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ORIGINAL ARTICLE

Interpretation of the Elecsys Toxo IgG avidity results for very low and very high index: study on 741 sera with a determined date of toxoplasmosis

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Abstract Initial results with the Elecsys Toxo IgG Avidity assay showed some potential for interpretation of a very low or very high index result. We aimed to examine these new insights into interpretation using a large panel of serum samples and to define the optimal thresholds. A total of 741 patient serum samples with known date of infection (from a few weeks to more than 9 months after infection), were analysed with the Elecsys Toxo IgG Avidity assay. Values ≥80% (threshold defined by the manufacturer) were reported in 289 sera; 288 sera were sampled more than 4 months after infection. Thus, avidity values ≥80% excluded an infection less than 4 months. Avidity values ≥90% were reported in 112 sera sampled more than 9 months after infection. Thus, avidity values ≥90% excluded infection less than 9 months. Moreover avidity values ≤15% were reported in the 62 sera sampled less than 3 months after infection. Thus avidity values ≤15% excluded infection more than 3 months.

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Introduction

Toxoplasmosis, caused by the parasitic protozoan *Toxoplasma gondii*, is widespread. The infection is generally asymptomatic in immunocompetent individuals, whereas intrauterine transmission of the parasite from mother to fetus during gestation can result in severe fetal and neonatal complications [1]. When primary infection occurs during pregnancy, the transmission rate is approximately 25% in the first trimester, 54% in the second trimester, and 65% in the third trimester for untreated women [2]. The diagnosis of toxoplasmosis acquired during pregnancy must be followed by measures to prevent fetal infection, such as treating the mother with spiramycin or pyrimethamine-sulfadiazine. Fetal infection can be detected using fetal ultrasound and/or amniotic fluid analysis [3]. The greatest concern about diagnosing primary toxoplasmosis in pregnancy is deciding whether the pregnant woman has acquired acute infection, or whether infection has occurred before conception, in order to initiate early therapy to prevent complications to the fetus [4]. Serologic diagnosis of acute toxoplasmosis is based on the presence of specific immunoglobulin M (IgM) and/or IgA antibodies, and/or a significant increase in specific IgG antibody levels. However, the persistence of specific IgM antibodies detected by classical serologic techniques has complicated the interpretation of serologic tests, as IgM anti-*Toxoplasma* antibodies may persist in the serum for years after acute infection in some patients [5–7].

The first technique to determine approximately the date of infection is the comparison of the results of two serologic tests with different antigenic targets and kinetics. Another technique is the determination of IgG avidity which excludes a recent infection [4]. The avidity test measures the functional affinity of specific IgG antibodies, which is initially low after

primary antigenic challenge and increases during subsequent weeks and months via antigen-driven B-cell selection. Protein-denaturing reagents are used to dissociate the antibody–antigen complex. The avidity index is calculated using the ratio of IgG antibody titers of dissociating reagent-treated and untreated samples. Different techniques which measure the avidity index have been developed and evaluated [8–12]. The maturation of avidity is slow and varies according to the avidity technique used. A high avidity index allows the exclusion of a recent infection and helps to prevent unnecessary follow-up and treatment. Nevertheless, a low or intermediate avidity index is inconclusive as a chronic infection cannot be excluded [4]. Different tests have been developed and their results have been compared in several studies [13–18]. The results show that acute toxoplasmosis cannot be reliably diagnosed based on low IgG avidity alone [14].

A new test, the Elecsys Toxo IgG Avidity assay (Roche Diagnostics GmbH, Mannheim, Germany), was shown to be a valuable tool for excluding recent *T. gondii* infection [19]. When Elecsys Toxo IgG avidity values are very low or very high, a recent or a chronic infection may be considered. Therefore, these new findings may pave the way for additional uses for IgG avidity assays and assist in the provision of more definitive interpretation than is possible currently. However, testing a larger panel of serum samples is required to confirm or refute these interesting preliminary results and to determine the two threshold values.

