IMPORTANCE OF SPECIFIC IgM SEROLOGY IN DIAGNOSIS OF PERINATAL INFECTIONS

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ABSTRACT

Specific Immunoglobulins M (IgM) are first produced as a primary immune response in viral, bacterial, or parasitic infections.

Using ELISA-Captia-IgM or IgM ELISA with IgG/rheumatoid factor sorbent, having superior sensitivity and specificity, IgM serology can provide early diagnosis in pre- and post-natal infections, with the possibility of timely treatment, of major importance, especially in asymptomatic cases where lesions are not yet manifest.

Also, a longer undetectable level of specific IgM antibodies following treatment is a microbial sterilization indicator.

Although the sensitivity and specificity of IgM tests are not absolute, their utility remains valid, especially when combined with other tests such as IgG ELISA, IgG antibody avidity test.

Some suggestive examples of syphilis, toxoplasmosis, rubella, CMV infection, underscore the importance of IgM antibody detection tests for the diagnosis of these perinatal infections.

Keywords: ELISA, IgG, IgM, Avidity Index (AI), CMV, rubella, syphilis, toxoplasmosis.

REZUMAT

Imunoglobulinele M specifice sunt produse primele ca răspuns imun primar în infecțiile virale, bacteriene sau parazitare.

Beneficiind de reactivi perfecționați (ELISA-Captia-IgM, ELISA-IgM cu sorbent pentru IgG/Factor Reumatoid), cu sensibilitate și specificitate înalte, serologia IgM poate asigura un diagnostic precoce în infecții pre/postnatale, cu posibilitatea instituirii unui tratament în timp util, cu importanță majoră mai ales în cazurile asimptomatice, în care leziunile nu sunt încă manifeste.

De asemenea, dispariția persistentă a IgM specifice în urma tratamentului reprezintă un indicator de sterilizare microbiană.

Deși testele IgM nu sunt absolute, utilitatea lor rămâne valabilă, mai ales în condițiile asocierii altor teste, cum ar fi ELISA IgA/E, testul de aviditate IgG, PCR.

Câteva exemple semnificative privind sifilisul, toxoplasmoza, rubeola, infecția cu CMV, subliniază importanța testelor IgM pentru diagnosticul acestor infecții perinatale.

Cuvinte-cheie: ELISA, IgG, IgM, IAV, CMV, rubeolă, sifilis, toxoplasmoză.

INTRODUCTION

The serological diagnosis of an acute or chronic infection in pregnant woman, mother and newborn is routinely made by detecting the presence or evolution of IgG, IgM and complementary IgA antibodies, using ELISA technique, an assay with high sensitivity and specificity.

This serology diagnostic technique is easy to apply to the adult, but is difficult to interpret in the newborn due to the transplacental passage of maternal IgG antibodies, amplified in the last months of pregnancy.

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Starting at 3 months of pregnancy, expression of CD16 receptors for Fc IgG on syncytiotrophoblast continues to increase and bound IgGs are internalized by placental cells and then released into the fetal circulation [1].

Their half-life in the newborn's blood is about 23 days, the titre continuously decreasing. If the IgG titre is stable for 6 months or longer, it is likely that these IgGs are produced by the fetus/newborn.

To eliminate this time frame and to therapeutically intervene as quickly as possible in the newborn/infant, it is necessary to detect specific IgM antibodies, if possible.

Even if specific IgM antibodies circulate in the blood of the pregnant women due to infection during pregnancy, they do not cross the placenta and their presence in the baby's blood signifies a production of their own in response to the infection transmitted by the mother.

It is very useful to associate the avidity test of IgG for the diagnosis of congenital infections late in the first year of life when maternal IgGs with a high degree of avidity have disappeared, making place of the baby low-avidity IgG (recent date IgG).

Physical properties of immunoglobulins

The five classes of immunoglobulins differ by molecular weight, by number of peptides per molecule, heavy chains, molecular form, antigen-binding valence, serum concentration (Table 1).

The biological properties of immunoglobulins

The major biological characteristics of immunoglobulins are dependent on the physical properties but to a great extent also on the immune system physiology. Thus, IgGs are the most abundant intra/extravascular antibodies, are bound to macrophages and PMN, and cross placenta having antitoxic and antimicrobial activity. IgA is a major immunoglobulin in mucosal secretions and does not cross the placenta. IgM is the first line of defense antibody in bacteremia and is the first produced in the primary immune response by the adult and also by the fetus or the newborn. IgE protects external surfaces and plays a role in parasitic infections as well as in atopic allergies. IgDs are present on the surface of lymphocytes in genesis and IgM release (Table 2).

