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Parasitology

Serological diagnosis of *Toxoplasma gondii* infection☆ Recommendations from the French National Reference Center for Toxoplasmosis

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ABSTRACT

Toxoplasmosis manifests no clinical signs in 80% of cases in immunocompetent patient, causing immunization characterized by the persistence of cysts, particularly in brain, muscles, and retina. Assessing the serological status, based on testing for serum toxoplasma IgG and IgM antibodies, is essential in cases that are increasingly at risk for the more severe disease forms, such as congenital or ocular toxoplasmosis. This disease also exposes immunosuppressed patients to reactivation, which can lead to more widespread forms and increased mortality. By interpreting the serological results, we can estimate the risk of contamination or reactivation and define appropriate prophylactic and preventive measures, such as hygienic and dietetic, therapeutic, biological, and clinical follow-up, according to the clinical context. We hereby propose practical approaches based on serological data, resulting from a consensus of a group of experts from the French National Reference Center Network for Toxoplasmosis, according to both routine and specific clinical situations.

1. Introduction

In France, a national program for prevention of congenital toxoplasmosis has been set up in 1978 (Villena et al., 2010), and the incidence of toxoplasmic seroconversion during pregnancy in 2012 was estimated at 1.9/1000 susceptible pregnant women (Ambroise-Thomas et al., 2001; Bellali et al., 2013; Nogareda et al., 2014). For the same year, the global prevalence of congenital toxoplasmosis was estimated at 2.58 per 10,000 live; births in France and 204 cases of congenital toxoplasmosis were observed. Of these, 52.5% were diagnosed at birth, and approximately 90% were asymptomatic. Updated findings can be found on the National Reference Center on Toxoplasmosis Web site: http://cnrtoxoplasmose.chu-reims.fr/.

The systems currently in place for monitoring congenital toxoplasmosis in European countries greatly vary and are principally dependent on prevalence rates. A recent investigation aimed to describe these different systems in Europe (Bénard et al., 2008). The results showed that, of the 28 countries investigated, only 4 possessed a specific monitoring system for congenital toxoplasmosis: Italy, Denmark, Germany, and France.

2. Immune response to toxoplasma infection

In 1948, Sabin and Feldman developed a serologic assay called the dye test (Sabin and Feldman, 1948) for conducting serological diagnosis of toxoplasmosis, an infection caused by an intracellular apicomplexan parasite infecting all nucleated cells in the human body. This assay has radically altered our view of this confidential clinical disease, which was previously associated only with a few cases of congenital and ocular toxoplasmosis. The 2 researchers later discovered, along with others, that toxoplasmosis was, in fact, the most widespread parasitic infection in the world that primarily manifested no clinical signs in 80% of cases (Montoya and Liesenfeld, 2004). One section of the population particularly at risk during an acute infection are pregnant women, due to toxoplasma's ability to cross the placental barrier and infect the vulnerable fetus before it can acquire immunity. The other groups at risk are immunosuppressed patients that have never been in contact with toxoplasma parasites (Belanger et al., 1999; Weiss and Dubey, 2009). In its acute infection form, toxoplasma spreads through all the organs, and

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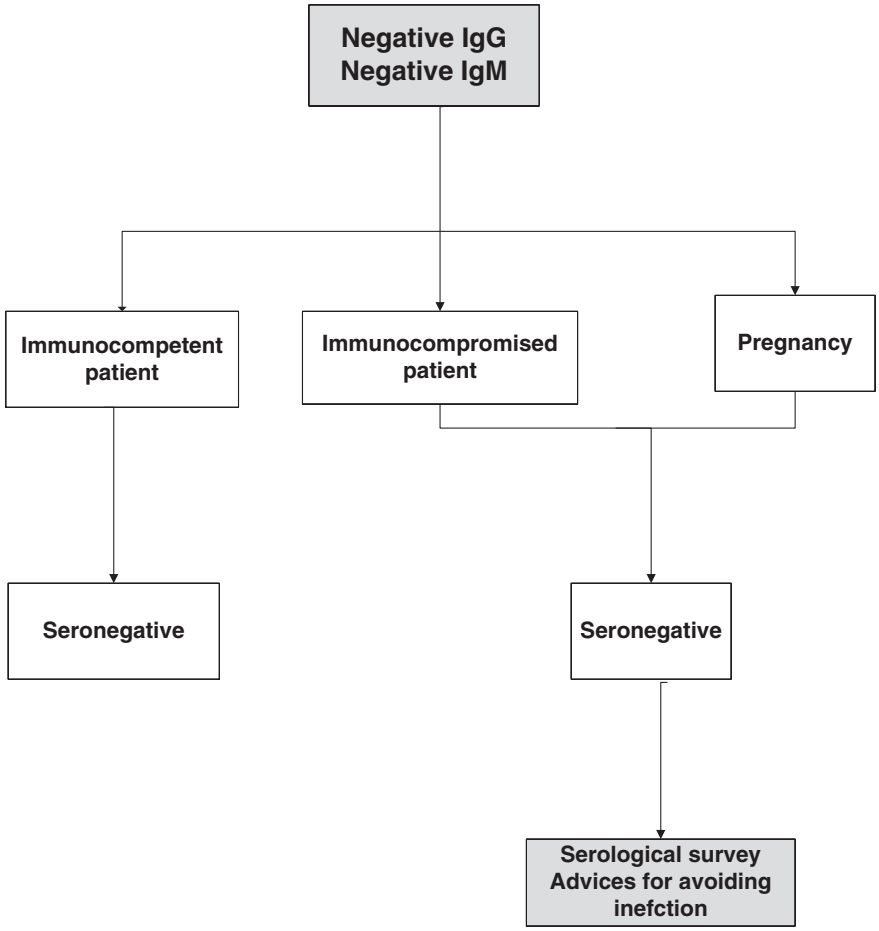

Fig. 1. Advice algorithm for toxoplasma serology with negative IgG and IgM. Adapted from Villard et al., 2011.

the parasite is seen to clear from the body in less than 1 week due to innate immunity and the rise of specific acquired immunity, including humoral immunity (Robert-Gangneux and Darde, 2012). All isotypes are produced by stimulated B cells: namely, IgM, IgG, IgA, and IgE. These antibodies are functionally important as they are known for blocking cell invasion, lysing parasites by activating complement pathways and the catalytic activity of NK and CD8+ T cells, or by phagocytosis. In immune-privileged organs, such as the muscles, brain, and eyes, this parasite remains in cysts in the form of bradyzoites for the rest of the patient's life. Reactivation may thus occur in immunosuppressed patients, such as those diagnosed with AIDS, which can lead to a disseminated toxoplasmosis with a high rate of mortality if no specific treatment is administered (Petersen et al., 2006). However, in the retina, reactivation can occur in perfectly immunocompetent patients, leading to retinochoroiditis (Burnett et al., 1998). Nevertheless, whether there are any clinical signs, detecting toxoplasmic antibodies is key to the serological diagnosis of toxoplasmosis. The ability to identify *T. gondii* infection is still today primarily based on serological assay detection of IgM, IgG, and IgA levels. IgMs are the first antibodies to appear, usually 1 week after the infection. Their levels rise until peaking after 1–3 months. A slow decrease then occurs over the next 9 months until negativation. However, one part of the patient population (9–27%) has been found to exhibit a persistent IgM antibody response that remains for 2 years or more (Bobic et al., 1991; del Bono et al., 1989; Gras et al., 2004). Toxoplasmic IgG appears after 2 weeks of infection and peaks at 3 months. It then remains at a plateau level for 6 months and after 1 year starts to slowly decrease to lower levels until the end of infected subject's life due to the persistence of latent cysts in immune-privileged organs. The antigen binding avidity of these IgG antibodies rises slowly during the first 4 months. The kinetic of IgA production is similar to those of IgM, the peak of IgA is reached later than IgM, and the IgA antibodies persist over 3 or 4 months following infection (Bessieres et al., 1992). The toxoplasma immunological status can be determined by serological screening using various methods that must be adapted to the clinical situation, as well as to the prevalence of toxoplasmosis in the setting where the assays are performed.

