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Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity

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Summary. - The development of IgG avidity assays has revolutionised serological diagnosis of *Toxoplasma* infections. The measurement of IgG avidity has shown its power in various clinical settings, especially in situations where timing and differentiation of primary and secondary infections is crucial. However, no laboratory test performed alone is self-sustained, whereby the diagnostic strategy of choice is sequential (or combinatorial) use of high-quality IgG, IgM, IgA and IgG-avidity assays. The impact of IgG avidity measurement will be discussed in five clinical scenarios: acute acquired infection, primary infection during pregnancy, congenital toxoplasmosis, ocular toxoplasmosis and *Toxoplasma* infection in immunocompromised patients. All in all in toxoplasmosis, superior diagnostic performance is achieved by appropriate combinations of serological, culture-based and PCR techniques.

Key words: Toxoplasma, IgG avidity, diagnosis, serodiagnostic assay, congenital toxoplasmosis, ocular toxoplasmosis, toxoplasmosis during pregnancy, enzyme immunoassay.

Riassunto *(Sierodiagnosi della toxoplasmosi. L'impatto della valutazione dell'avidità delle IgG).* - Lo sviluppo dei saggi di avidità delle IgG ha rivoluzionato la diagnosi sierologica delle infezioni da *Toxoplasma*. La misura dell'avidità delle IgG ha mostrato il suo potere in vari ambienti clinici, specialmente in situazioni in cui diviene cruciale il tempismo e la distinzione tra infezione primaria e secondaria. Comunque, nessun test condotto da solo si autosostiene, e quindi la strategia diagnostica di scelta è l'uso sequenziale (o combinatoriale) di saggi di qualità elevata per IgG, IgM e IgA e IgG avidità. L'impatto della misurazione dell'avidità delle IgG potrebbe essere discusso in cinque scenari clinici: infezione acquisita acuta, toxoplasmosi congenita, toxoplasmosi oculare e infezione da *Toxoplasma* nei pazienti immunocompromessi. Tutto sommato, per la toxoplasmosi è richiesta una prestazione diagnostica superiore attraverso combinazioni appropriate di tecniche sierologiche, colturali e di PCR.

Parole chiave: Toxoplasma, IgG avidità, diagnosi, saggio sierodiagnostico, toxoplasmosi congenita, toxoplasmosi oculare, toxoplasmosi in gravidanza, saggio immunoenzimatico.

Introduction

Toxoplasma gondii has a worldwide distribution and is one of the most prevalent infectious agents in humans. In immunocompetent individuals, acute infection is usually asymptomatic and spontaneous recovery is the rule. However, primary infection during pregnancy constitutes a great diagnostic challenge, by predisposing the offspring to the risk of congenital toxoplasmosis [1]. The most common manifestation of congenital toxoplasmosis is retinochoroiditis, even if some cases of ocular toxoplasmosis have been reported. In immunocompromised patients, toxoplasmosis is almost always due to reactivation of a latent infection.

The conventional single-serum assays do not make a clear distinction between a recent primary and chronic infection. The tendency of specific IgM to persist for a long time even at high levels has been verified in several studies [2-6]. After its introduction in serodiagnosis of toxoplasma infections [7], the measurement of IgG avidity has proved to be a highly useful procedure, especially in combination with conventional serological assays.

Antibody affinity and avidity

The binding affinity of an antibody for its antigen is, according to the law of mass action, the equilibrium constant K = ka/kd in the reaction: Ab+Ag<ka/kd>Ab x Ag, where ka and kd are the respective rate constants of association and dissociation, Ab is the antibody, Ag is the antigen, and Ab x Ag is the immune complex formed. The term intrinsic affinity is only applicable to

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uniform and immunologically monovalent reactants and is confined within *in vitro* conditions. The term avidity (or functional affinity) denotes the net antigen-binding force of populations of antibodies, and is preferable to the term affinity [8].

As a rule, IgG avidity is initially low after primary antigenic challenge and, by antigen-driven B cell selection, increases during the subsequent weeks and months. IgG antibodies representing pre-existing immunity as well as those obtained at any stage after secondary response exhibit high avidity. The proportion of high-avidity IgG increases progressively after primary immunization, even if the low-avidity IgG might persist at a low level [9, 10].

Different IgG avidity assays

A number of different methods have been used in measurement of IgG affinity/avidity; however most of those are laborious and unsuitable for diagnostic laboratories. During the 1980s modifications of EIA were developed, in which a protein-denaturing agent is included either in the serum diluent [11-13] to prevent formation of the antigen-antibody complex, or in the post-IgG washing fluid to dissociate the antigen-antibody bond [14, 15].

