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ORIGINAL ARTICLE

Interpretation of very low avidity indices acquired with the Liaison XL Toxo IgG avidity assay in dating toxoplasmosis infection

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Abstract

Congenital toxoplasmosis is an important cause of complications in pregnancy. Toxoplasmosis is often asymptomatic and thus serological tests are usually performed to screen for it. A first serum which exhibit both IgG and IgM may be due to nascent toxoplasmosis seroconversion, non-specific IgM reaction, or residual IgM. The IgG avidity test has been proposed to identify latent infections. A high index excludes recent toxoplasmosis whereas an intermediate or low index only suggests a recent infection, the caveats being that some people with latent *Toxoplasma gondii* infection show IgG with low or intermediate avidity. In this study, we investigated the ability of the Liaison XL Toxo IgG avidity (DiaSorin, Saluggia, Italy) assay to confirm recent infection when IgG avidity index is very low (≤ 0.1). Four thousand two hundred ninety-seven sera exhibiting both IgG and IgM were included and avidity was performed on the Liaison device according to the manufacturer's recommendations. One hundred twenty-six sera on the 297 sera which exhibited very low IgG avidity indices (≤ 0.1) could be exploited: 97% of sera with IgG avidity indices < 0.05 actually corresponded to recent infection (less than 3 months). A similar but less pronounced trend was observed for the sera exhibiting indices between 0.05 and 0.1 (69% corresponded to recent infections). The IgG avidity index data we obtained with the Liaison XL Toxo device are similar to those obtained with other devices. This body of consistent results underlines the interest of very low IgG avidity indices as a sign of probable recent toxoplasmosis.

Keywords Congenital toxoplasmosis · Recent infection · Low IgG index avidity

Introduction

Toxoplasmosis is a globally distributed zoonotic infection caused by the protozoan parasite *Toxoplasma gondii*. It can be transmitted vertically, resulting in congenital toxoplasmosis. This latter is an important cause of complications in pregnancy such as miscarriages and severe fetal or newborn impairments. The vertical transmission risk increases with gestational age: from 25% in the first trimester, to 65% in the third trimester in untreated women. However, fetal impairment tends to be worse when transmission occurs early in pregnancy [1–4]. Toxoplasmosis is often asymptomatic in pregnant women and thus serological tests to detect immunoglobulin G (IgG) and immunoglobulin M (IgM) are usually performed to screen for it. The presence of IgM may be due to nascent toxoplasmosis seroconversion, a non-specific IgM reaction or residual IgM. Consequently, a first serum that exhibits both IgM and IgG is problematic as concerns diagnosis [2, 5–8]. In such cases, the IgG avidity test first described in 1989 by Hedman et al. has been proposed to identify latent infections on initial testing: a high index excludes recent toxoplasmosis. In contrast, an intermediate or low index only suggests a recent infection, the caveat being that some people with latent *T. gondii* infection show IgG with low or intermediate avidity [6, 7, 9, 10]. Pregnant women in France benefit from serological toxoplasmosis screening during the first trimester and those who are seronegative monthly follow-up until delivery. The objective of this screening program, in place since 1978, is to promptly identify and treat maternal infection [1, 2, 11]. Indeed, recent studies performed in France and other

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European countries have shown that early treatment of toxoplasmosis during pregnancy lowers transmission and sequelae rates [12, 13]. In a meta-analysis of individual patient data, Peyron et al. demonstrated that transmission rates were significantly reduced by half when treatment was initiated at 3 weeks versus 8 weeks after the estimated date of maternal infection. A short delay between maternal infection and treatment initiation was also associated with a significant decrease in ocular lesions over a 2-year follow-up period [11, 14]. Considering these observations on the importance of early treatment, an IgG avidity index value offering a sure diagnosis of recent toxoplasmosis would be of great value. Several studies have already demonstrated the interest of defining new IgG avidity index thresholds, linking particularly very low indices to recent toxoplasmosis on Elecsys (Roche Diagnostics) and Architect (Abbott) devices [3, 15]. The aim of our study was to investigate the ability of the Liaison XL Toxo IgG avidity (DiaSorin, Saluggia, Italy) assay to confirm recent infection when IgG avidity index values are very low. To our knowledge, our work here is the first to look at very low IgG index avidity on the Liaison XL device.