The National Reference Center for Toxoplasmosis has established flowcharts providing basic interpretation of toxoplasmic serology in seronegative pregnant women based on the detection of IgM and IgG alone. These flowcharts are embedded in the French program of prevention of congenital toxoplasmosis.

This study sought to define other flowcharts of immunocompetent patients in both routine cases and specific clinical situations, such as congenital toxoplasmosis, immunosuppressed situations, and ocular toxoplasmosis.

3. Methods

It is crucial to choose the right methods at the right cost. However, the choice of method may be informed by the prevalence rates in the country (Stillwaggon et al., 2011) and local or national recommendations for the follow-up and may also depend on the observed clinical situation, for example, involving pregnant women, newborns, immunosuppressed patients, or those presenting with decreased vision. The methods' performance is highly variable (Bobic et al., 2009; Petersen et al., 2005; Villard et al., 2012, 2013), and it can be difficult to choose between the historical assays, such as dye test, hemagglutination, agglutination, immunoglobulin M immunosorbent agglutination (ISAGA),

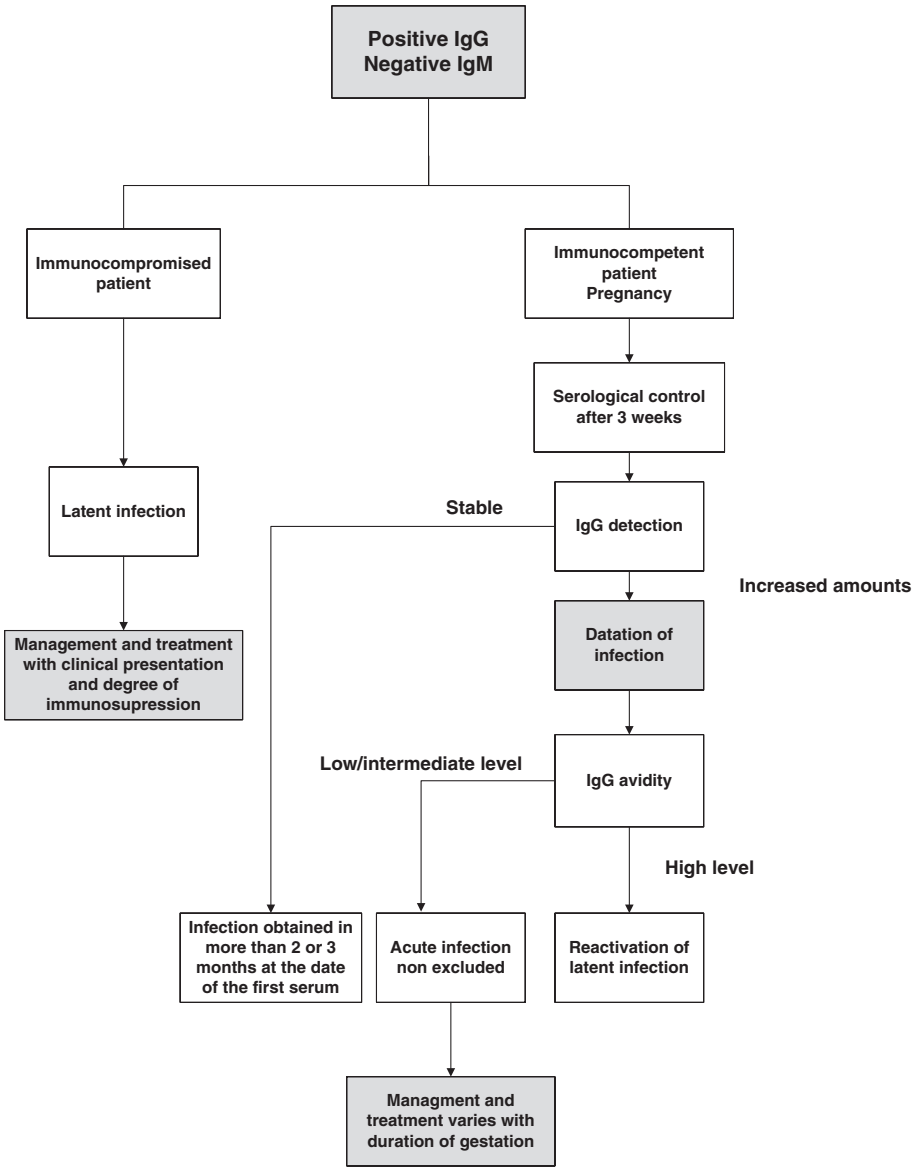

Fig. 2. Serological monitoring for toxoplasma serology with positive IgG and negative IgM. Adapted from Villard et al., 2011.

indirect fluorescent antibody test (IFAT), or the most recent automated ones or even the more confidential methods such as immunoblot (Ashburn et al., 2001; Evans and Ho-Yen, 2000; Franck et al., 2008; Johnson et al., 1989).

These methods can be separated into 2 groups in order to optimize the interpretation of serological data, the first included fast or automated screening and the second used for confirmatory tests. Screening methods are either low on expense or limited to a small amount of serum, e.g., hemagglutination and agglutination, or are automated in cases of a larger screening process like enzyme-linked immunosorbent assay (ELISA) or CLIA (Chemiluminescence immuno assay). Confirmation methods are mostly in-house, complex, or costly methods, such as dye test, IFAT, immunoblot, and ISAGA. These last methods should be handled exclusively in reference centers.

Finally, the last degree of complexity in the serological diagnosis of toxoplasmosis is the global interpretation of the data acquired by these different methods. Interpreting these data can be a difficult exercise, and the vast choice of available complementary methods adds to the complexity of the diagnosis. The conclusions drawn from the resulting data can lead to primary prophylaxis; specific treatment; serological follow-up; and more invasive investigation for toxoplasma forms, such as amniocentesis, bronchoalveolar lavage, or spinal fluid sampling.

4. Routine serological situations

4.1. Absence of IgG and IgM (Fig. 1)

The goal of serology testing in pregnant women is thus to assess if they are at risk, sparse false negatives are allowed, while a false positive is unacceptable as any hygienic and dietetic preventive measures would not be provided. This screening method is based on the detection of toxoplasmic IgG, and the assays should therefore achieve a specificity of over 99.9%.