Table 1. Physical properties of immunoglobulin classes [2, 3]

Properties	IgG	IgA	**IgM**	IgD	IgE
Coefficient of sedimentation	7S	7S, 9S, 11S	**19S**	7S	8S
Molecular weight	150 000	160 000 and dimer	**900 000**	185 000	200 000
No. of base units (4 peptides/u)	1	1, 2	**5**	1	1
Heavy chain	**γ**	**α**	**μ**	**δ**	**ε**
Light chains k+**λ**	k+**λ**	k+**λ**	**k+λ**	k+**λ**	k+**λ**
Molecular format	γ_2k_2, $\gamma_2\lambda_2$	$(\alpha_2k_2)_{1-2}$ $(\alpha_2\lambda_2)_{1-2}$ $(\alpha_2k_2)_2S$ $(\alpha_2\lambda_2)_2S$	$(\mu_2k_2)_5$ **(J)** $(\mu_2\lambda_2)_5$ **(J)**	$\delta_2k_2(\delta_2\lambda_2?)$	ε_2k_2, $\varepsilon_2\lambda_2$
Valence for antigen binding	2	2,4	**5(10)**	2	2
Normal serum concentration	8 - 16 mg/ mL	0,9 - 4,5 mg/mL	**0.6 – 2.8 mg/mL**	0 – 0.4 mg/ mL	0.017 – 0.45 mg/mL
Percent of the total immunoglobulins	80	13	**6**	0 - 1	0,002
Percentage of carbohydrate content	3	8	**12**	13	12

Table 2. The immunoglobulins biological properties [2]

	IgG	IgA	IgM	IgD	IgE
Major characteristics	The most abundant intra and extra-vascular Ig with antimicrobial and antitoxic activity	Major Ig in the serum mucosal secretions; protecting surfaces in contact with the external environment	- The first Ig produced in the immune response - The first produced by newborn - Very effective agglutinating activity - First line of defense in bacteremia	The majority are present on the surface of lymphocytes (together with IgM)	- Protection of external surfaces - Role in parasitic infestations - Responsible for symptoms of atopic allergies
Fixing C ' Classic path Alternative path	 ++ -	 - +	 +++ -	 - -	 - -
Placental crossing	++	-	-	-	-
Fixing on mast and basophil homologous cells	-	-	-	-	+++
Macrophages and neutrophils binding	+++	+	-	-	+

Immunoglobulins M exhibit some special characteristics:

- They circulate in the form of pentameric and hexameric forms, the last form being up to 20X more effective in C' mediated cell lysis.
- Monomeric IgM is the major receptor together with IgD used by B lymphocytes to recognize the antigens.
- IgMs are multivalent: 10 valences for small haptens and 5 for large antigens, resulting in high avidity.
- They are effective as agglutinating and cytolytic antibodies: a single IgM is equivalent to 100-1000 IgG.
- IgMs are first synthesized in the primary immune response by the fetus and the newborn (Fig. 1).

The biological characteristics of IgM also affect serodiagnosis. The fact that they are synthesized first, but they have short half-life (5 days) is especially important in terms of sensitivity of the tests. This is influenced by the moment when they are detected, as their titres decrease, except in some cases, quite quickly.

The IgM detection is complicated by the presence of transplacental maternal IgG antibodies. This is avoided when using capture type tests or those involving the IgG/FR sorbent (Table 3).

Ensuring high specificity/sensitivity for ELISA IgM detection assays

There are two technical ways: either using an IgG/RF sorbent or using a capture format test. In the first variant, the test serum is treated with a human anti-IgG serum that can precipitate up to 15 mg IgG/mL, with recovery of nearly 100% IgM.

This sorbent eliminates not only the IgGs that compete with IgMs but also the negative IgM results. At the same time, the sorbent also eliminates RF, i.e. IgG anti IgM, that could produce false positive IgM results. It should be noted that fetal RFs are produced by fetal immunization with maternal IgG allotypes. In conclusion, absorption of IgGs from serum samples is mandatory for all IgM tests (Figs. 2 and 3).