The aim of this study was to investigate the ability of the Elecsys Toxo IgG Avidity assay to confirm a recent infection when the avidity values are very low, to exclude an infection in the last 9 months when avidity values are very high, and to define the optimal thresholds in the two cases on a large panel of serum samples with known dates of infection.

Materials and methods

Serum samples

A total of 824 patient serum samples were obtained from routine screening performed by parasitology–mycology laboratories at three university hospitals in France: Grenoble ($n = 199$), Marseille ($n = 325$) and Paris ($n = 300$). All serum samples included in the study were taken from pregnant women with past or recent seroconversion. Each center classified the samples into ten groups according to the time of infection as follows: within the first month after infection and between the 1st and 2nd, 2nd and 3rd, 3rd and 4th, 4th and 5th, 5th and 6th, 6th and 7th, 7th and 8th, 8th and 9th, and after the 9th month of infection. The date of infection was either established from the dates of consecutive negative and positive results, or from the first positive sample (positive IgM and negative or weakly positive IgG followed by a two-fold or greater increase) based on the kinetics of antibody production. Serum samples with stable, low IgG rates between two consecutive samples, and without IgM, and serum samples collected at time of delivery from women with a periconceptional seroconversion, were estimated as being collected more than 9 months after infection. The pregnant women sampled one to nine months after infection (282/741 sera, 38%) received antiparasitic treatment, mainly spiramycin, at the time of sampling.

Diagnosis of *T. gondii* infection was previously established by testing the samples using routine techniques for the detection of anti-*T. gondii* IgG and IgM antibodies and, if necessary, the determination of IgG avidity. In Grenoble, IgG and IgM antibodies and IgG avidity were determined using Vidas Toxo IgG II and IgM assays (bioMérieux, Marcy l'Etoile, France), homemade IgG and IgM indirect immunofluorescence assays and, if necessary, an IgM immunosorbent agglutination assay (Toxo ISAGA IgM; bioMerieux), LDBio-Toxo II IgG immunoblot (LDBio, Lyon, France) and Vidas Toxo IgG Avidity assay (bioMerieux). In Marseille the techniques used were Architect Toxo IgG and IgM assays (Abbott Diagnostics, Wiesbaden, Germany), Toxo-HAI assay (Fumouze Diagnostics, Le Malesherbes, Levallois Perret, France) and, if necessary, the LDBio-Toxo II IgG immunoblot, Toxo ISAGA IgM assay, and Vidas Toxo IgG Avidity assay. In Paris the techniques used were Platelia Toxo IgG and IgM assays (Bio-Rad, Marnes-la-Coquette, France), homemade IgG and IgM indirect immunofluorescence assays, an IgM immunosorbent agglutination assay (Toxo ISAGA IgM) and, if necessary, Platelia Toxo IgG Avidity assay and LD-Bio Toxo II IgG immunoblot.

Avidity measures

The Elecsys Toxo IgG Avidity assay is a one-step double-antigen sandwich assay. The samples were tested with the Elecsys Toxo IgG Avidity assay using a Cobas e411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). In accordance with the technical specifications, the Elecsys Toxo IgG Avidity assay was performed on all serum samples with an IgG titer greater ≥6 IU/mL. Interpretation was as follows: index < 70%, low avidity; 70% ≥ index < 80%, intermediate avidity; index ≥80%, high avidity (excludes primary infection within the last 4 months).

Statistical analyses

A descriptive analysis of the serum samples' characteristics and IgG avidity was performed using means and standard deviations for quantitative data and frequencies for qualitative data. To confirm the characteristics of the test based on the threshold of 80% defined by the manufacturer, sensitivity (ratio of chronic toxoplasmosis serum samples [≥4 months] with an avidity result ≥80% to the total number of chronic

toxoplasmosis samples), specificity (ratio of acute toxoplasmosis serum samples [<4 months] with an avidity result <80% to the total number of acute toxoplasmosis samples), negative predictive value (NPV), and positive predictive value (PPV) were assessed. From the serum samples included, two optimal thresholds were determined.

