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Classification System and Case Definitions of *Toxoplasma gondii* Infection in Immunocompetent Pregnant Women and Their Congenitally Infected Offspring

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Classification systems and case definitions provide the foundations upon which clinical and epidemiological studies are based. The European Research Network on Congenital Toxoplasmosis acknowledged the lack of such a system or definitions within its field of interest and established a working group to address the issue. Congenital *Toxoplasma gondii* infection was defined as occurring in four separate patient groups: pregnant women, fetuses, infants, and individuals > 1 year of age. The likelihood of *Toxoplasma gondii* infection was separated into five mutually exclusive categories: definite, probable, possible, unlikely, and not infected. Inclusion within a specific category is dependent upon the case definition, which is in turn derived from criteria based on serological, parasitological, and clinical information. Notes are included within the classification not only to clarify the definitions, but also to improve the reliability and quality of diagnosis. The goal is to construct a system that encompasses all aspects of congenital toxoplasmosis, which is applicable to different countries and health services, suitable for large epidemiological studies, aids the diagnosis and management of individual cases, and lends itself to computerisation.

Toxoplasma gondii infection in the immunocompetent person is normally of little consequence: it is frequently asymptomatic, with lymphadenopathy occurring in only about 20% of cases. This may be accompanied by other symptoms and signs such as pyrexia, myalgia, night sweats, sore throat, and, occasionally, hepatosplenomegaly (1). Clinical disease usually resolves within a few weeks or months. Rarely, symptoms persist for many years due to chronic active infection (2).

A primary infection in the pregnant woman potentially presents a much more serious problem, because in about 40% of cases, the parasite is transmitted to the fetus. If the fetal infection occurs early in pregnancy, then miscarriage or severe disease can result. The classic triad of signs, i.e. hydrocephalus (or microcephaly), retinochoroiditis, and cerebral calcification may be present, but any combination may be found. If infection occurs in the last trimester, the neonate is usually born with a subclinical infection and clinically will appear normal (3). However, most of these children will develop signs of congenital infection, usually retinochoroiditis, by the second decade of life (4, 5). As acute primary infection in immunocompetent pregnant women is usually asymptomatic, detection is invariably based upon serology. Thus,

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in the absence of a screening programme for *Toxoplasma gondii* infection, many infections will be missed (6).

Serological diagnosis of primary maternal infection is clear cut when there is evidence of seroconversion, but a more common scenario is to find specific toxoplasma IgM antibody consistent with recent infection in the first serum sample. This antibody can be detected for one year or longer after the infection (3). Timing the onset of infection, therefore, is very important, and this usually necessitates the services of a specialist toxoplasma laboratory where other tests such as the dye test (7), IgG avidity (8, 9), or differential agglutination (10) may be employed.

Fetal infection can only be established definitely by culture of the organism (the term culture is taken to include both tissue culture and mouse inoculation) or histopathological demonstration of parasites in fetal tissues or by demonstration of specific toxoplasma DNA in amniotic fluid, fetal blood, or tissues. The diagnostic value of a finding of specific toxoplasma IgM and/or IgA in fetal blood is reduced by the risk of contamination by maternal blood.

Although there have been no randomised control trials, there is general agreement that treatment of *Toxoplasma gondii* infection during pregnancy and in the newborn reduces both the risk of maternal-fetal transmission and later sequelae in infected children (1). Debate continues, however, about the most appropriate treatment regimen, as placebo-controlled trials in this particular patient group are considered by most workers in the field to be unethical. The exact benefit of treatment, however, cannot be determined with certainty, as a true comparison of current and previous studies of efficacy of treatment is not possible because neither case definitions nor a uniform classification system that includes diagnostic criteria exist for *Toxoplasma gondii* infection.

The European Research Network on Congenital Toxoplasmosis, whose members come from 53 centres in 18 European countries, recognised the current increasing interest in this particular area and established a working group (comprising medical microbiologists, epidemiologists, and clinicians) in order to produce a classification system and case definitions for *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring, encompassing all aspects of congenital toxoplasmosis and harmonising reports and studies. (It is important to note that this classification system is not applicable to immunosuppressed individuals.) The goal was to design a system that would be relatively simple in its construction and easily understood so that it could be used as an aid by medical staff who do not have specialised knowledge of *Toxoplasma gondii* infection. The classification system has been designed with the intention that it be suitable not only for large epidemiological studies but also for the collection of data by a local toxoplasma centre.