The absence of IgG and IgM antibodies rules out the possibility of a recent infection more than or equal than to 7 days in absence of recent contamination of less than 1 month, and it is advisable to provide hygienic and dietetic preventive measures (AFSSA, 2006), for pregnant women and immunocompromised patients in these cases. For those, a serological follow-up is required, depending on the clinical situation. Public health authorities in France have

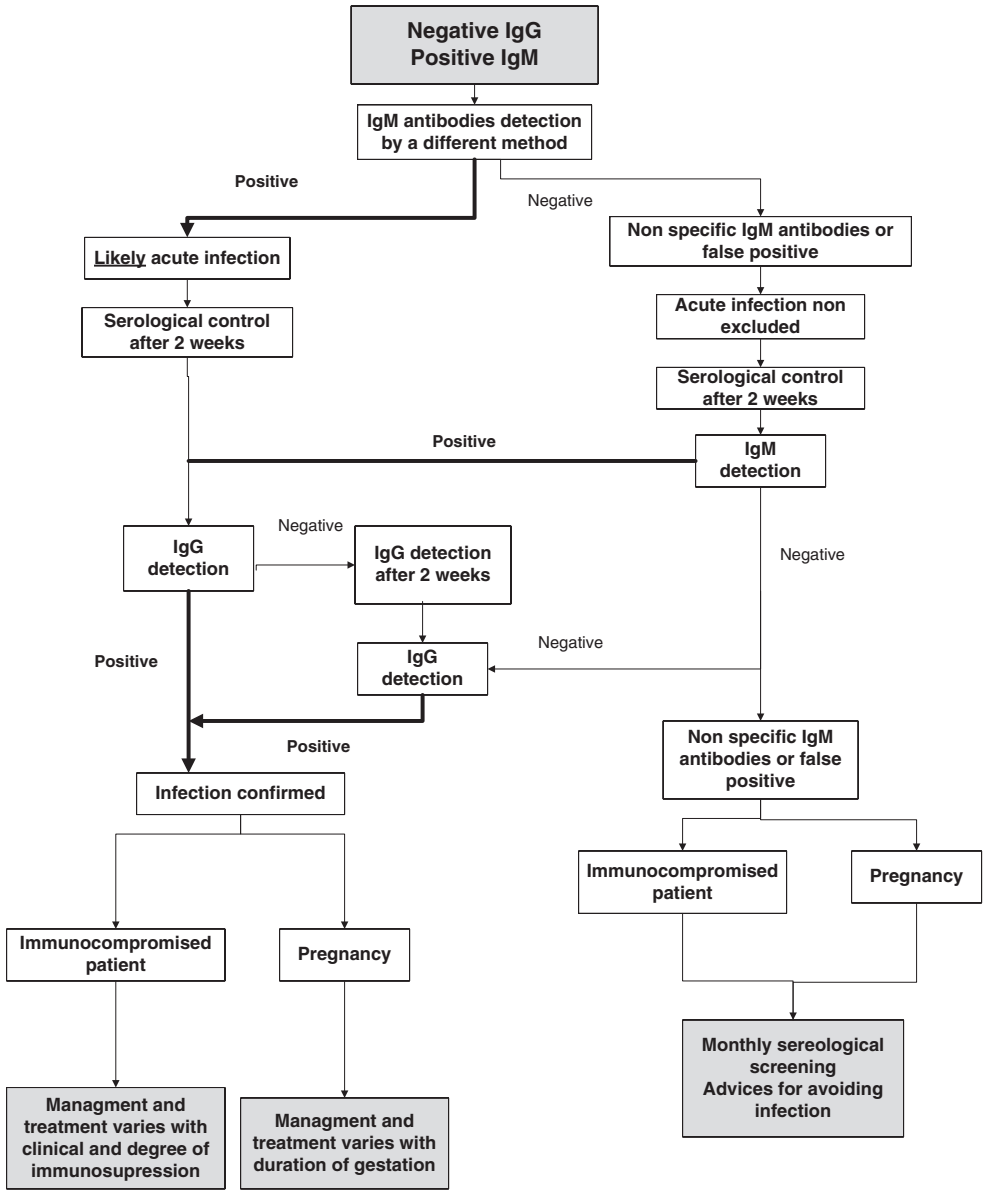

Fig. 3. Serological monitoring for toxoplasma serology with negative IgG and positive IgM. Adapted from Villard et al., 2011.

implemented a congenital toxoplasmosis prevention program that includes a monthly serological screening until delivery (Nogareda et al., 2014) and for immunocompromised patients, every 6 months.

4.2. Presence of IgG and absence of IgM (Fig. 2)

The presence of specific IgG and absence of IgM immediately points to a previous infection, and any kind of serological follow-up can thus be discontinued, as the immunity is assumed to protect the fetus from any reinfection. However, in some cases, the IgM antibodies may be evanescent or even negative (Fricker-Hidalgo et al., 2013). It is typically recommended to perform a second serology 3 weeks later in order to track any potential increase in IgG levels. Stable IgG levels indicate a diagnosis of chronic toxoplasmosis. A significant increase in IgG levels depending on used techniques leads to tests to determine IgG avidity. In cases of high IgG avidity, reinfection or reactivation is strongly suspected. Serological follow-up is unnecessary if this situation occurs in an immunocompetent subject. On the contrary, if IgG avidity is low or equivocal in a pregnant woman, the date of contamination cannot be specified, and adapted management must be initiated, depending on gestational age.

4.3. Absence of IgG and presence of IgM (Fig. 3)

Toxoplasma parasites reach the placenta extremely quickly, and early treatment reduces the risk of mother-to-child transmission, also enabling the disease severity to be reduced (Desmonts et al., 1990; Moncada and Montoya, 2012). The methods should therefore enable very early detection of IgM, the first isotype to appear, and thus, well-adapted management can be started based on amniocentesis antenatal diagnosis and treatment. In this context, the sensitivity of the reagents detecting IgM is heightened. IgG antibodies are, however, crucial in confirming seroconversion and infection. The detection of IgM associated with absence of the IgG should evoke a recent infection, requiring confirmation by a second sampling 2 weeks later. First, the specificity of IgM should be confirmed by means of a reference method using a different technical background, such as ISAGA-IgM or immunofluorescence. If the IgM is not thus confirmed, the presence of nonspecific IgM is strongly suspected or a false-positive reaction. The result of the second sampling conducted 2 weeks later is crucial. Then if another isotype IgG/IgA is detected in addition to IgM, acute infection is confirmed (Gutierrez et al., 1997). If IgM levels remain stable and no IgG