Materials and methods

Serum samples

Sera were collected in the Pitié-Salpêtrière Hospital (Paris, France) and in several laboratories located throughout France between January 2016 and October 2017. These sera were sent to our laboratory, an element of the national reference center for toxoplasmosis, mainly because they exhibited both IgG and IgM. Consequently, determining the date of the *Toxoplasma* infection was necessary (the presence of IgM was attested by at least one serological method) and IgG avidity was determined in these cases.

Methods

Determination of IgG and IgM titers

The Liaison XL system is a fully automated, magnetic microparticle chemiluminescent system within which light responses are measured as relative light units (RLUs) [16]. All serological tests to determine the presence of specific IgG and IgM on sera were performed following the manufacturer's recommendations. IgG results were expressed as international units per milliliter (IU/mL) and interpreted as negative when < 7.2 and positive when ≥ 8.8 as per the Liaison XL threshold values. IgM results were expressed as an index (AU/mL) and interpreted as negative when < 6 and positive when > 8, also as per the Liaison XL threshold values.

Dates of the infection

Dates of infection were established either from the dates of consecutive negative and positive results or from the first positive sample (positive IgM and weakly positive IgG followed by a twofold or greater increase on a second sample taken at least 2 weeks later) based on the kinetics of antibody production, with stable IgG rates—to conclude to a stability of IgG, a period of 3 weeks is necessary—and twofold increases in IgG rates respectively excluding and indicating infection within the 2 months preceding the date of the first sample collection. Concerning seroconversion and for case selection, we arbitrarily defined 3 months as the maximum period of time between the collection of negative and positive samples.

Avidity measures

To determine the IgG avidity index, the Liaison XL method relies on the progressive increase of the avidity of the antibody for its target antigen during the course of natural immunity following infection. The strength of antibody binding can be evaluated by introducing a washing step using a dissociating buffer, i.e., urea on the Liaison device. This washing step removes low-avidity antibodies associated with recently acquired infections [1]. The resulting titer of detectable IgG is used to calculate a ratio of titers obtained from urea-treated and urea-untreated samples. IgG must be ≥ 8.8 IU/mL to enable the calculation of the IgG avidity index. Importantly, however, the manufacturer's technical specifications state that the results from samples exhibiting a titer of IgG < 15 IU/mL have to be interpreted cautiously. We considered an index ≥ 0.300 as representative of high avidity, an index ≥ 0.200 but < 0.300 as representative of intermediate avidity, and an index < 0.200 as representative of low avidity. Finally, if a urea-treated sample exhibited a result below 1.5 U/mL, leading to a luminometry index outside of the measurement limits, it was considered low avidity. According to the manufacturer's specifications, an IgG avidity index higher than 0.300 excludes *Toxoplasma* infection within the last 4 months [16]. The date of *T. gondii* infection could not be confidently established in cases where the IgG avidity index was below 0.200 since cases of latent infection with an absence of IgG avidity maturation have been observed under that threshold as well [6]. Thus, in this study, we focused on sera exhibiting a very low IgG avidity index, i.e., ≤ 0.100.

Results

A total of 4297 sera exhibiting both IgG and IgM were included in the study. IgG avidity indices were high (≥ 0.300) for 3072 sera (71%) and intermediate or low (< 0.300) for 1225. Among these latter, 297 sera exhibited very low IgG avidity

indices (≤ 0.1). This subset of sera comprised 79 sera with avidities ≤ 0.01 (outside of the measurement limits of the technique) and 218 with IgG avidity indices ranging from 0.0103 to 0.0998. Of these 297 very low IgG avidity sera, data were lacking for 171, i.e., only one sample was collected or an interval greater than 3 months separated the collection of two samples. Thus, 126 patient sera had exploitable data and their dates of infection were determined as explained above. The results are presented in Fig. 1 and Table 1.