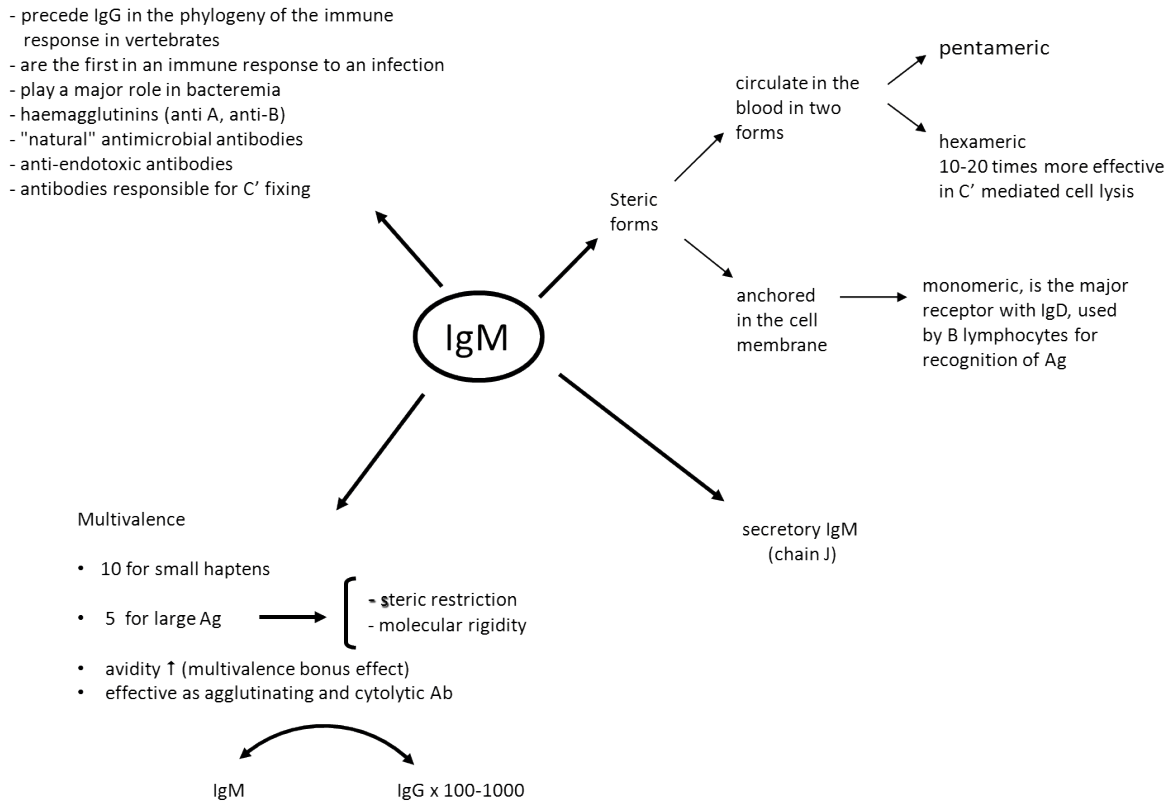

Fig. 1. Characteristics of immunoglobulins M

Table 3. Immunoglobulins characteristics that affect serologic diagnosis

Ig class	Placental crossing	Half life	Test sensitivity	Test specificity
IgG	Yes	23 days	> 90%	> 98% (complicated by maternal transplacental IgG)
IgA	No	7 days	65 – 95%	> 97%
IgM	No	5 days	90 – 98%	> 98%
IgE	No	2.3 days	75 – 95%	> 95%