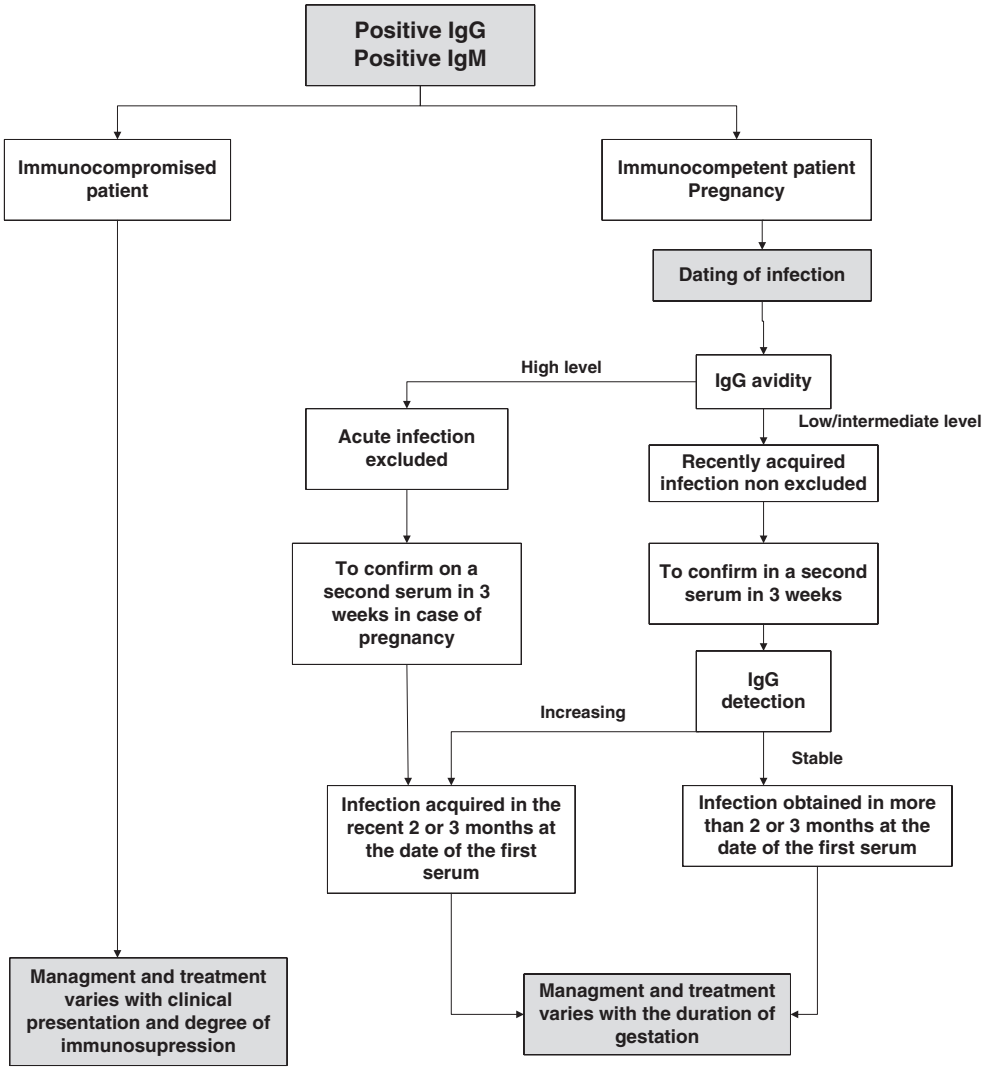

Fig. 4. Serological monitoring for toxoplasma serology with positive IgG and IgM. Adapted from Villard et al., 2011.

seroconversion is observed in a no-treatment context, a serological follow-up should be performed, tracking any IgG seroconversion over the following 2 weeks. The appearance of low concentrations of IgG antibodies is variable according the used tests. A recent study demonstrated a better sensibility with the reactive of immunoblot in the early detection of the IgG and confirmation of toxoplasmic seroconversion (Jost et al., 2011). Acute toxoplasmosis in a pregnant woman necessitates adapted management depending on gestational age. If the patient is immunosuppressed, the treatment method is defined based on clinical background and immunosuppression level.

4.4. Presence of IgG and IgM (Fig. 4)

The above-mentioned presence of IgM detected in the disease's chronic stage has led to efforts to distinguish between the acute and chronic stages in cases where IgM is present in the serum, and measuring IgG avidity is a precious tool in achieving this. The discovery that IgG matures over time resulted in using this property to distinguish an acute phase, based on poorly competent IgG, from a chronic stage, with highly competent IgG (Villard et al., 2013). This method has enabled us to more clearly establish the date of contamination in 1 serum by ruling out recent infection, i.e., typically defined as manifesting in the previous 4 months (Ashburn et al., 2001; Holliman et al., 1994). It has also aided in more precisely determining the risk of transmission to the fetus with regard to pregnancy cases (Evans and Ho-Yen, 2000). Some reference centers (especially in the United States) use differential agglutination (AC/HS) in conjunction with avidity (Montoya et al., 2007). The AC/HS test's window for excluding a recently acquired infection appears to be remarkably longer than that for the avidity test (Dannemann et al., 1990).

During pregnancy, it is necessary to determine as precisely as possible the date of contamination in order to evaluate the risk of transplacental transmission and fetal infection. In the absence of any previous biological test findings, IgG avidity must be assessed, enabling a precise definition of the date of infection (Candolfi et al., 2007). If IgG avidity is high, a recent infection lasting less than 16–20 weeks depending on the commercial assay can be ruled out. If IgG avidity is low, or equivocal, a recent infection cannot be excluded, and a follow-up analyzing IgG kinetics can provide the precise date of infection. An IgG titer remaining stable over a 2-week period constitutes a marker of a latent infection lasting at least 2 or 4 months depending of used techniques and individual variability. A significant increase, defined as at least a 2-fold increase in a no-treatment context, is a marker of acute infection and necessitates adapted management depending on gestational age and the estimated date of infection.

4.5. Presence of equivocal titer of IgG without IgM (Fig. 5)

This kind of serological profile raises the problem of precisely characterizing a subject's immune status which depends on the threshold

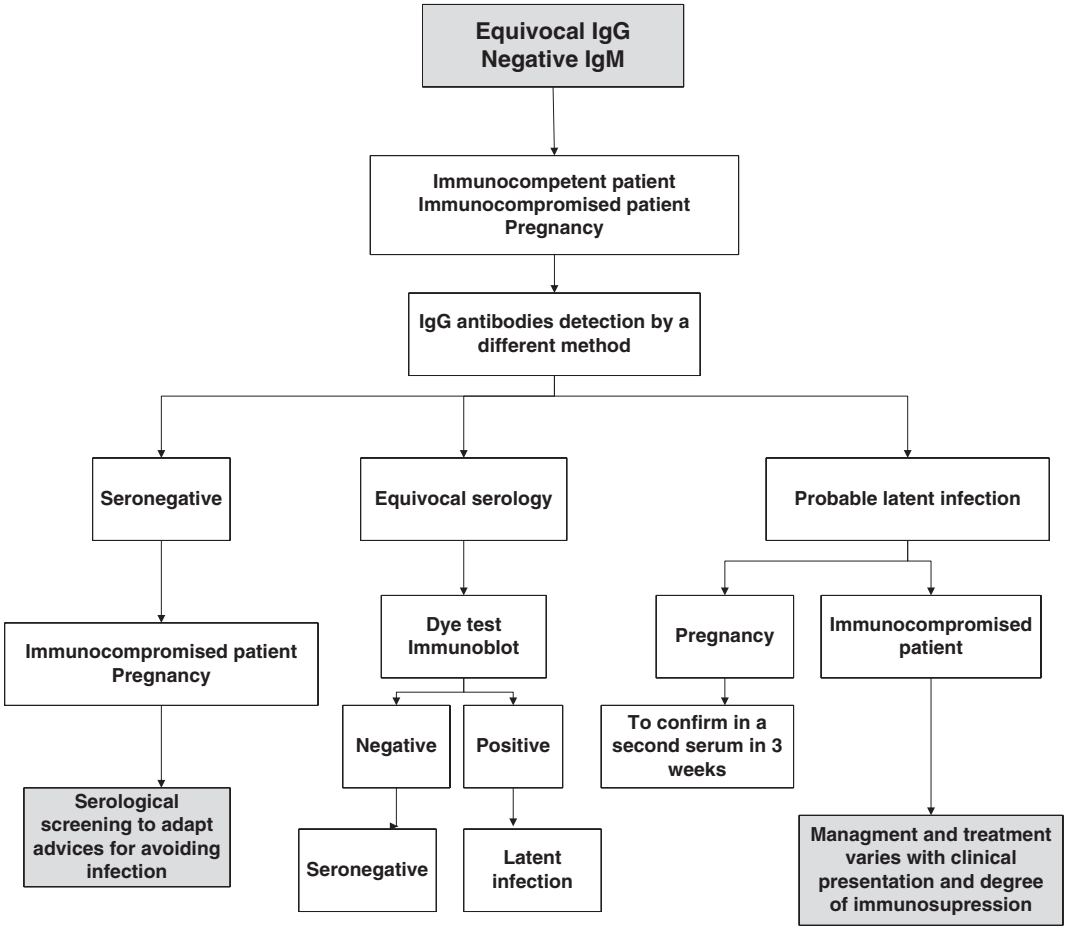

Fig. 5. Advice algorithm for toxoplasma serology with equivocal IgG and negative IgM. Adapted from Villard et al., 2011.

of the technique. In the specific case of pregnancy, the immune status directly influences whether a serological follow-up is initiated. It is therefore highly recommended to monitor the IgG titer using a different method (Lesle et al., 2011). A reference method like dye test or immunoblot should be conducted in an expert laboratory to confirm for low titers of IgG with immune enzymatic tests (Fig. 6). As a result, pregnant women and immunocompromised patients are provided with hygienic recommendations or prescribed serological follow-up, according to French national guidelines and with consideration of clinical status in immunocompromised patients.