Patient Groups

Congenital *Toxoplasma gondii* infection involves a range of clinical presentations. It entails infection of the mother, transmission of the parasite to the fetus, and the consequences of infection in the neonate or the older child/adult. It was decided, therefore, that the best approach would be to define infection as occurring in four separate patient groups: (i) the pregnant woman, encompassing a period of two months prior to conception through parturition; (ii) the fetus which includes tissue recovered in utero and after a miscarriage, an abortion, or a stillbirth; (iii) the infant; and (iv) individuals > 1 year of age for whom no information is available for the first year of life.

Categories of Infection

Five mutually exclusive categories of the probability of infection can be considered: (i) definite infection; (ii) probable infection; (iii) possible infection; (iv) unlikely to have been infected; and (v) not infected.

The case definitions (presented below) for inclusion in the "definite" and "not infected" categories are considered absolute and leave no room for doubt. The other three categories are subjective. The case definitions have been allocated to each specific category in the context that in "probable infection" strong evidence of infection is available, but absolute proof is lacking, while in "possible infection" the evidence is suggestive but incomplete; and in the "unlikely to have been infected" category there is little to support the diagnosis, but infection cannot be completely excluded. When applying this classification system, it must be appreciated that an individual case can initially be allocated to one category, but addition-

Table 1: Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring.

Patient group	Category of infection	Case definition
1. Primary maternal infection during pregnancy	1.1 Definite	1.1.1 Seroconversion – both samples taken after conception.[a]
		1.1.2 Positive culture from maternal blood.[b]
		1.1.3 Confirmed congenital infection in offspring.
	1.2 Probable	1.2.1 Seroconversion – first sample taken within 2 months before conception.
		1.2.2 Significant rise of IgG titres, and presence of IgM and/or IgA.[c]
		1.2.3 High IgG titres, presence of IgM and/or IgA, and onset of lymphadenopathy during pregnancy.[c]
		1.2.4 High IgG titres and presence of IgM and/or IgA in second half of pregnancy.[c]
	1.3 Possible	1.3.1 Stable high IgG, without IgM, in second half of pregnancy.[c]
		1.3.2 High IgG and presence of IgM and/or IgA in first half of pregnancy.[c]
	1.4 Unlikely	1.4.1 Stable low IgG, with or without IgM.[c]
		1.4.2 Stable high IgG, without IgM, in early pregnancy.[c]
	1.5 Not infected	1.5.1 Seronegative (during pregnancy).
		1.5.2 Maternal preconception seropositive sample.
		1.5.3 Positive IgM and/or IgA without appearance of IgG.[c]
2. Fetal infection	2.1 Definite	2.1.1 Positive culture from fetal tissue, fetal blood, or amniotic fluid.[b]
		2.1.2 Histopathological demonstration of parasites in fetal tissue.
		2.1.3 Demonstration of toxoplasma DNA in amniotic fluid or in fetal blood or fetal tissue.
	2.2 Probable	2.2.1 Positive IgM and/or IgA in fetal blood.[d]
		2.2.2 Persistent ultrasound findings and definite or probable primary maternal infection during pregnancy.[e,f]
	2.3 Possible	2.3.1 Persistent ultrasound findings and possible primary maternal infection during pregnancy.[e,f]
		2.3.2 No positive fetal findings, but definite primary maternal infection during pregnancy.[f]
	2.4 Unlikely	2.4.1 No positive fetal findings, but possible primary maternal infection during pregnancy.[f]
	2.5 Not infected	2.5.1 Seronegative mother.
		2.5.2 Positive IgM and/or IgA in mother without appearance of IgG.[c]
		2.5.3 Maternal preconception seropositive sample.
3. Congenital infection in infants	3.1 Definite	3.1.1 Positive culture from cord blood or body tissues obtained within the first 6 months of life.[g]
		3.1.2 Confirmed histopathological demonstration of parasites in body tissues obtained within the first 6 months of life.[g]
		3.1.3 Rise in IgG titres within the first 12 months of life, with or without clinical signs of the classic triad.
		3.1.4 Persistently positive IgG beyond the first 12 months of life, with or without clinical signs of the classic triad.[h,i]
		3.1.5 Positive IgM within the first 6 months of life.[i,j]
		3.1.6 Positive IgA within the first 6 months of life.[k]

(continued on next page)

Table 1, continued: Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring.