Discussion

The retrospective study we present here is the first to investigate the ability of the Liaison XL Toxo IgG avidity assay to confirm recent *T. gondii* infection via very low IgG avidity indices (≤ 0.1). Ninety-seven percent (70 of 72) of exploitable sera with IgG avidity indices ≤ 0.05 actually corresponded to recent infection (less than 3 months): consequently, if we choose a threshold of 0.05, the PPV is 97%. A similar but less pronounced trend was observed for the 54 sera exhibiting indices between 0.05 and 0.1, with 37 of them (69%) corresponding to recent infections. In addition, beyond the small number of patients for whose date of toxoplasmosis was greater than 2 months, we cannot exclude the fact that there are infections of less than 3 months. Unfortunately, the sera available in our laboratory did not enable us to answer this question. Only 126 of the 297 (42%) sera with very low IgG avidity indices (≤ 0.1) could be exploited in our study; the sampling situations for the other 171 did not permit their assessment. The explanation for this problem resides in the fact that many of the laboratories sent us sera only when they were unable to reach a conclusion themselves. As soon as they got a second serum sample, the date of the infection was much easier to establish and they no longer required our center's expertise. However, our inability to assess all the sera caused no evident bias, and it is unlikely that it drastically changed the proportion of recent infection in our series. Furthermore, our findings are congruent with those published using other reagents and devices. Equally, the finding that 71% of sera from patients presenting both IgG and IgM showed a high IgG avidity index and then excluded *Toxoplasma* infection within the last 4 months is consistent with the fact that the presence of IgM can be due to residual IgM: Gras et al. showed that IgM-positive test ($n = 446$, method: ISAGA) results persisted beyond 2 years by 27% of women [5].

Indeed, an increasing body of data supports the association of very low IgG avidity and recent infection, whatever the device or method used to measure avidity indices [3, 15]. For example, Murat et al. examined the ability of the Elecsys and Architect assays to determine the time of *T. gondii* infection in a group of pregnant women at different stages of infection [15]. Concerning the Elecsys assay, they reported that in all but one of their samples ($n = 32$), an avidity value less than 19% corresponded with an infection dating to less than 3 months (the exception was a very low avidity value taken 5 to 6 months after infection onset). For them, the Architect system performed similarly: all but one of their samples ($n = 18$) with avidity < 17% corresponded to sera collected less than 2 months after infection (the exception here was a low avidity sample taken more than 9 months after infection). These results suggest that very low avidity values obtained with the Elecsys or Architect systems are almost always associated with recent infections. Fricker-Hidalgo et al. also determined threshold values using the Elecsys Toxo IgG avidity method and reported that values ≤ 15% excluded infections

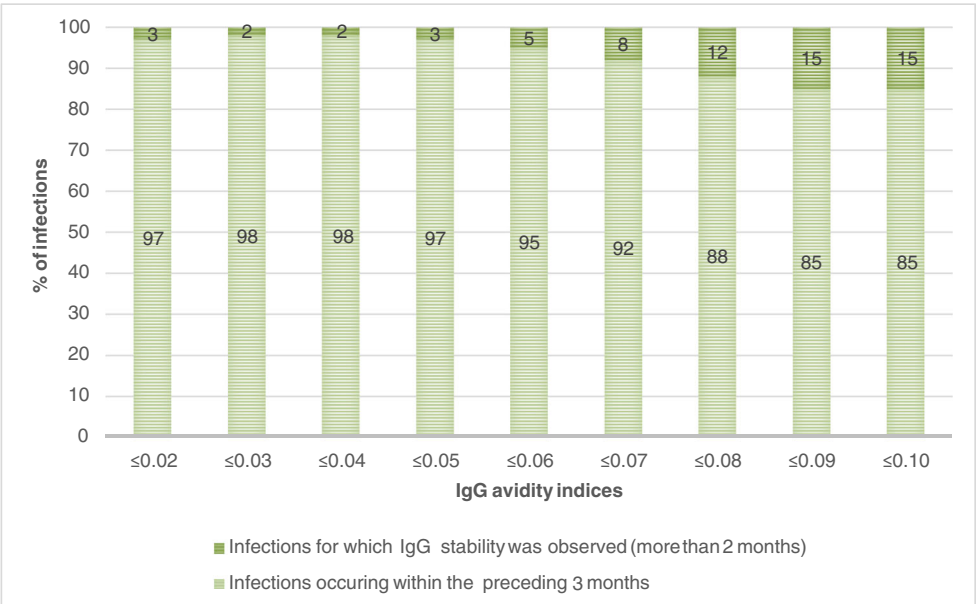

Fig. 1 Date of toxoplasmosis infection according to the thresholds of IgG avidity indices