Finally, universal monthly maternal screening for congenital toxoplasmosis, with follow-up and treatment designed according to French protocols, has been found to be cost-saving if the methods are well adapted to the local seroprevalence (Stillwaggon et al., 2011).

5. Specific clinical situations

Some specific clinical situations, apart from the serological screening of immunocompetent patients, require specific methods to be implemented, supported by specific coverage.

5.1. Prenatal and postnatal follow-up in a suspected case of congenital toxoplasmosis (Figs. 7 and 8)

Maternal infection leads to the risk of a transplacental passage of toxoplasma parasites to the fetus and may cause congenital toxoplasmosis, resulting potentially in abortion or fetal death (Villena et al., 2010). Severe symptoms, such as hydrocephalus, microcephaly or encephalitis, or sequelae, such as visual impairment, intracranial calcifications, or mental retardation, can be observed in affected infants (Montoya and Liesenfeld, 2004; Weiss and Dubey, 2009). The severity and percentage of infection depend on the date of contamination during pregnancy. It is crucial to determine the immunological status of the pregnant woman. In his 1974 study, Desmonts reported that those who were seropositive prior to pregnancy did not transmit infection to the fetus (Desmonts et al., 1960), though some exceptions were observed in cases of recontamination by more virulent strains (Desmonts et al., 1990). When toxoplasma DNA is detected in the amniotic fluid, the treatment is changed to a pyrimethamine and sulfonamide association, with postnatal clinical follow-up. If no prenatal diagnosis is established or if the data cannot confirm congenital toxoplasmosis (CT), a serological and parasitological diagnosis is performed at birth, and serological follow-up is initiated.

In France, the national program to prevent congenital toxoplasmosis created in 1978 and the surveillance system by the French National Institute of Public Health Surveillance with The National Reference Centre for toxoplasmosis established in 2007 is associated with a lower rate of severe forms of congenital toxoplasmosis. In 2012, prevalence of CT was estimated at 2.58 per 10,000 births with a prevalence of severe forms of 0.09 per 10,000 births. The majority of infections are asymptomatic (Villena et al., 2010).

Postnatal screening and follow-up of neonates can be a complementary management strategy to prenatal diagnosis or an alternative in countries with no regular serological screening of pregnant women. Screening at birth can be associated with parasite detection in the amniotic fluid, placenta, or cord blood serum, along with detection of IgM, IgA, or IgG in the cord blood serum and newborn serum. Positive PCR alone in placenta may be related to residual DNA from dead parasites or placental persistence with no fetal transmission (Ajzenberg et al., 2002; Fricker-Hidalgo et al., 2007; Robert-Gangneux et al., 2011).

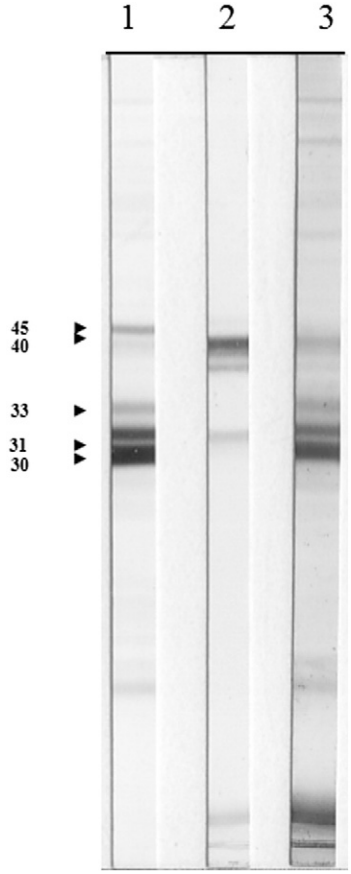

Fig. 6. IgG immunoblot. 1. Positive control with 5 bands corresponding to 30, 31, 33, 40, and 45 kDa. 2. False-positive serum with an equivocal titer of IgG. 3. Positive patient with the 5 bands.

A quantity of IgG, and sometimes IgM or IgA, antibodies may be in the first 10 days, of maternal origin. IgG has a half-life of 3 weeks; and IgM and IgA, 10 days. Classical methods are unable to distinguish between these maternally transmitted IgG antibodies or contamination-resulting IgM and IgA antibodies and those that are newly synthesized newborn antibodies. Follow-up is necessary to confirm CT. Specific neonate antibody isotypes should be tracked using methods able to detect low levels of these antibodies, which should also confirm their neonatal origin (Magi and Migliorini, 2011). A comparative immunoblot test for specific IgG and IgM analysis from paired mother-newborn at birth and from follow-up sera of neonates can confirm a CT diagnosis. Different band patterns from mothers and neonates at birth indicate a specific antibody neosynthesis in the newborn serum, also confirming CT. A comparison of this profile with IgM can rule out contamination of the cord serum with maternal serum, especially for the detected IgM antibodies (Fig. 8).

The sensitivity of immunoblot at birth is 48–50% for IgG, reaching 65–79% when combined with Western blot (WB) detection of IgM (Tissot Dupont et al., 2003) and achieving as high as 95.8% in the combination of WB IgM with prenatal and serological neonatal tests during the first month of life (L'Ollivier et al., 2012; Pinon et al., 2001).

IgG is the principal isotype that should be followed up by classical methods, ensuring a precise determination of its titer (IU/mL or any other units). IgG displays a long half-life of 3 weeks and should be monitored until its disappearance.

Any modification of the classical curve of IgG decay, either in terms of stabilization or increase, is a clear indication of CT. However, this process is long and costly. The detection of IgA or IgM during this period enables a shorter follow-up while offering a clear indication of CT.

5.2. Suspicion of ocular toxoplasmosis (Fig. 9)

Ocular toxoplasmosis (OT) is a major cause of posterior uveitis worldwide. This disease is a form of chorioretinitis that can be the result of a congenital (Silveira et al., 2001) or acquired disease due to acute infection, often in the context of an acute disease outbreak (Holland, 1999; Montoya and Remington, 1996; Stanford et al., 2006) or otherwise a reactivation of latent disease embedded in the retina and choroid (Mets et al., 1996), leading to multiple lesions that can cause blindness if widespread, especially when involving the macula area (Arevalo et al., 2010; Bodaghi et al., 2001). OT should be always confirmed by detection of either toxoplasma DNA using PCR or of a local production of IgG and/or IgA antibodies. This confirmation enables a targeted treatment against toxoplasma and its subsequent inflammation to be initiated in order to limit the cytolysis of retinal tissues and development of larger scars, thereby improving visual acuity outcome.