Also the avidity result can be calculated in different ways, i.e. by the shift of antibody titration curve due to a single concentration of protein denaturant [11-13], or by the concentration of protein denaturant that reduces the EIA absorbance by 50% [14], or by the proportion of IgG that is resistant to a single concentration of protein denaturant, expressed either by the ratio of IgG-EIA titres or by the ratio of IgG-EIA absorbances from single dilutions of serum. These latter approaches are the most popular in diagnosis. The benefit of comparison of end-point titres (with/without urea) is that the IgG avidity result (expressed in percent) is independent of IgG concentration. In general, IgG-avidity tests for various pathogens have shown their value both in the diagnostic front line and in confirmation of results by other (e.g. IgM) tests in distinguishing primary from secondary infections and in assessing the time of the initial antigenic challenge [13-21].

Avidity measurement in serodiagnosis of *Toxoplasma* infections

An assay measuring the antigen-binding avidity of IgG antibodies against *T. gondii* was developed in 1989 [7]. By this technique acute infection can be diagnosed using a single serum specimen, and further, primary infection can be distinguished from secondary (recurrent or reactivated) infections. The quantity of *Toxoplasma*-IgG has been shown to be an unreliable indicator of recent infection [1] and the long-term persistence of *Toxoplasma*-specific IgM-antibodies poses problems in timing of infection, especially in pregnant women.

Simple indices (with/without urea) obtained from single dilutions of serum have turned to be insufficient for toxoplasma-IgG avidity determination [7, 22, 23]. Because the end-point titration-based IgG-avidity technique is laborious and reagent consuming, a logistic procedure for avidity calculation has been developed and computerized [24]. Its diagnostic performance is similar to that of the reference method while being twice as cost-effective.

Today, several commercial IgG avidity assays are available. Not many cross-evaluations of their diagnostic performances have been published. A high correlation between two IgG assays modified in-house for avidity determination was observed with immuno-compromised patients and healthy adults [25]. A high correlation was also observed between two commercial IgG-avidity assays, with immunocompetent pregnant women ([26] Alvarado-Esquivel, unpublished data). In another study, a poor correlation was found between 3 commercial IgG-avidity assays [27]. The latter report, however, has been criticized for a number of important points [28]. Of note, avidity results may be strongly affected by the protein composition of the antigen preparation [29].

In the following, we will discuss the impact of IgG avidity measurement in five clinical scenarios: acute acquired infection, primary infection during pregnancy, congenital toxoplasmosis, ocular toxoplasmosis and *Toxoplasma* infections in immunocompromised patients.

Acute acquired *Toxoplasma* infection

The vast majority of acquired infections in healthy individuals are benign and either asymptomatic or with vague symptoms. Lymphadenopathy is the most common manifestation in non-pregnant and pregnant individuals, causing 3-7% of clinically significant cases [30, 31]. Uncommonly, lymphadenopathy may persist for months and may thus be confused clinically and/or histologically with neoplastic diseases (such as lymphoma and carcinoma), particularly of the head, neck and breast. For the clinician, such cases present a diagnostic challenge.

The diagnosis of acute *T. gondii* infection should be based upon the demonstration of a rise in antibody (IgG or Dye Test) titres in serial serum samples, but often only one sample is available. Further, *Toxoplasma*-specific IgM and IgA antibodies tend to

Table 1. - Diagnostic tests for toxoplasmosis in different clinical scenarios

Clinical scenario	Diagnostic tests
Immunocompetent patients	
Acute infection suspected	*Toxoplasma*-IgG and toxoplasma-IgM antibodies, followed by *toxoplasma*-IgG –avidity (if toxoplasma-IgM positive)[(a)]
Immunity	*Toxoplasma*-IgG- antibodies.
Ocular toxoplasmosis	Serology (IgG and IgM antibodies) for verification of past exposure; seldom useful to show acute infection.
Congenital toxoplasmosis	Serology (IgG, IgM and IgA antibodies) of the infant and the mother. *Toxoplasma*-PCR (and culture, if available) from blood, urine, CSF.
Immunosuppressed patients	Serology (IgG and IgM antibodies) for verification of past exposure; a second sample is needed to show reactivation. *Toxoplasma*-PCR (and culture, if available) to verify ongoing active infection; blood, CSF.

(a) Additional information may be obtained by measuring IgA and IgE antibodies.

persist long. IgG avidity has shown its usefulness in diagnosis of acute *Toxoplasma* infections in several studies [7, 32-35]. As a sign of primary infection even seroconversion can in some cases be simulated by a sero-reversion following latent infection [36]. On the other hand, immunosuppressive conditions may affect the kinetics of IgG avidity maturation [37]. Pregnancy, possibly augmented by anti-toxoplasma pharmacotherapy, may have a similar effect [33]. Therefore, the current consensus is that measurement of IgG avidity serves best in ruling out (by high avidity) rather than ruling in (by low avidity) a recently acquired infection [33-35, 38, 39]. This also accentuates the importance of careful determination of cut-off values in commercial IgG avidity assays. In cases of equivocal (borderline) avidity results, *follow-up* samples should be collected.

