as well as amniocentesis could also be offered to these pregnant women on a case-by-case basis in order to prevent and/or manage congenital toxoplasmosis [1]. In these situations, additional thresholds allowing the exclusion of infections <9 months would be of great value. As mentioned above, such thresholds have already been suggested, but for only two commercial assays and they need to be confirmed in further studies [8,12].

Interpretation of grey-zone avidity results during pregnancy is more complex. Currently, unless there is perfect stability of antibody titres in 2 sera taken 2-3 weeks apart, they are most often interpreted as non-informative in dating the infection, whatever the gestational term. As an infection during pregnancy therefore could not be firmly ruled out, antiparasitic treatment and prenatal diagnosis of congenital toxoplasmosis are implemented according to national or local guidelines. However, if obtained on a first-trimester sample, it may be helpful to investigate a grey-zone avidity result with a different avidity testing method, i.e the VIDAS assay which displayed the best performances for the diagnosis of past infections, which could possibly give a high avidity result instead [11,30,31]. Some

authors also suggested that grey-zone avidity results obtained with the Architect and VIDAS assays could exclude a recent infection of <4 months, but again, it needs confirmation [16,31].

As mentioned above, a low avidity result must be considered as non-informative for dating the infection, and must lead to the implementation of measures for prevention and diagnosis of congenital toxoplasmosis (see above) whatever the gestational term, without delay. These measures are especially required in case of a very low avidity result as it has been shown to be highly suggestive of a very recent infection, and/or in presence of both high Tg-specific IgM titres and IgG which could also indicate a recent infection [8,12,25].

As a result, because of complex interpretation, and with the exception of a high avidity result in the very beginning of pregnancy, avidity testing in pregnant women must be referred to an expert toxoplasmosis laboratory.

IgG maturation and host-parasite dynamics can be altered in immunocompromised patients, making interpretation of avidity results even more difficult [11]. Therefore, in that context, screening for toxoplasmosis infection during pregnancy should rather rely on monthly serological testing +/- PCR, whatever the serological status.

Alternatives to avidity testing /perspectives

Despite its aforementioned limits, avidity testing remains an essential tool: to date, it is the most reliable diagnostic tool for the discrimination between acute and past toxoplasmosis in a single serum.

Performing Tg-specific IgA testing in association with avidity testing in pregnant women could also help to distinguish between a recent and a chronic Tg infection. Indeed, in a recent study, Olariu *et al* showed that the prevalence of IgA antibodies decreased when AI increased. IgG+, IgM+ and IgA+

pregnant women were more likely to have had a recent infection than IgG+, IgM+ and IgA- ones [37]. However, a recent infection could not be excluded on the absence of Tg-specific IgA alone, and these antibodies can persist for months after infection [37,38].

Improvements in avidity testing would probably consist of the use of recombinant antigens or chimeric peptides [39,40]. Kinetics of IgG maturation differ against native versus recombinant antigens, as well as against one recombinant antigen and another [40]. Using this property could help to discriminate better between recent and past infections. To date, two commercial fully automated avidity testing assays are already based on recombinant antigens: the Architect/Alinity (SAG1, GRA8) and the Elecsys ones (SAG1) (Table 1) [29]. Another qualitative assay for the determination of Tg-specific IgG avidity, although less common, uses recombinant antigens: recomLine Toxoplasma IgG [Avidity] (Mikrogen). Other potentially interesting recombinant antigens, used alone or in association, have been identified including MIC3, GRA1, GRA6, GRA7, SAG2 and ROP1 [41–45]. Recombinant chimeric peptides, composed of different epitopes selected from Tg antigens, have also been generated and tested for their potential in toxoplasmosis serological diagnosis [39,40,46]. Among them, the recombinant multi-epitope peptide (rMEP) developed by Dai *et al* showed promising results for the discrimination of recent from late infections [46].

Conclusion

Tg-specific IgG avidity testing is an essential tool to help to date an infection when both IgM and IgG are detected in a single serum in pregnant women (Figure 1). Although used in diagnosis for about 30 years, it has scarcely evolved. The same difficulties in its interpretation have persisted over the years, requiring recourse to expert toxoplasmosis laboratories. Definition of additional thresholds to exclude an infection of less than 9 months would be of particular value during pregnancy when both IgG and IgM are detected. In addition, confirmation of a recent *T. gondii* infection by a low avidity result would be useful in avoiding unnecessary treatment of pregnant women. To date, only a very

few studies have suggested such thresholds; confirmation of these results and additional assays are both required.

Transparency declaration:

• Conflict of interest disclosure:

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Table 1: Automated commercial Tg-specific IgG avidity testing assays