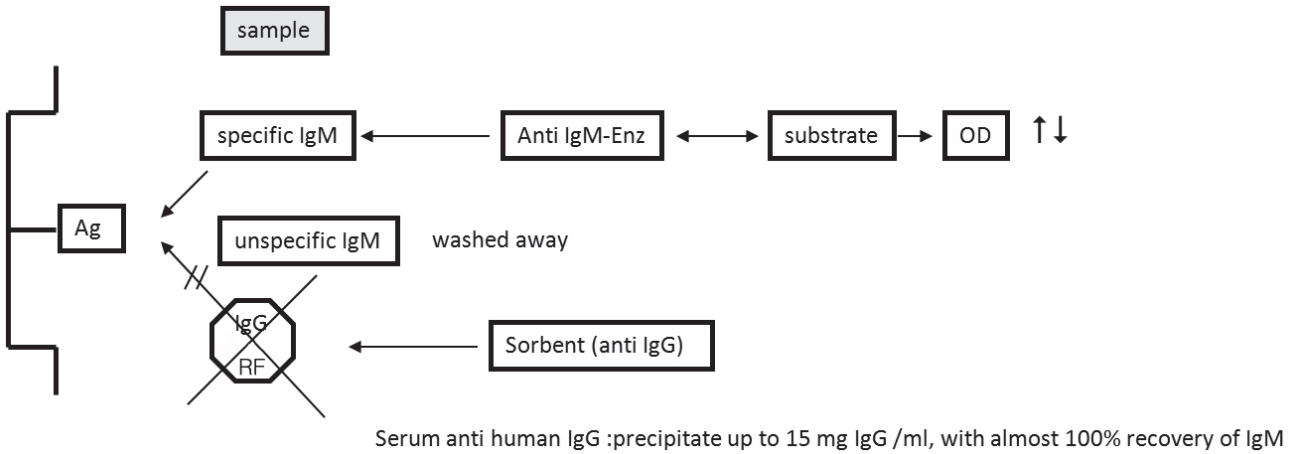

Fig. 2. IgM ELISA with sorbent for IgG and RF elimination

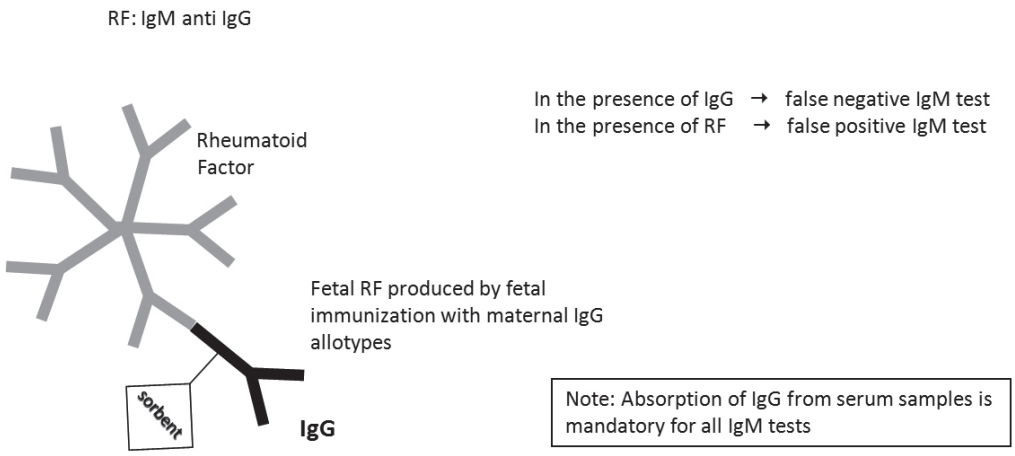

Fig. 3. Anti IgG/RF sorbent increases the IgM sensitivity and specificity test

The second variant, ELISA capture, uses the fixation of IgM antibodies (specific and non-specific) in the plate well for the test sample, and the antigen-enzyme conjugate will only select specific IgMs, resulting in the color reaction produced by the enzyme substrate being due exclusively to captured specific IgMs (Fig. 4).

Immune status in the newborn

In the newborn, lymphoid organs (lymph nodes, spleen) are relatively underdeveloped, except in the case of an intrauterine infection. Capacity of graft rejection and antibody synthesis are sufficiently apparent. The intrinsic Ig level is lowered in the absence of a congenital infection, excepting transplacentally transferred IgG (Fig. 5).

Immunoglobulins M are intrauterine and extrauterine products at low but significant level, they reach the adult level at the age of one year and do not cross the placenta.

Immunoglobulins A, E and D have very low values in the newborn's circulation and reach 20-25% of the adult level at one year of life.

The transplacentally transferred IgG level decreases rapidly in the first 2-3 months after birth by catabolism, with a half-life of about 23 days. In parallel, IgG own synthesis takes place and will almost completely replace maternal IgG at the age of 7-8 months [4] (Fig. 6).

Cytomegalovirus (CMV)

CMV is a DNA (240 Kb) β herpes virus, with 35 structural proteins, which can cause serious disease in infants and adults. CMV can persist in the human body for years and may cause recurrent infections or be transmitted to other individuals. CMV infections are very common: 60-85% of the population has been infected and most cases are asymptomatic.

One up to 3% of women are infected during pregnancy and in one out of every two cases the infection is passed on to the fetus.