Table 1 Date of toxoplasmosis infection across the very low IgG avidity index intervals

IgG avidity index threshold	≤ 0.02 (a)	[0.02–0.03]	[0.03–0.04]	[0.04–0.05]	[0.05–0.06]	[0.06–0.07]	[0.07–0.08]	[0.08–0.09]	[0.09–0.1]
Seroconversion	19	5	6	7	3	1	3	5	4
Significant increase in IgG	18	3	6	6	6	6	1	5	3
Total number of recent toxoplasmosis cases	37	8	12	13	9	7	4	10	7
Stability of IgG (b)	1	0	0	1	2	3	5	5	2

(a) Includes cases where the IgG avidity index could not be determined; (b) patients for which the date of toxoplasmosis was greater than 2 months (stability of IgG between two samples): for these patients, no prior serology was available (date is based only on available serological analysis in our laboratory). Consequently, we cannot differentiate a latent infection with no maturation of the avidity index of the IgG from a recent infection. No supplementary information could have been obtained as concerns a potential treatment with spiramycin. However, this one does not affect the kinetics of IgG production

dating to more than 3 months [3]. And furthermore, Candolfi et al. reported that low avidity values lent support to the diagnosis of acute infection [17]. Fricker-Hidalgo et al. and Murat et al. also investigated the meaning of very high avidity and its link with latent toxoplasmosis [3, 15]. They reported strong trends between very high avidity values obtained with the Architect or Elecsys assays and sera collected more than 9 months after infection onset. Particularly, avidity values > 90% were exclusively reported in sera collected more than 9 months after infection. Considering these observations, it appears that both very low and very high avidity indices can date infections in pregnant women whose sera exhibit IgG and IgM antibodies for toxoplasmosis.

From a clinical point of view, our results and those obtained by other teams on different devices should encourage manufacturers to adapt their recommendations for interpreting the results of avidity assays. This is all the more important given the literature's consistent reports of good outcomes in the setting of congenital toxoplasmosis when treatment is initiated shortly after maternal infection. Several studies have, for example, shown that rapid treatment decreases vertical transmission and improves outcomes [11, 18]. Therefore, we suggest that when physicians observe a very low IgG avidity index on a serum sample from a pregnant woman, they should initiate treatment immediately, without waiting for the results of a confirmatory sample. Indeed, this latter must be taken at least 2 weeks after the first and thus imposes a delay in treatment, potentially deleterious for the patient and her child. A very low avidity index (≤ 0.05) should also be seen as an indication for reinforced ultrasound surveillance of fetal development given that our study shows that very low avidity is highly suggestive of recent infection.

Since the 1960s, spiramycin (reduces maternal parasite burden with little crossing of the placental barrier) until delivery has been the standard of care in France for pregnant women diagnosed with toxoplasmosis. Prenatal testing is also proposed to these patients. However, in this setting, the sixteenth week of gestation and the fourth week after the maternal infection must be attained before amniocentesis can be done. Currently in France, spiramycin can be switched to pyrimethamine-sulfonamide (sulfadoxine or sulfadiazine) in combination with folinic acid, but only in the case of a positive antenatal result [1]. However, in other countries such as Austria and Germany, pyrimethamine-sulfadiazine is commonly used for prophylaxis after 16 weeks of gestation. A recent randomized clinical trial by Mandelbrot et al. sought to compare the efficacy of pyrimethamine and sulfadiazine versus spiramycin to reduce placental transmission [19]. Those authors observed a lower transmission rate with pyrimethamine and sulfadiazine (18.5%) compared to spiramycin (30%) but the difference did not reach statistical significance. Consequently, a definitive conclusion will require further randomized studies. Their data nonetheless contribute evidence in favor of using pyrimethamine and sulfamide for the prevention of toxoplasmosis transmission after the first trimester of pregnancy.

To conclude, the IgG avidity index data we obtained with the Liaison XL Toxo device are similar to those obtained with other devices. This body of consistent results underlines the interest of very low IgG avidity indices as a sign of probable recent toxoplasmosis. Nevertheless, this result should be verified by controlling the serology 3 weeks later (when no prior negative serology is available) to confirm acute infection. The high positive predictive value of very low avidity for recent toxoplasmosis should be taken into account for new recommendations on toxoplasmosis treatment in pregnant women.

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