	bioMérieux Vidas® Toxo IgG Avidity	**Biorad Platelia™ TOXO IgG Avidity**	**Diasorin Liaison® XL Toxo IgG Avidity**	**Vircell *Toxoplasma* Virclia® IgG Avidity monotest**	**Technogenetics Immunodiagnostic Systems IDS-iSYS/TGS TA Toxo IgG Avidity**	**Abbott Architect®/Alinity® Toxo IgG Avidity**	**Roche Elecsys® Toxo IgG Avidity**
Principle of reaction	ELFA	Indirect EIA	CLIA	CLIA	Indirect CLIA	CMIA	CLIA
Dissociation or blocking agent	Urea	Urea	Urea	Urea	ND	Recombinant proteins	Recombinant proteins
Antigens	*Toxoplasma* Lysate Antigen	ND	*Toxoplasma* Lysate Antigen	ND	*Toxoplasma* Lysate Antigen	P30 (SAG1) P35 (GRA8)	P30 (SAG1)
Automation	Semi-automated	Manual and automated	Automated	Automated	Automated	Automated	Automated
Requirements of the avidity reaction	IgG ≥ 8 IU/mL IgG >15 IU/mL: to be diluted	IgG ≥ 9 IU/mL	IgG ≥ 8.8 IU/mL IgG <15 IU/mL: interpretation with caution	Antibody index ≥1.1	Protocol depending on the IgG titre (1,5-5;5<IgG<50 ;>50)	IgG ≥ 1.6 IU/mL	IgG ≥ 6 IU/mL IgG >500 IU/mL: to be diluted
CE-IVD	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Interpretation Low avidity Grey zone High avidity	Index <0.20 0.20≤AI<0.3 ≥0.30	Index <0.40 0.40≤AI<0.5 ≥0.50	Index <0.20 0.20≤AI<0.3 ≥0.30	Index <0.4 0.4-0.5 >0.5	Index ≤0.1 0.1<AI≤0.15 >0.15	Percentage <50% 50-59.9% ≥60%	Percentage <70% 70-79% ≥80%
Meaning of : Low avidity	Not a proof of recent infection	Suggestion of a recent infection of <20 weeks	Suggestion of a primary infection acquired within the last 4 months	In favor of recent primo-infection of less than 4 months	Suggestion of a primary infection acquired within the last 4 months	Cannot be used to diagnose an acute infection	No clinical interpretation
High avidity	Strongly suggests an infection of >4 months	Suggestion of a past infection of >20 weeks	Exclusion of a primary infection acquired within the last 4 months	In favor of past-infection of more than 4 months	Exclusion of an infection acquired within the last 4 months	Strong indication of an infection of >4 months	Exclusion of an infection acquired within the last 4 months

References	[5,11,26,27,31, 47]	[11,21,30]	[11,27,47]		[48]	[11,26,27,29,31,47]	[8,12]

ND: No data

- **See previous results** (if available)
- **Testing of previous blood serum samples** (if available)

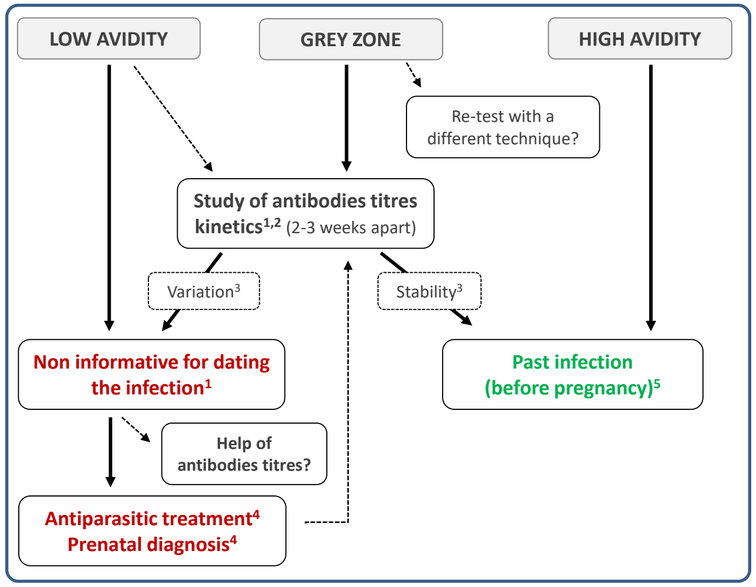
Study of antibodies titres

High-titre IgM Low-titre IgG	High-titre IgM High-titre IgG	Low-titre IgM Low or high-titre IgG
↓	↓	↓
Suggestion of a **Very recent infection?**	Suggestion of a **Recent infection (several weeks)?**	Suggestion of a **Past infection?**

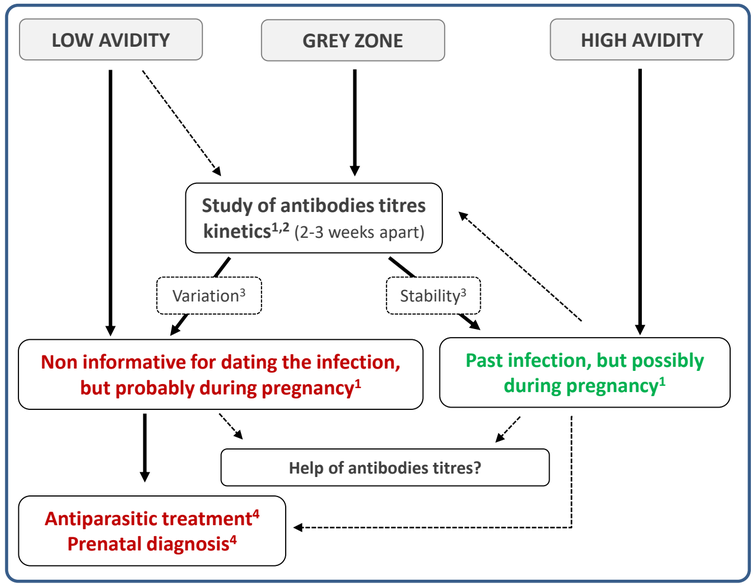
IgM +
IgG +

IgG avidity testing

1st trimester



2nd and 3rd trimesters



[1]Refer to an expert toxoplasmosis laboratory
[2]Anti-*Toxoplasma* treatment may alter antibodies titres kinetics
[3]No consensual definition of a significant variation of antibodies titres (depends on the technique)
[4]According to local and national guidelines
[5]Possible periconceptional infection