Patient group	Categorie of infection	Case definition
	3.2 Probable	3.2.1 Positive culture from placental tissue.[b]
		3.2.2 IgM positive between 6 and 12 months, but no previous serological test results for comparison.
		3.2.3 Retinochoroiditis and/or hydrocephalus/cerebral calcification and definite primary maternal infection during pregnancy, no other results available.[l]
	3.3 Possible	3.3.1 Retinochoroiditis and/or hydrocephalus/cerebral calcification, but no infant serological tests or knowledge of maternal infection.[l]
		3.3.2 One of the clinical signs of the triad present, positive IgG, but no knowledge of maternal infection.
	3.4 Unlikely	3.4.1 Continuous decline in IgG titre without IgM and/or IgA, with or without clinical signs up to the first 6 months of life, without treatment.
	3.5 Not infected	3.5.1 Seronegative within the first 12 months of life, without treatment.[m]
		3.5.2 Seronegative 6 months after finishing treatment.
4. Individuals over 1 year of age (for whom no information is available for the first year of life).	4.1 Definite	4.1.1 Not applicable.
	4.2 Probable	4.2.1 Typical retinochoroiditis and IgG positive patient, definite primary maternal infection during pregnancy.[l]
		4.2.2 Typical retinochoroiditis with hydrocephalus and/or cerebral calcification.[l]
	4.3 Possible	4.3.1 Typical retinochoroiditis and IgG positive patient, maternal IgG positive or unknown.[l]
	4.4 Unlikely	4.4.1 Not applicable.
	4.5 Not infected	4.5.1 Seronegative offspring.
		4.5.2 Seronegative mother.
		4.5.3 Maternal seropositive sample preconception.

[a] Should be confirmed by third sample.
[b] Culture includes both tissue culture and mouse inoculation.
[c] At least two samples taken three weeks apart during pregnancy.
[d] Risk of contamination by maternal blood reduces diagnostic value.
[e] Ventricular dilation and/or echogenic intracerebral lesions.
[f] If ultrasound alone is performed, classification is not possible.
[g] Primary infection of infant up to 6 months of age considered very unlikely.
[h] IgG titres may be modified by treatment.
[i] Ideally, infants should be tested monthly.
[j] Excluding sample within the first two days of life.
[k] Excluding samples in the first ten days of life.
[l] Retinochoroiditis should be confirmed by an ophthalmologist.
[m] Very rarely, trace levels of IgG may be found for a few more months.

General Note: Ideally, tests should be confirmed by another method or by another laboratory. It is the responsibility of the laboratory performing the test to define high and low IgG titres, but a titre equal to or greater than 300 IU is generally considered high. Specific anti-toxoplasma chemotherapy may modify a rise in IgG titres.

al information can, in due course, result in its re-allocation to another category. It is also hoped that this categorisation will enable clinicians to identify key elements in the diagnosis of congenital *Toxoplasma gondii* infections.

Case Definitions

The agreed criteria upon which case definitions are based are narrow, but absolute. If the appropriate criteria are not available, then an individu-

al case cannot be placed within the classification system. The case definitions are derived from three sources of information, detailed below.