Primary infection during pregnancy

On account of the diversity or absence of symptoms, the diagnosis of *Toxoplasma* infection during pregnancy has to be based on maternal serology. The effects of maternal *Toxoplasma* infection on the fetus are determined by the stage of pregnancy; primary infection in early gestation may result in severe clinical disease, whereas congenital toxoplasmosis following third trimester maternal infection is usually subclinical at birth [40].

Concerning the role of IgM, in general, most investigators would agree that the best use of IgM antibody detected by a sensitive test is when it is absent; thus a woman with IgG but without IgM is extremely unlikely to have toxoplasmosis acquired recently [41]. An accurate diagnosis is particularly important for pregnant women: it must be obtained early, for timely therapeutic decisions. Because the level of specific IgG as such is an unreliable indicator of infection acuteness, the mothers presenting with antibodies in their first sample constitute the greatest challenge in diagnosis and therapy.

We have shown that, specific IgG of low avidity is an excellent single-serum indicator of recent primary *Toxoplasma* infection [39], as verified by other studies [34, 41-44]. Even under screening conditions as assessed by the third-trimester sera and retrieval of the first-trimester antibody status, even the positive predictive value of a low avidity result was very high. Also the predictive value of a high-avidity result against toxoplasma infection during early pregnancy was excellent. The best IgG- avidity assays are also highly sensitive. With the avidity technique we were able to show that, among women presenting with *Toxoplasma* IgM at the time of pregnancy, a high proportion had not actually been infected recently. At present, in light of our 12-year experience in using IgG avidity in daily diagnosis, we continue to recommend as primary tools an IgG assay and a sensitive IgM test. The role of subsequent IgG-avidity measurement is to confirm or to dispute the IgM diagnosis (Table 1). A similar strategy has been recommended by other large-scale studies [34, 41, 42]. When maternal primary infection is diagnosed, either by IgG seroconversion, or by IgM positivity combined with low avidity of IgG, the mother shall be referred without delay for

obstetric consultation and amniotic fluid sampling for PCR. Demonstration of *Toxoplasma*-DNA by PCR in amniotic fluid is useful for verification of fetal infection [45, 46]. In maternal serology, high avidity of specific IgG during the first trimester is a strong indicator against maternal primary infection; the fetuses of such women are at low risk for congenital toxoplasmosis and no interventions are needed in such pregnancies.

In general, the purpose of the primary, sensitive assay is to select a study group of reasonable size which still includes all the suspects. The second, confirmatory test has to be specific. We positioned the avidity assay behind the IgG and IgM tests in order to identify each subject with conventional markers of infection. Also, because the avidity test is dependent on the presence of specific IgG , the paucity of the IgG very early after infection favours IgM examination in the first sample.

Congenital toxoplasmosis

Congenital infection results from maternal primary infection during pregnancy. The rate of mother-fetus transmission increases with gestational age from 20% to 70% [1, 47]. Among the children with intrauterine infection, most are initially asymptomatic; however, by early adulthood the vast majority manifest as retinochoroiditis or neurologic defects [48-50].

Postnatal diagnosis can be complicated by transplacentally acquired maternal IgG or by variability of perinatal IgM findings [51, 52]. Not all congenitally infected children produce detectable levels of specific IgA or IgM. Therefore, a recommendable approach seems to be a combined measurement of IgM and IgA [53]. The isolation of the organism from amniotic fluid, placenta and fetal blood is specific, but may be insensitive and 4 to 6 weeks are required for diagnosis. Reports of the polymerase chain reaction (PCR) have been promising, but so far it has not gained a superior role in postnatal diagnosis. In antenatal diagnosis, however, PCR from amniotic fluid, performed by reference laboratories, has been shown to be both sensitive and specific [45, 46].

In our small series of congenitally infected children, all showed significant maturation of IgG avidity during postnatal follow-up [54]. However, compared to its decisive value in diagnosis of acquired infection, the value of IgG avidity measurement in postnatal diagnosis is less clear. During the first months of life, maternal IgG decreases, whereas the endogenous IgG of the infected newborn persists or increases. Avidity result in the neonate thereby represents that of a mixture of the maternal and the child's own IgG, and is therefore dictated by several factors such as the sampling time and both the titre and the avidity of the mother's and the child's IgG. Yet, postnatal measurement of IgG avidity may give valuable additional information. Another interesting, unexplored possibility is the use of neonatal avidity measurement as a primary screening tool for maternal infections during pregnancy.