The serology testing in these settings is unique due to the fact that the assays need to detect intraocular *T. gondii* antibody production in the aqueous humor. Biological diagnosis should be carried out using aqueous humor samples or is less frequently conducted in vitreous samples by means of paracentesis. The serum should always be sampled simultaneously.

If the serum tests are negative for toxoplasma antibodies, all investigations should be stopped in immunocompetent patients. When toxoplasma antibodies are detected, a PCR assay and detection of IgG antibodies are conducted on ocular fluid. A positive PCR confirms the toxoplasmic origin and enables early specific treatment to be initiated, rapidly (Bastien, 2002; Bourdin et al., 2014). Furthermore, by genotyping the strain involved in the disease on DNA, we can improve our understanding of the impact of *T. gondii* strain genotypes on toxoplasmic retinochoroiditis (Ajzenberg et al., 2009; Fekkar et al., 2011). In the presence of a negative PCR, classical criteria should be applied to the serum to determine if it is an acute infection or recurrence (Gilbert and Stanford, 2000). In the event of an active infection, there is the risk of blood-retinal barrier break-down, due to high choroiditis and antibodies leaking from the serum, potentially leading to false-positive results. Two major methods can be used in combination to determine local toxoplasma antibody production versus systemic toxoplasma, namely, specific IgG. The first is the Goldmann–Witmer coefficient (GWC) (Desmonts, 1966). This coefficient is calculated based on the determination of the specific versus total IgG levels in the serum and aqueous humor. Its sensitivity is around 50% (Garweg et al., 2000; Robert-Gangneux et al., 2004; Talabani et al., 2009). The second is a similar method using ELISA to compare the levels of toxoplasma-specific antibodies versus mumps virus–specific antibodies, instead of measuring total IgG (Turunen et al., 1983; Villard et al., 2003).

Yet in some cases, if the blood-retinal barrier is ruptured, the GWC (Desmonts et al., 1960) is unable to distinguish between systemic and local toxoplasma-specific antibodies in the aqueous humor. This is why using WB to determine a toxoplasma-specific antibody recognition profile is of great value in this context. As well as having been developed for the diagnosis of congenital toxoplasmosis, a WB analysis of serum and paired aqueous humor sampled the same day is able to determine the recognition profile. Any observed difference, namely, 1 or more different bands, signifies the presence of toxoplasma-specific ocular antibodies, revealing an OT (Talabani et al., 2009; Villard et al., 2003). While the sensitivity of WB is similar to that of GWC, the former achieves higher specificity (>95%) and is less influenced by rupture of the blood-retinal barrier (Robert-Gangneux and Darde, 2012). The combination of GWC, PCR, and WB has been shown to improve the sensitivity of biological diagnosis, which can reach up to 83% (Fekkar et al., 2011; Villard et al., 2003). If both the serology and PCR are negative, other causative infectious and noninfectious diseases should be investigated (Kijlstra et al., 1989).

5.3. Follow-up of immunosuppressed patients (Fig. 10)

The incidence of opportunist toxoplasmosis is probably underestimated and depends highly on the prevalence of infection, well known in Europe

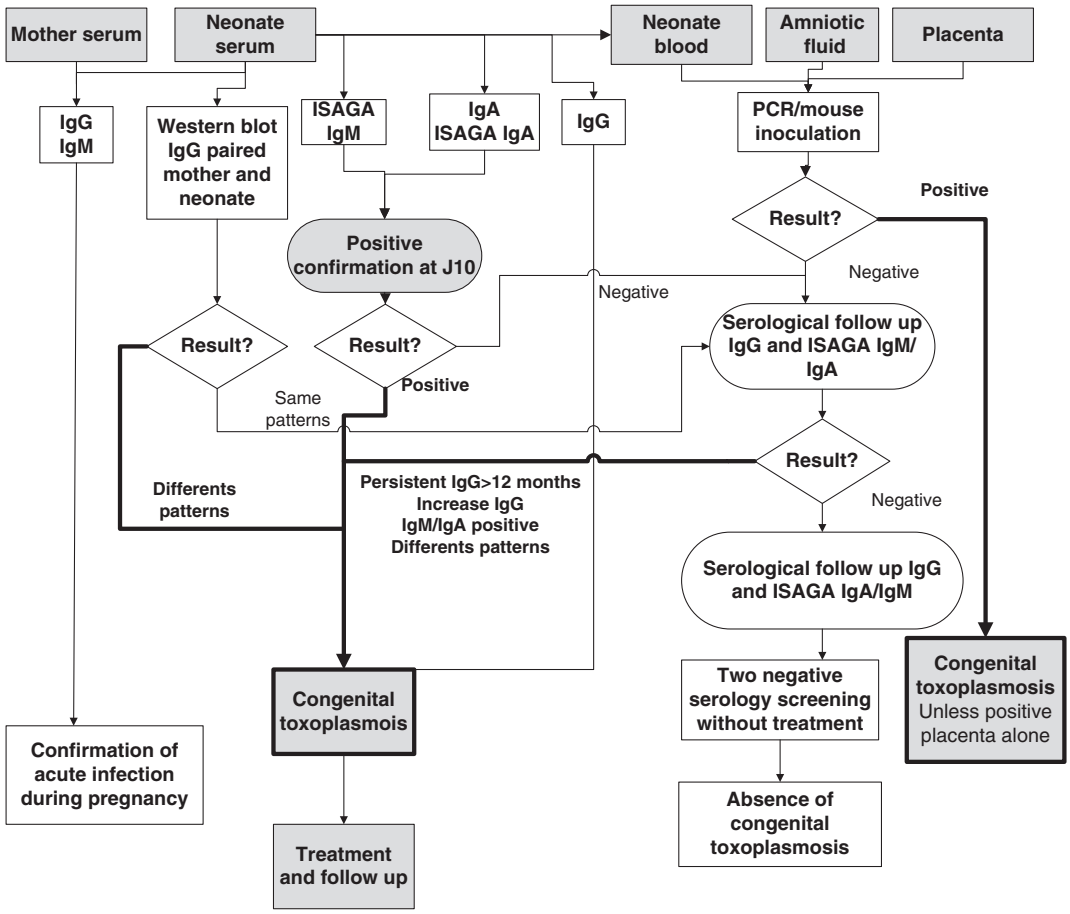

Fig. 7. Advice algorithm for toxoplasma serology in children born from mother with confirmed or highly suspected contamination during pregnancy.

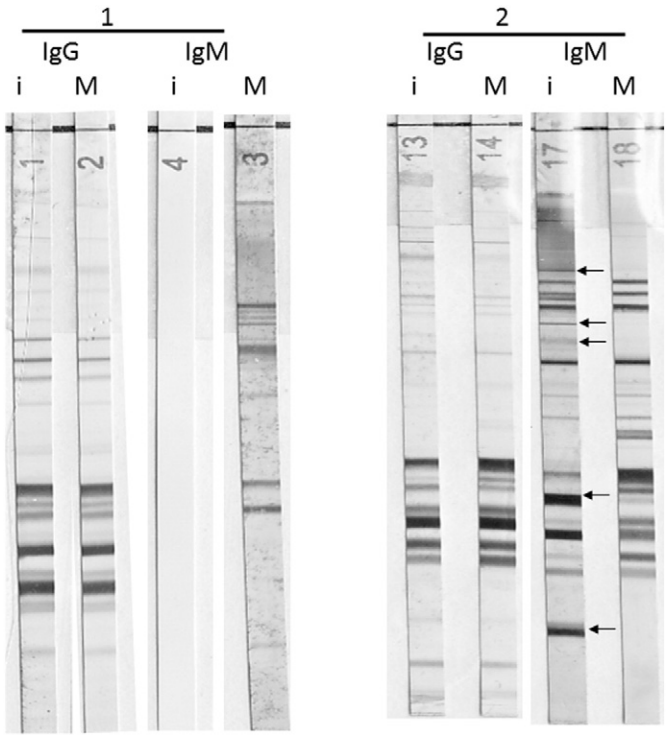

Fig. 8. Comparison of immunoblot IgG and IgM with paired mother (M) and infant (i) at birth. 1. Absence of IgM and same profile of IgG in favor of passive transmission of maternal IgG. 2. Same profile for IgG and different profile IgM (arrows) confirming congenital toxoplasmosis.