1. *Serological Status.* This is determined by the result of specific toxoplasma antibody analysis enabling comparison of antibody levels in at least two consecutive serum samples obtained three or more weeks apart. Ideally, these findings are confirmed by a third sample. With fetal blood sampling, of course, only one sample is normally available. In view of the different types of serological tests employed in laboratories from different countries, the working group decided that it would be inappropriate to delineate diagnostic laboratory criteria and that the interpretation and significance of the results of serological testing should be the responsibility of the laboratory performing those tests. However, IgG levels equal to or greater than 300 IU are generally considered high. Ideally, test results should be confirmed by another method and/or another laboratory. It is recommended that, whenever possible, the involvement of a specialist toxoplasma laboratory should be sought both for individual patients and for research studies.
2. *Parasitological Status.* This is determined either by the histopathological demonstration of parasites in tissues, the culture of the organism by tissue culture, or mouse inoculation, or by the detection of specific toxoplasma DNA from a variety of tissues or body fluids.
3. *Clinical Status.* In the pregnant woman, this will be determined by the presence or absence of clinical features. The best marker of acute infection is lymphadenopathy, usually cervical, and this is the only clinical finding used in this section of the classification. In the fetus, an assessment of clinical status depends upon the use of ultrasound and the demonstration of persistent findings of ventricular dilation and/or echogenic intracerebral lesions. Less specific ultrasound findings such as increased thickness of the placenta, ascites, pleural effusions, and echogenic intrahepatitic lesions are not used in the classification.

It is important to note that if there is no knowledge of maternal serological characteristics, and the only investigation performed has been fetal ultrasound, then it is not possible to use this system alone to classify fetal infection.

In the neonate, the classic triad of signs, i.e. hydrocephalus or microcephaly, retinochoroiditis, and cerebral calcification, may be present, but any combination may be found. These are the only signs and symptoms used in the classification. In individuals over 1 year of age, the appearance typical of toxoplasmic retinochoroiditis is the usual finding and should be confirmed by an ophthalmologist. (Definitions of ocular toxoplasmosis are not included in this classification system.)

The classification system is presented in Table 1. Brief notes are included in order to clarify the case definitions and improve the reliability and quality of the diagnosis.

Discussion

Classification systems and case definitions are regarded as important tools by both epidemiologists and clinicians, since the accurate description of a condition not only improves the quality, collection, and analysis of specific data, but also permits meaningful comparisons to be made between studies from different centres and geographical areas. For example, it enables researchers to refer similar categories of patients to specific tests or treatment protocols. Clinically, such systems clarify diagnostic requirements, thus, leading to informed decisions about the management of individual patients, as in the case of HIV infection in adults and adolescents (11). An important criterion in producing a case definition in this classification is the result of serological investigation. As there are many different types of serological assays for toxoplasma with varying specificities and sensitivities, the working group concluded that the classification system should not recommend particular assays or require specific antibody test results in order to make a serological diagnosis. The responsibility for confirming the reliability and accuracy of the results rests with the laboratory performing the test. It must be stressed, however, that the laboratory should have experience in this field and use appropriate methods with quality control and quality assurance schemes (where available).

Results should be confirmed by another serological method and/or by another laboratory recognised in the field of *Toxoplasma gondii* infection. A diagnosis based on a solitary serum sample is considered inadequate and, whenever possible, must be confirmed by testing a second sample in parallel. Because of the implications of the finding of seroconversion for the pregnant woman, the

working group recommends that this be confirmed by a third sample. Other working groups in the Research Network are evaluating diagnostic methods for *Toxoplasma gondii* infection, and it is anticipated that their reports will be published in due course.

Because the immunological response to *Toxoplasma gondii* infection may vary among individuals, and since different assay systems can detect antibodies for varying periods of time, case definitions of a "definite" primary infection during pregnancy are very narrow; namely, seroconversion when both serum samples are taken after conception, positive from maternal blood, and confirmed congenital infection in the offspring. Recurrent parasitaemia in an immunocompetent individual is considered a very rare phenomenon and, thus, is not included in the classification system. It is recognised that the demonstration of a rise in titres, the presence of acute-phase antibodies, and IgG avidity testing may strongly suggest a primary maternal infection occurring during or just before pregnancy, but, as the results are not absolute, such infection must be classed as "probable." A maternal preconception sample (taken 2 or more months before conception) that is only IgG seropositive, results in a "not-infected" classification. Except for the onset of lymphadenopathy during pregnancy, clinical symptoms in the pregnant woman are relatively nonspecific and cannot be considered as contributing to the diagnostic classification.