The first threshold allowed the exclusion of acute infection for patients with chronic toxoplasmosis (seroconversion >9 months). The specificity was defined as the ratio of the number of avidity results lower than the threshold for toxoplasmosis <9 months to the number of toxoplasmosis cases <9 months. Sensitivity was defined as the ratio of the number of avidity results higher than the threshold for chronic toxoplasmosis (>9 months) to the number of chronic toxoplasmosis cases. The second threshold allowed confirmation of recent seroconversion for patients with acute toxoplasmosis (seroconversion in the last 3 months). The specificity was defined as the ratio of serum samples with an avidity result higher than the threshold for toxoplasmosis >3 months to the number of toxoplasmosis cases >3 months. Sensitivity was defined by the ratio of the number of avidity results lower than the threshold for acute toxoplasmosis (<3 months) to the number of acute toxoplasmosis cases. Thresholds and their 95% percentile bootstrap confidence interval values, sensitivity and specificity, PPV, and NPV were assessed.

Results

IgG avidity results and time of infection

IgG results from 83 samples were <6 IU/mL. The recommendations of the manufacturer was that IgG results of all samples must be positive (titer ≥6UI/mL) to be assayed by Elecsys Toxo IgG Avidity. Thus Elecsys Toxo IgG Avidity assay was performed on the 741 serum samples with positive results by Elecsys Toxo IgG (≥6 IU/mL).

The results of the Elecsys Toxo IgG Avidity assay are detailed by different sample groups in Table 1. The percentage of low avidity results in each serum sample group ranged from 96.1 to 98.4% for samples collected between 0 and 4 months after infection, from 62.5 to 85.7% for samples collected between 4 and 9 months after infection, and was 16.3% for samples collected more than 9 months after infection. The higher percentage of intermediate avidity (30.8%) was observed for serum samples collected around 6 months after infection. The percentage of high avidity results was 70.4% for samples collected more than 9 months after infection. These data showed that the avidity changed from low index and high index for few serum samples collected 4–9 months after infection and for a large number of serum samples collected after 9 months.

The Elecsys Toxo IgG avidity index median showed an increasing trend (Fig. 1a), but the values were scattered. Indeed, the index medians increased with the time of infection except at points 3–4 months, 5–6 months, 7–8 months, and 8–9 months. It could be explained by the low numbers of sera analysed in these four groups ($n = 26$, 7, 24 and 8, respectively). Moreover the increase of avidity index was observed at different times after infection depending on the patients. Results from the 741 serum samples were classified according to the threshold of 80% and the gray zone (70% ≤ values < 80%) defined by the manufacturer and the time of infection (Table 2). The Elecsys Toxo IgG Avidity assay gave low avidity values for 382/741 (51.5%) serum samples; 267/382 (69.9%) of these samples were collected less than 4 months after infection, and 115/382 (30.1%) were collected more than 4 months after infection. The avidity index values were in the gray zone for 70/741 (9.4%) serum samples, 5/70 (7.1%) of which were collected <4 months after infection, and 65/70

Table 1 Number (percentage) of serum samples classified according to time between infection and sample with low, borderline, and high avidity index determined by Elecsys Toxo IgG Avidity assay

Time since infection (months)	Number of samples with Elecsys Toxo IgG avidity result			
	Low avidity <70%	Gray zone 70% ≤ v < 80%	High avidity ≥80%	Total
0–1	58 (96.6%)	1 (1.7%)	1 (1.7%)	60
1–2	127 (98.4%)	2 (1.5%)	0	129
2–3	57 (98.2%)	1 (1.7%)	0	58
3–4	25 (96.1%)	1 (3.8%)	0	26
4–5	14 (82.3%)	2 (11.8%)	1 (5.9%)	17
5–6	6 (85.7%)	1 (14.2%)	0	7
6–7	9 (69.2%)	4 (30.8%)	0	13
7–8	16 (66.6%)	4 (16.7%)	4 (16.7%)	24
8–9	5 (62.5%)	1 (12.5%)	2 (25.0%)	8
>9	65 (16.3%)	53 (13.3%)	281 (70.4%)	399
TOTAL	382	70	289	741

(92.9%) of which were collected >4 months after infection. The avidity index values were high for 289/741 (39%) serum samples. The time between infection and collection of serum samples was more than 4 months for 288/289 (99.7%) samples with the exception of serum from one patient, which was taken 1 month after infection (Elecsys Toxo IgG avidity index, 86.7%). On analysis of a sample collected one month later (Bio-Rad avidity assay), the avidity index was low (13%).