and North America yet poorly studied in the rest of the world. Toxoplasmosis is an opportunistic infection that carries the risk of complications in AIDS patients and those having received hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT). In the case of immunodeficiency, 2 different populations are at risk: toxoplasma-naive patients are at risk for acute severe and lethal toxoplasmosis, in which the diagnosis of infection is an emergency (Aubert et al., 1996; Chandrasekar and Momin, 1997; Luft and Remington, 1992; Petersen et al., 2006), and patients with latent toxoplasmosis who are at risk for reactivation and secondary disseminated infection. Diagnosis in these patients is often challenging, firstly owing to the nonspecific clinical features of toxoplasmosis. Serology is essential to estimate if the patients are at risk for reactivation or not and could be valuable in emergency diagnosis (Bretagne et al., 2000; Costa et al., 2000). Moreover, serological data should take into account the toxoplasma status of both donors and recipients (Fig. 8). Nevertheless, in this population, serology is often of limited interest due to immune deficiency impacting the B-cell count and interfering treatments, such therapeutic immunoglobulin containing anti-toxoplasma antibodies. Immunological deficiency may lead to multiorgan failure in some cases or a rapid spread of the pathogen. Parasite detection by PCR is therefore strongly recommended when serology is negative, with PCR offering high sensitivity in these settings (Martino et al., 2005).

5.3.1. AIDS-positive patients (Fig. 10A)

In AIDS-positive patients, the cellular immunity is weakened and profoundly impaired in the late stages of infection. When the CD4+ T count is <100 cells/μL, there can be a risk of disease reactivation or severe acute toxoplasmosis. Toxoplasmic encephalitis following a reactivation of latent cysts is the most predominant disease (Luft and Remington, 1992; Weiss and Dubey, 2009). Active retroviral therapy

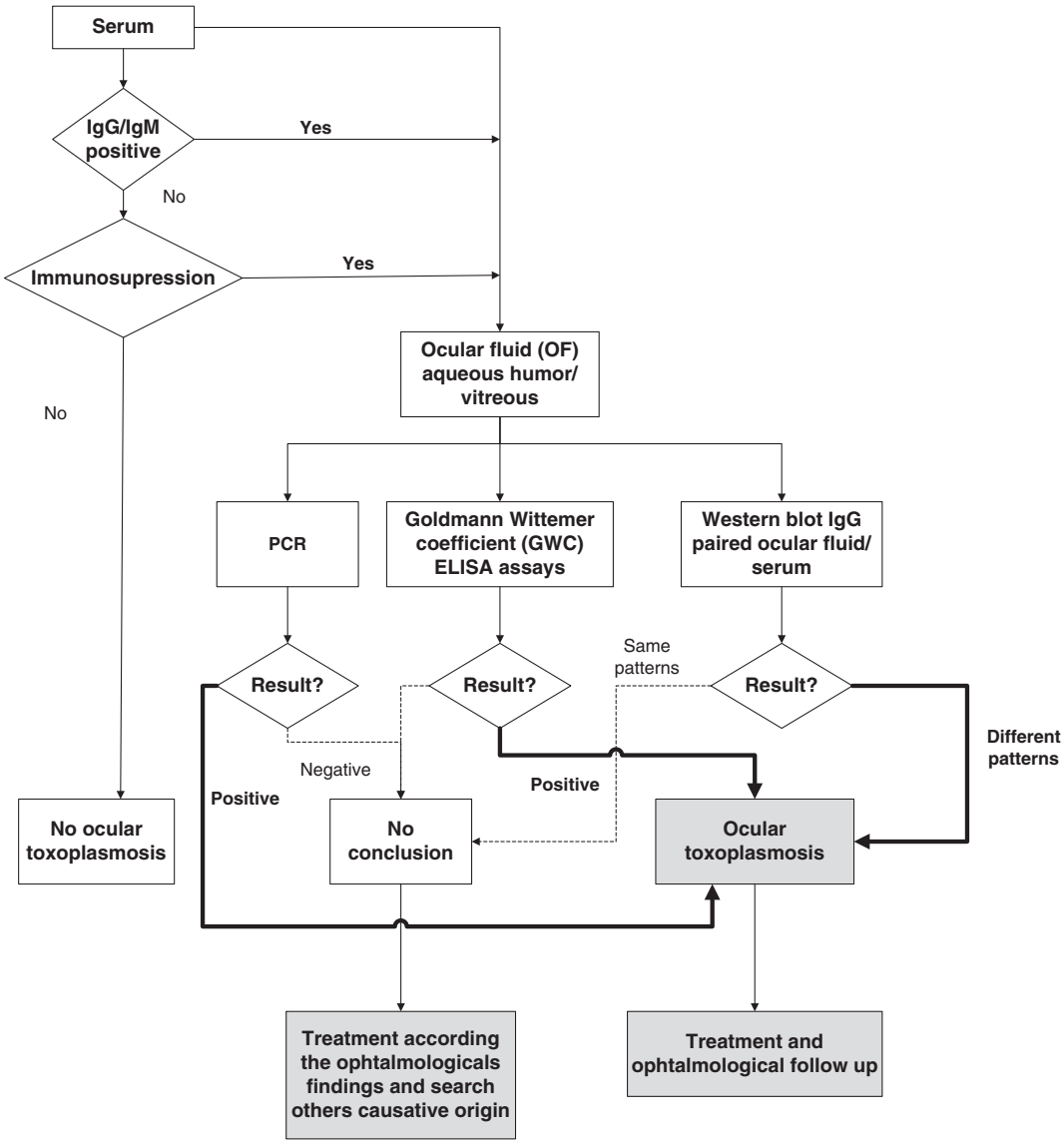

Fig. 9. Advice algorithm for toxoplasma serology in patients with a suspected toxoplasma chorioretinitis.

can restore the T-cell immune response and cause the incidence of toxoplasmosis to fall dramatically. Other organs can be secondarily infected by disseminated infection, including the lungs, eyes, liver, and bone marrow (Rabaud et al., 1996). Latent chronic infection is diagnosed by positive in peripheral blood, and prevention methods using cotrimoxazole are recommended for patients with higher risk of toxoplasmosis. When serology is negative, there is a risk of acute toxoplasmosis, and hygienic and dietetic preventive measures are advised, with serological follow-up according to the CD4+ cell count.

5.3.2. Transplant patients (Fig. 10B and C)

Serological screening of donors and recipients prior to transplantation enables the mismatch and identification of patients at higher risk for toxoplasmosis to be clearly defined.