Classification of fetal infection is possible only if amniotic fluid, fetal blood, or fetal tissue are used for the demonstration of *Toxoplasma gondii* infection, or if fetal ultrasound screening is combined with the results of serological tests in the mother. The diagnostic value of serological tests in the fetus (12) is reduced because of the risk of contamination from maternal blood during cordocentesis, and this is reflected within the classification. Gammaglutamyltransferase activity and leucocyte, eosinophil and platelet counts in fetal blood are nonspecific tests, and merely provide indirect evidence of infection. Recent work has clearly demonstrated the vital role that specific toxoplasma DNA detection [in particular the polymerase chain reaction (PCR) test] in amniotic fluid can play in the diagnosis of fetal infection (13). The theoretical possibility that contamination by maternal blood may invalidate this PCR test does not appear to have been borne out in practice. The PCR test of amniotic fluid may well result, in time, in cordocentesis becoming a redundant investigative procedure in this particular context. However, because of the risk of false results, this test should be limited at present to specialised laboratories with considerable experience in the use of PCR and the detection of *Toxoplasma gondii* infection.

The definitive diagnosis of congenital toxoplasmosis in infants or babies born to mothers with known or suspected *Toxoplasma gondii* infection in pregnancy can be difficult. Material for parasitological examination or serial blood samples for serological testing is not always available. In addition, the serological diagnosis is complicated by the presence of maternal toxoplasma IgG, which crosses the placenta and can be detected in the infant's blood for up to the first year of life or even a little longer. Therefore, only the detection of parasites in neonatal tissue or body fluids, the presence of specific IgM antibodies within the first six months of the infant's life, a rise in specific IgG antibodies within the first 12 months of life, or the persistence of specific IgG antibodies after 12 months of age or longer can be construed as being indicative of a congenital infection (3). The presence of specific toxoplasma IgA within the first six months of life is also taken as an indication of congenital infection (14); however, very rarely a false-positive result may occur in the first days of life (unpublished data). Primary infection in a child under 6 months of age is considered very unlikely. It is important to note that specific anti-toxoplasma chemotherapy with pyrimethamine and sulphonamides may modify the IgG response of the infant and the mother (3), and that this may apply to other anti-toxoplasma chemotherapeutic agents as well. In such cases the child should be followed for at least six months after treatment. If it remains seronegative, the child is classified as "not infected". Untreated children can be classified as "not infected" if they become seronegative within the first 12 months of life. (Very rarely, trace levels of IgG may be found for a few more months in such infants.)

The clinical signs of the classic triad of hydrocephalus, retinochoroiditis, and cerebral calcification presenting together or in any combination are considered to have particular diagnostic value and are used in the case definitions. Other manifestations of congenital infection, which may include jaundice, rash, pyrexia, lymphadenopathy, and hepatosplenomegaly, are not as specific and are not considered to have such a significant diagnostic role. Therefore, these have not been included in the classification system.

There will be patients aged 1 year and over who present with a potential diagnosis of congenital toxoplasmosis and for whom no information regarding the first year of life is available. In these circumstances, it is impossible to categorise such a *Toxoplasma gondii* infection as "definite" congenital infection. An individual who is seronegative or has a mother who either is seronegative or had a seropositive preconception sample is classified in this system as "not congenitally infected."

Retinochoroiditis typical of *Toxoplasma gondii* infection is an important clinical finding, but such an observation should be confirmed by an ophthalmologist. Case definitions specific to ocular toxoplasmosis are not, however, included in this classification system.

Reactivation of a latent *Toxoplasma gondii* infection has been reported in immunosuppressed pregnant women, but this classification system is applicable only to immunocompetent individuals.

The European Research Network on Congenital Toxoplasmosis believes that the classification system presented here will facilitate the development of uniform clinical and laboratory diagnoses. This should be of benefit not only to future research into congenital toxoplasmosis, but also to clinicians who will be caring for mothers or children with suspected *Toxoplasma gondii* infection. The network recognises that this classification system and the case definitions may be adapted for specific epidemiological studies, for clinical use, and for setting local laboratory diagnostic criteria. However, ideally, any local modifications should be minor, since otherwise the benefits accruing from a common classification system will be diminished or lost.

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