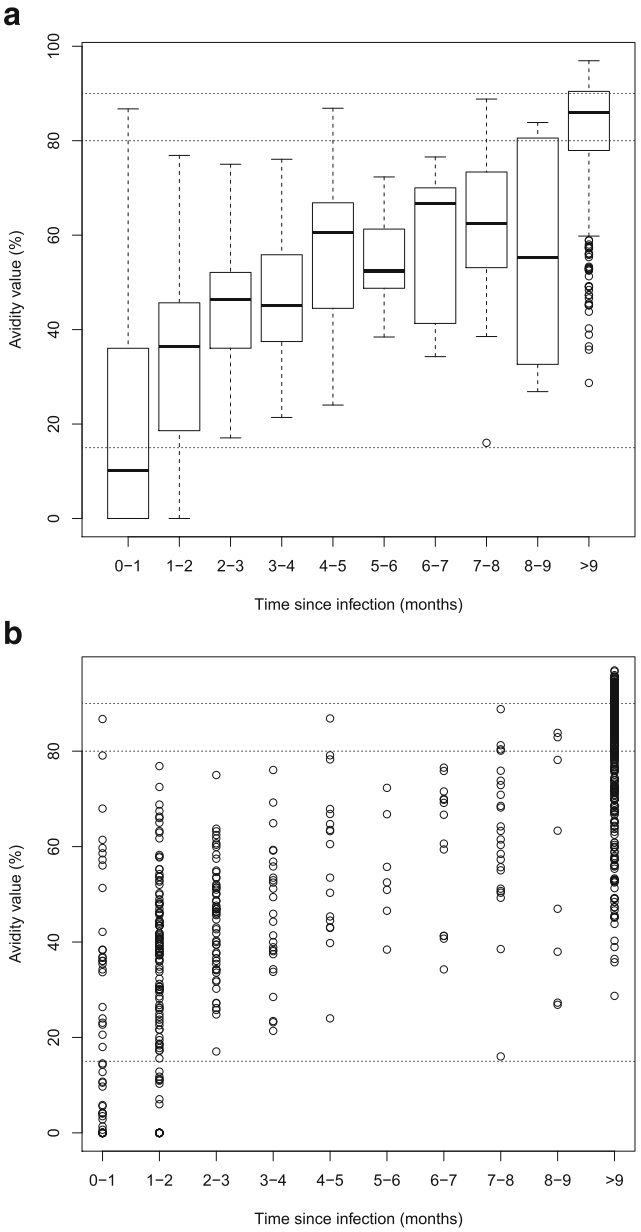

Fig. 1 Elecsys Toxo avidity results of 741 serum samples from pregnant women according to time since infection. **a** For each time point after infection, the median value is represented by a wide horizontal bar, while the median ± 1 standard deviation is represented by narrow horizontal bars. **b** Each dot represents one serum sample. The time from onset of infection to sampling of serum is given to the nearest month. Interpretation of avidity test results: <70%, low; 70 to 70% ≤ values < 80%, borderline; ≥80%, high

Table 2 Elecsys Toxo IgG avidity results from 741 serum samples classified according to the threshold of 80% (classical interpretation: low avidity <70%, gray-zone 70% ≤ values < 80%, and high avidity ≥80%), and time since infection <4 months and >4 months

Time since infection	Number of samples with Elecsys Toxo IgG avidity result			
	Low avidity <70%	Gray zone 70% ≤ v < 80%	High avidity ≥80%	Total
Infection <4 months	267	5	1	273
Infection >4 months	115	65	288	468
Total	382	70	289	741

The threshold of avidity value defined by the manufacturer was 80%. The avidity values higher than 80% excluded an infection less than 4 months. Sensitivity was 61.5% and specificity was 99.7%; the PPV of high avidity was 99.7%, and the NPV of low avidity was 60.2% (Table 2).