In SOT recipients (Fig. 10B), the highest risk of reactivation of toxoplasma in the donor graft and disseminated secondary infection occurs in cases involving chronically infected seropositive donors (D+) and toxoplasma-seronegative recipients (R−), especially in cases of heart or heart-lung transplant. The immunosuppressive status leads to the rupture of cysts contained in transplant and a disseminated active infection (Fernandez-Sabe et al., 2012; Morris et al., 2010; Rogers et al., 2008). Clinical symptoms usually occur within the first 3 months after transplantation. The incidence of toxoplasmosis in seronegative heart recipients receiving an organ from a seropositive donor (mismatch D+/R−) has been reported as high as 50–75% in the absence of prophylaxis (Gallino et al., 1996), and prevention with cotrimoxazole is recommended for patients at a higher risk for toxoplasmosis (Cavattoni et al., 2010; Derouin and Pelloux, 2008). Cases of transmission in noncardiac SOT recipients are less frequent. Transmission from a seropositive donor to a seropositive recipient (D+/R+) is possible, though it is difficult to differentiate between a transplant-resulting transmission and a reactivation of latent infection in the recipient (Robert-Gangneux et al., 2000). In R−/D− transplantation, there is no risk of parasite transmission. Seronegative recipients are susceptible to acute toxoplasmosis infection via consumption of contaminated food and hygienic and dietetic measure must be implemented in these cases.

Toxoplasmosis following allogeneic HSCT remains a cause of severe infection associated with a high mortality rate. The highest risk of transmission resulting from reactivation is observed in seropositive recipients following allogeneic HSCT (Bories et al., 2012; Derouin and Pelloux, 2008). The onset of clinical symptoms generally occurs within the first 2 months after transplantation. In autologous HSCT, transmission is rare (Geissmann et al., 1994).

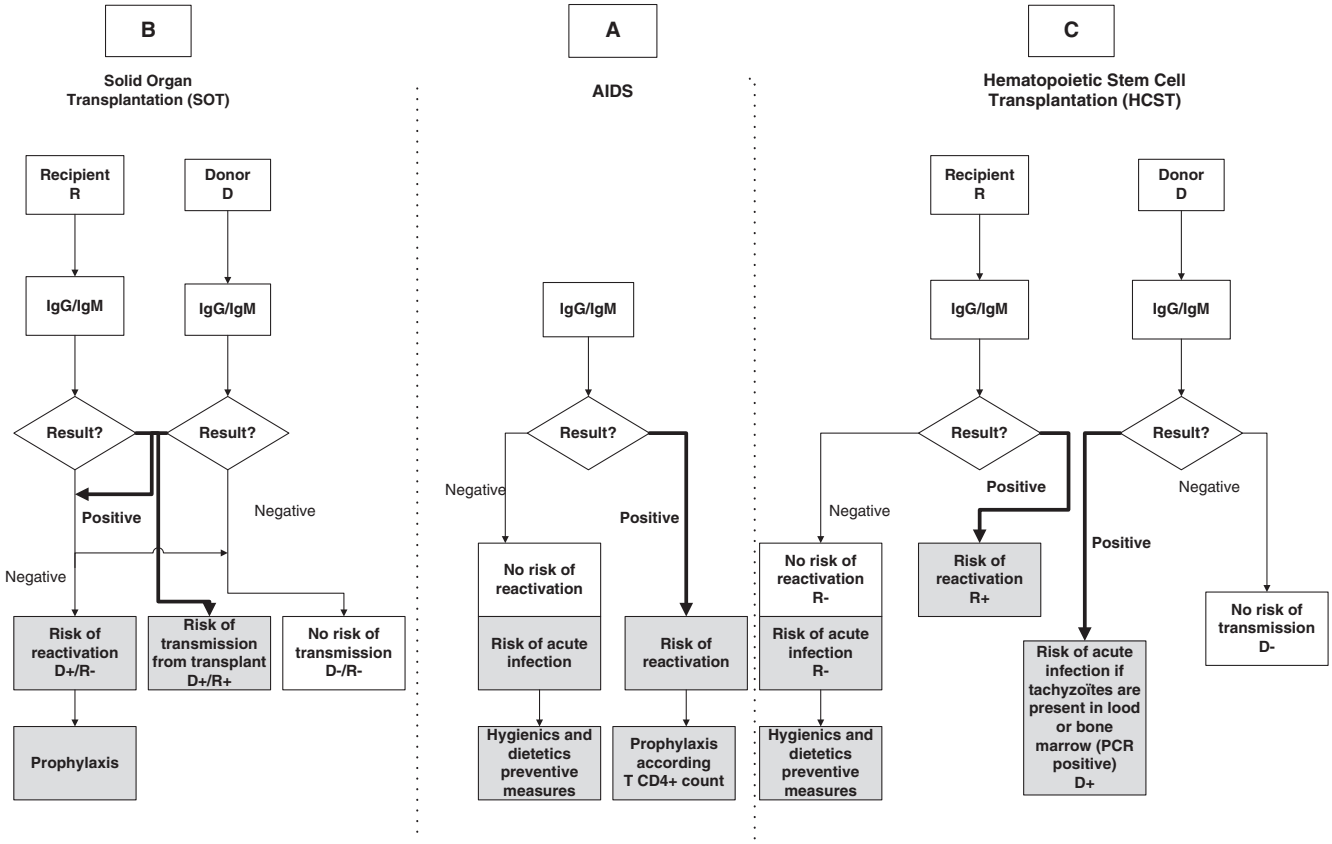

Fig. 10. Advice algorithm for toxoplasma serology in immunosuppressed patients. Patients with AIDS (A), SOT (B), and HSCT (C).

The incidence of disseminated toxoplasmosis following bone marrow transplantation is estimated at 0.6–6% in seropositive allogeneic transplant recipients, resulting in a mortality rate of 60–90% (Small et al., 2000). Serological screening of donors and recipients prior to transplantation (Fig. 10C) enables the identification of patients at higher risk for toxoplasmosis, essentially consisting of seropositive recipients, and a reactivation of latent infection (R+/D− or R+/D+). A prevention strategy with cotrimoxazole can then be initiated for these patients. There is no risk of transmission in HSCT cases in R− recipients with a seropositive and chronically infected donor (R+), nor is there any risk of transmission in seronegative recipients (R−) receiving transplant from a seronegative donor, although these patients are susceptible to acute toxoplasmosis infection through consumption of contaminated food and hygienic and dietetic measures must therefore be implemented (Fig. 8C). The transmission of *T. gondii* to a seronegative recipient from a seropositive donor (R−/D+) is possible if the donor had a recent and active acquired infection, with tachyzoites present in the blood. There is no risk of transmission in cases of HSCT in mismatch R−/D+ (chronically infected). Serological follow-up combined with PCR after allogeneic HSCT is recommended in all patients at risk for toxoplasmosis (Fricker-Hidalgo et al., 2009).

6. Conclusions

Despite initial screening providing the medical benefits of early treatment and appropriate medical care, it is a costly method, and the availability of these tests according the clinical and epidemiological grounds must be established. Serological diagnosis becomes a highly complex issue as soon as we look at less developed locations that lack the technology. We should highlight the fact that all medical laboratories should refer their more complex cases to a reference center, assuming that this center has the reference methods necessary to solve these complex cases. In France, a National Reference Center was created in 2006 based on a network of reference laboratories. This means that experts can share their methods and skills, providing help and support to all French medical laboratories and health professionals.

Finally, universal monthly maternal screening for congenital toxoplasmosis, with follow-up and treatment designed according to French protocols, has been found to be cost-saving if the methods are well adapted to the local seroprevalence (Stillwaggon et al., 2011; Wallon et al., 2013).

Conflict of interest

The authors declare that they have no conflicts of interest.

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