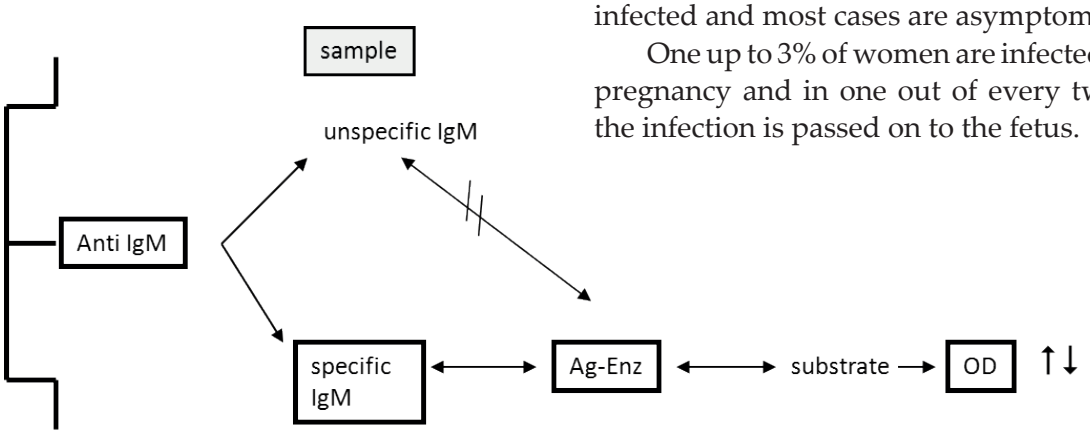

Fig. 4. Capture format of ELISA IgM antibodies

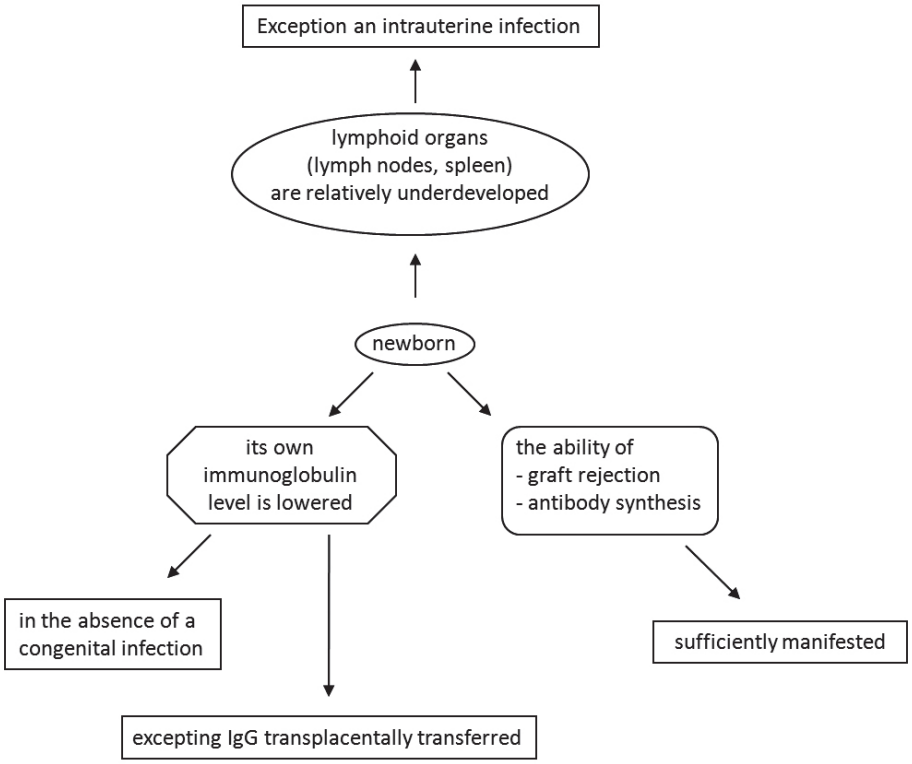

Fig. 5. Newborn immune status

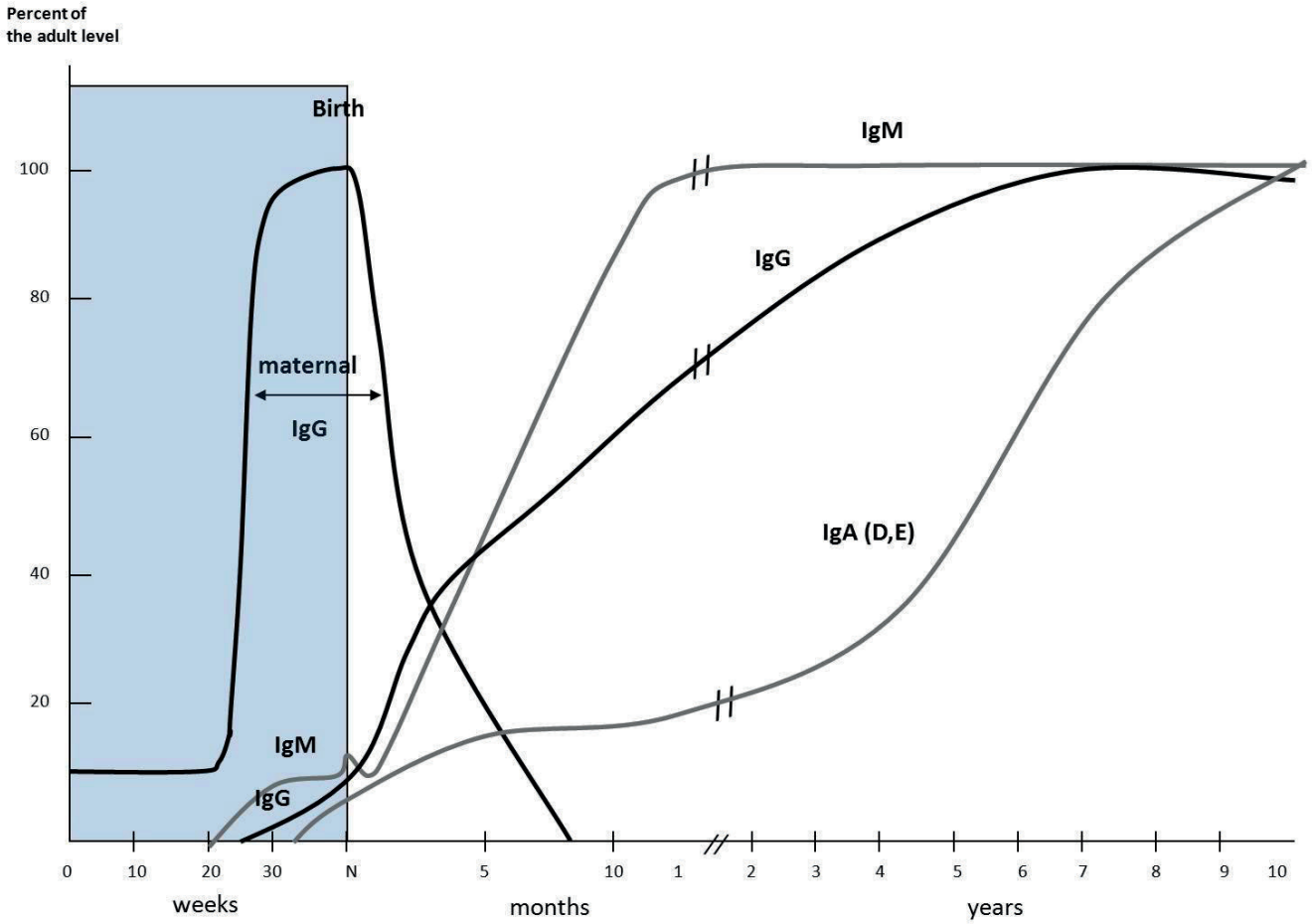

Fig. 6. Evolution of serum immunoglobulin levels in humans [4]

In primary maternal infection the transmission of CMV infection is 25 times higher than in an old maternal infection. The infection is often asymptomatic but in about 5% of cases, the consequences for the fetus can be very severe: hepatosplenomegaly, hydrocephalus, microcephaly, prematurity. Fetal death is frequent [5, 6]. Even in cases of asymptomatic infection, approximately 10% of infants show sensory-neural side effects, such as deafness or partial/total blindness. Symptomatic Central Nervous System CMV infection is present almost exclusively in primary maternal infection. The anti-CMV IgM antibodies, present in approximately 70% of primary infections, generally persist for 16-20 weeks after infection and may reappear irregularly during re-activation.

The detection of anti-CMV IgM antibodies is useful in the diagnosis of current primary infection, particularly in pregnant women and infants [7 - 10].

Prenatal CMV diagnosis

The actual incidence of intrauterine infection is not known exactly. Demonstration of CMV IgG seroconversion is limited: serial samples are required. It is important to determine CMV IgM antibodies (ELISA, blotting). CMV IgMs are present in approximately 70% of the primary infections and may persist for 4-5 months with recurrence in case of reactivation. Their significance, somewhat relative to persistence, can be improved by corroborating with the IgG avidity test, but considering that IgG avidity maturation is not complete until 4-5 months after primary infection. Amniocentesis could also be performed in 21-25 weeks of pregnant women with primary infection. In this case, the detection of CMV can be performed by virus culture and/or by amniotic fluid PCR [11, 12].

Postnatal CMV diagnosis