In as much low avidity in the neonate suggests maternal primary infection during pregnancy, the child may or may not be infected. During postnatal follow-up a significantly increasing or decreasing avidity result is a pathological finding and indicates antibody synthesis of the newborn and congenital toxoplasmosis. On the other hand, while high avidity during the first weeks of life represents past immunity of the mother, it does not necessarily rule out maternal primary infection during early pregnancy; the highly avid maternal antibodies might conceal endogenous antibody production in the newborn. Since the diagnosis of both maternal and congenital *Toxoplasma* infections requires various serodiagnostic assays and often PCR or isolation, it should be centralised into reference laboratories and university hospitals.

Of particular interest is the IgG -avidity maturation kinetics in children infected during early pregnancy. For comparison, children with congenital rubella syndrome following maternal first-trimester infections exhibit persistence of low avidity of IgG during infancy [55, 56] and even at an older age [57]. The mechanism of this phenomenon is unclear, but tolerance or anergy of helper T-cells is a possibility. During the first trimester T-lymphocytes cannot yet distinguish foreign antigens from self, a fact that could lead to defective avidity maturation in the antibodies of B-cell origin.

Ocular toxoplasmosis

Ocular toxoplasmosis involves principally the uveal tract, manifesting especially as posterior uveitis; *T. gondii* is the most common cause of posterior uveitis. Although the manifestations of ocular toxoplasmosis may be protean [58], retinochoroiditis is the most common lesion. One attack lasts approximately 4 months, and an average lifetime rate is 2.7 attacks [59]. Although the peak incidence of toxoplasmic retinochoroiditis occurs between 15 and 20 years of age, it may occur beyond 60 years [60].

Toxoplasmic retinochoroiditis is usually considered the result of congenital rather than of acquired *Toxoplasma* infection; only about 1.5% of patients with acquired toxoplasmic lymphadenopathy have retinochoroiditis [61-64]. *Toxoplasma* antibodies in the aqueous humour have been detected in patients with active retinochoroiditis [65-67], but, unfortunately,

serological diagnosis of active ocular toxoplasmosis is insensitive [68-70], possibly due to local antibody production. Therefore, the value of serology is limited in ocular toxoplasmosis, and the role of IgG avidity measurement is merely to confirm the presence of a latent infection and to raise the suspicion of an ongoing reactivation [67, 71].

Acquired infection in an immunocompromised patient

Toxoplasmosis in immunocompromised patients is usually due to reactivation of latent infection [72]. However, the parasite may be transmitted by blood or blood products and by transplanted organs [73, 74]. In contrast to the majority of immunocompetent patients, the immunocompromised may have serious clinical manifestations. Although the heart, muscle, skin, and lungs [75] may all be affected, the brain is usually the source of most concern [76, 77]. Toxoplasmic encephalitis is estimated to be the most common focal brain lesion in AIDS, and about 30% of seropositive AIDS patients will ultimately develop toxoplasmic encephalitis [78, 79]. Specific anti-toxoplasma treatment is indicated in immunocompromised patients.

The measurement of IgG avidity may be of some help in diagnosis of *Toxoplasma* infections in immunocompromised patients. Holliman *et al*. assessed the performance of IgG avidity assay in HIV infected patients [42]. They did not observe significant differences between avidity levels in HIV infected patients with or without cerebral toxoplasmosis. We described the first liver transplant recipient with a *Toxoplasma* reactivation verified by IgG avidity [80]. Our patient had been *Toxoplasma*-seropositive with high avidity referring to past immunity already before the first transplantation. Serological follow-up showed a diagnostic rise in *Toxoplasma*-IgG, negative *Toxoplasma*-IgM, and constantly high IgG avidity, indicating a reactivation before the second transplantation. Both donors were *Toxoplasma* seronegative. Polymerase chain reaction (PCR) showed *Toxoplasma*-DNA in blood and in liver biopsy before death of the recipient.

We recommend that solid organ and hematopoietic stem cell transplant candidates be screened for *Toxoplasma* antibodies. The antibody status of the recipient should be known already before transplantation, because in these patients toxoplasmosis may result from reactivation of a latent infection. Furthermore, the possibility of toxoplasmosis is easily forgotten, because of nonspecific symptoms, e.g. fever. The current procedure in our unit for liver transplant recipients includes not only prophylactic use of trimethoprim-sulphamethoxazole, but also determination of *Toxoplasma* serostatus before transplantation. This knowledge helps the clinician to be alert and to react promptly upon clinical suspicion of toxoplasmosis.

Concluding remarks

The development of IgG avidity assays has revolutionized serological diagnosis of *Toxoplasma* infections. The measurement of IgG avidity has shown its power in various clinical settings, especially in situations where timing and differentiation of primary and secondary infections is crucial. However, no laboratory test performed alone is self-sustained, whereby the diagnostic strategy of choice is sequential (or combinatorial) use of high-quality IgG, IgM, IgA and IgG-avidity assays. All in all, superior diagnostic performance is achieved by appropriate combinations of serological, culture-based and PCR techniques.

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