Threshold determination to confirm an infection had occurred within 3 months or >9 months

Figure 1b shows that the threshold of 90% could be used to exclude an infection in the last 9 months with 100% specificity, 28.1% sensitivity, 100% PPV, and 54.4% NPV (Table 3). The threshold of 15% could be used to exclude an infection more than 3 months with 100% specificity, 25.1% sensitivity, 100% PPV, and 72.7% NPV (Table 4).

Discussion

The potential for the Elecsys Toxo IgG Avidity assay to exclude an infection in the previous 9 months may allow exclusion of an infection during pregnancy, avoiding serious adverse consequences which can cause emotional distress for the patient [19]. This interpretation of the results could be valuable in countries without monthly screening. In our study, the threshold defined to exclude acute toxoplasmosis in the case of chronic infection was an avidity index result of 90%. This sensitivity was low

Table 3 Elecsys Toxo IgG avidity results from 741 serum samples classified according the threshold of 90% and time since infection <9 months and >9 months

Time since infection	Number of samples with Elecsys Toxo IgG avidity result		
	<90%	≥90%	Total
Infection <9 months	342	0	342
Infection >9 months	287	112	399
Total	629	112	741

Table 4 Elecsys Toxo IgG Avidity results from 741 serum samples classified according to the threshold of 15% and time since infection <3 months and >3 months

Time since infection	Number of samples with Elecsys Toxo IgG avidity result		
	<15%	≥15%	Total
Infection <3 months	62	185	247
Infection >3 months	0	494	494
Total	62	679	741

(28.1%) because the sera samples were mainly collected at the time of delivery from women with periconceptional seroconversion and the time between the infection and the sample were estimated closer to 9 months.

Furthermore, confirmation of recent infection can avoid erroneous classification of an acute infection as a chronic infection, especially when IgM results are very low or absent [20]. Many studies have verified the significance of findings of the IgG avidity test to discriminate acute infection [2]. However, no commercial kit has been validated for confirming recent infection because low avidity test results are not necessarily observed in acute infection. The possibility of confirming recent infection was studied with the Elecsys Toxo IgG Avidity assay. This study was performed on 273 serum samples collected from patients in the acute phase of toxoplasmosis, i.e. less than 4 months with known time of infection. The threshold defined was an avidity index of 15% and the number of serum samples with an avidity result <15% was 62/273 samples collected <4 months after infection.

The sensitivity and specificity of avidity tests have been calculated in different ways in several studies. Candolfi et al. [18] investigated patients with acute toxoplasmosis to assess sensitivity and patients with chronic toxoplasmosis to assess specificity. Thus, sensitivity was high (100%) and represented the specificity evaluated in our investigation or other studies [7]. In another study, Villard et al. [14] estimated the proportion of low avidity results (sensitivity), the PPV, and the NPV of a low avidity result for the acute toxoplasmosis population. Similar results were calculated using the proportion of high avidity results seen in the latent toxoplasmosis population. Specificity was not calculated. Others authors [10, 15, 21] did not calculate sensitivity and specificity. Thus the comparison of the results of different studies is not always possible.

In conclusion, the Elecsys Toxo IgG Avidity assay (index ≥ 80) excluded an infection of less than 4 months with a sensitivity of 61.5% and a specificity of 99.7%. Threshold values of 90% and 15% could be used to exclude an infection more than 9 months and less than 3 months, respectively, with 100% specificity. Thus, using serum samples collected in the second or third trimesters of pregnancy, Elecsys Toxo IgG Avidity assay results ≥90% established that the infection occurred before pregnancy (more than 9 months). An Elecsys Toxo IgG Avidity assay result ≤15% confirmed a very recent infection of less than 3 months.

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Compliance with ethical standards

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Conflict of interest The Laboratory of Parasitology and Mycology have received funding from bioMérieux, Abbott Laboratories, and Roche Diagnostics.

Ethical approval The samples used in this study are part of the collection declared to the French Ministry of Health under the number DC-2008-582.

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