Postnatal diagnosis, beyond individual importance, has epidemiological value. In the case of intrauterine transmission, anti-CMV IgM detection is of particular importance, as IgMs do not cross the placenta. Detection of anti-CMV IgM antibodies has relative value compared to detection of CMV by PCR, viral culture from urine, saliva, blood (attention: inconsistent viremia) in the first 3 weeks of life. In case of intrapartum/postpartum transmission (breastfeeding), the virus is detectable in urine and saliva after 3 weeks [13].

Rubella

The virus that causes rubella is an RNA virus and member of Togaviridae family. Rubella is a mild self-limited disease in most individuals who acquired the infection after birth. Rubella is spread either by direct contact with nasopharyngeal secretions of an infected individual or through droplets. Congenital infections in infants result from transplacental spread of the virus associated with maternal viremia during primary infection in pregnancy. Because the antibody protects against significant viremia, neonates born to women without preexisting immunity are at risk if the mother has rubella during her pregnancy. Reinfection of women during pregnancy may occur, but the risk of fetal infection is very low. A primary infection during pregnancy can cause significant problems to the fetus, including fetal death, miscarriage, or congenital anomalies (ophthalmological, cardiac, neurological).

90% of newborn congenital infections occur in pregnant women with primary rubella infection in the first trimester. 85% of newborns congenital abnormalities appear after the primary infection in the first month of pregnancy. Rubella infection after 4 months of pregnancy rarely produces congenital defects detectable at birth [14, 15, 16, 17].

Diagnosis of pregnant women

Clinical diagnosis can only be established for symptomatic infections: fever, rash, adenopathy, arthralgia, curvature. Virological diagnosis: nasopharyngeal cultures, one week before and two weeks after the eruption.

Serological diagnosis: rubella IgM antibodies detection. These are detectable at the onset of the eruption with a maximum of three weeks, after which they decrease rapidly, the time being important. The presence of high

titre of rubella IgM means recent infection diagnosis. In the case of a negative result, another sample will be tested [18 - 21].

For confirmation, rubella IgG antibodies detection is used as quickly as possible after the rash development and second sample is tested 2-4 weeks after the eruption (IgG seroconversion). This is also associated with the IgG avidity index. When specific IgMs are present together with IgG seroconversion and positive cultures, the risk of fetal infections is maximal. Since infection is often asymptomatic, serological control of pregnant women is mandatory [14, 22].

The diagnosis of the newborn

Virological diagnosis: virus isolation considering its presence for months in nasopharynx, urine, blood, CSF.

Serological diagnosis: determination of specific IgM anbodies in the first 2 months of life with maximum sensitivity. If rubella IgM level is too low, false negative results may occur. Rubella IgG determination has to be repeated: at birth, at 3 months, at 6 months. Their persistence for 6-12 months at a higher or equal birth titre is an indicator of intrauterine infection. Caution: postnatal infection should be eliminated [23].

Syphilis

The placenta is an effective barrier to *Treponema pallidum*: the trophoblastic epithelium and the Hofbauer cells - placental macrophages. Infection may be limited to placenta: villitis, vasculitis and thrombosis lead to slower fetal growth. The infection can pass through the capillaries of the villi in the fetal blood either contiguously or transported by the cells. This is the initial stage of fetal infection, analogous to the secondary stage of the adult. In the late maternal infection the probability of the fetal infection is low. But fetal infection can occur throughout the pregnancy, more frequently starting with weeks 9-10 of pregnancy. Beginning with week 18, the fetal immune system becomes functional and, consequently, clinical signs appear. *Treponema pallidum* is detectable in placenta and fetal tissues [1, 24].

Antepartum diagnosis

The prenatal diagnosis of pregnancy involves the semi-quantitative serologic diagnosis VDRL/RPR and TPHA assays. The information about the history of the infection, whether treated or not, is also necessary [25, 26].

Diagnosis of the newborn

If the mother is positive in the two tests, transplacental IgGs passage will cause the seropositivity of the newborn. In the absence of infection, the VDRL/TPHA tests will have a titre equal to or lower than that of the mother, with a continuous decrease. A 2-4-fold higher titre is indicative of intrauterine infection. Because specific IgM antibodies do not cross the placenta (high molecular weight), their determination by treponemal IgM ELISA is mandatory. In the case of newborn infection, the test is positive immediately after birth and may remain positive for another 6 months, in this case being also predictive of neurosyphilis. Positive ELISA IgM is confirmed by treponemal blotting IgM assay (antibodies to major antigens 15, 17, 45, 47 kDa) with 99-100% sensitivity/specificity. The serology is the only diagnostic alternative for asymptomatic infection [27 - 29].

Toxoplasmosis

Toxoplasma gondii, the human toxoplasmosis agent, is an obligatory intracellular protozoa with a biphasic life cycle: a sexual phase that occurs only in the cat's digestive epithelium (the only definitive host) and an asexual one. The latter occurs following an infection of an intermediate host (human, other mammals, birds). In the intermediate host, toxoplasma evolves in two phases: in the acute phase of the infection, as a mobile proliferating tachyzoite, and in the chronic phase, in the form of a cyst, containing a number of bradyzoites (brain, muscle).

The chronic form is the result of the action of a humoral and cellular intact immune system in response to the multitude of antigens in the various multiplication phases. Toxoplasmosis is transmitted by the ingestion of food contaminated by sporulated oocysts, through the contact with soil or other contaminated

materials with oocysts existent in the excretions of cats.

Most cases of congenital transmission result from the acquisition of maternal primary infection during pregnancy. The risk of transplacental transmission is highest when primary infection occurs during the last pregnancy trimester. In this case, the frequency of symptomatic neonatal infection is low. The lowest minimal risk of congenital infection occurs when the primary infection appears during the initial period of pregnancy, and the frequency of symptomatic neonatal infection is high.

Neonatal symptomatology includes the classic triad consisting of hydrocephaly, intracranial calcifications, chorioretinitis to which one or more of the following signs may be added: rash, lymphadenopathy, hepatosplenomegaly, jaundice, cataract, microophthalmia, optic atrophy, fever, anemia [30, 31].

Prenatal diagnosis

Diagnosis of primary infection in pregnant women

Most cases of *T. gondii* infection in immunocompetent pregnant women are inapparent. If the infection is symptomatic, it is usually manifested by lymphadenopathy, fatigue with or without fever. In acute infection, anti-Toxoplasma IgM and IgG antibodies occur within one or two weeks.

High titres of specific IgG suggest an acute infection, but can not distinguish between a newly acquired infection or one acquired prior to conception, or much earlier. The key question is whether acute infection occurred during pregnancy or before conception.

If the last variant is real then the presence of the pre-existing maternal immune response will protect the fetus.

Toxoplasma IgM ELISA can distinguish between recent infection and old infection. For more accurate dating of the infection, specific IgA and IgE ELISA are useful: half-life of IgA and IgE are 7 and 2.3 days respectively. After anti-Toxoplasma IgM and IgG antibody detection, the IgG avidity test may also exclude recent infection (over the last 4 months). Seronegative pregnancies should be tested monthly to detect a possible seroconversion. HIV positive pregnancies should be tested also for latent toxoplasmosis [32 - 34].

Diagnosis of fetal infection

After confirmation of primary infection in pregnancy, amniocentesis is performed for the PCR test (primers for the repeat B1 gene).

Also, cordocentesis may be used for the specific IgM/IgA antibodies detection, in the case of a possible fetal infection, close to birth [33, 35].

Postnatal diagnosis

IgG ELISA is positive with a 4-fold higher titre than that of the mother, reaching a maximum at 1-2 months of life. The titre descends continuously in case of passive transfer and becomes undetectable at 6-12 months.

In infected children, depending on the date of intrauterine infection, specific IgG antibodies level decreases immediately at birth (transfer antibodies) and then grows in 3-4 months. In the case of very close fetal infection specific IgG antibodies appear only at 2-3 months of life: false negative anti-Toxoplasma IgG antibodies.

IgM /IgA /IgE ELISAs are positive at 1-2 weeks with a maximum of 6-9 months of life.

Testing has to be repeated to follow up evolution to asymptomatic infants.

Synthesis of specific antibodies may be delayed 3-4 months depending on the evolution of the infection, which is influenced by the late fetal infection, maternal protective antibodies and the treatment.

In some cases, a CSF PCR test can be performed.

Newborns with evolutive HIV infection do not produce specific antibodies and the diagnosis is based on clinical data and microorganism isolation (difficult) [36, 37].

MATERIAL AND METHODS

A total of 119 serum samples collected from women before conception and pregnant women in different stages of pregnancy were tested differently with IgM and IgG ELISA for detection of specific antibodies. If specific IgG

was positive in the tests for diagnosis of CMV infections, rubella, toxoplasmosis, IgG avidity was also tested. For the diagnosis of syphilis, Treponemal IgM ELISA and the Fluorescent IgG Treponemal Antibody with Absorption (FTA-Abs) were used. Avidity tests were performed using the VIDAS (Enzyme Linked Fluorescent Assay) technique. Until testing, the collected sera were stored at -30^{0}C.

RESULTS AND DISCUSSION

CMV

For CMV infection, a number of seven serum samples from women before conception and a number of 55 serum samples from pregnant women with different stages of pregnancy were tested (Table 4). The seven serum samples from non-pregnant women were negative for CMV IgM antibodies, positive for CMV IgG antibodies, with an Avidity Index (AI) greater than 0.8, which indicates that CMV primary infection occurred more than 3 months ago. These cases represent the ideal situation in which there is no risk of fetal infection. The presence of anti-CMV IgG antibodies provides protection for the future mother and the fetus.

A total of 30 sera from pregnant women with varying pregnancy stages between 5-28 weeks were negative for CMV IgM antibodies and positive for CMV IgG antibodies with AI> 0.8. The pregnancies between 5 to 12 weeks present a minor risk of fetal infection. Those aged 13 to 28 weeks are at higher risk, especially the most advanced. In that case, the infection, although older than 3 months, may be assumed to overlap the beginning of pregnancy and CMV IgM antibodies are not detectable if they occurred 6 months ago.

The two 29-30 weeks pregnancies with CMV IgM antibodies positive and CMV IgG antibodies negative present a maximum risk of fetal infection, given that the infection occurred probably during the last trimester of pregnancy, and CMV IgG antibodies did not have enough time to appear.

In the group of 10 cases with 13-28 weeks of pregnancy, with CMV IgM antibodies and

Table 4. CMV serology: IgM, IgG and IgG Avidity index (Medical Analysis Laboratory, Cantacuzino Institute)

Age of pregnancy	No. of cases	IgM	IgG	AI (avidity index) / Primary infection	Conclusions
Pregnancy preparing	7	N	P	> 0.8/ > 3 months	ideal
5 - 28 weeks	30	N	P	> 0.8/ > 3 months	5-12 weeks Risk ↑ / Risk ↑↑ 13-28 weeks
29 - 30 weeks	2	P	N		Risk ↑↑↑
13 - 28 weeks	10	P	P	5 cases – indefinite 5 / > 0.8 > 3 months	Risk ↑↑ / Risk ↑
7 – 25 weeks	8	E	P	3 cases - indefinite 5 / > 0.8 > 3 months	Risk ↑ / Risk ↑
8 - 25 weeks	3	N	-	-	Risk for the pregnancy > 12 weeks
8 – 12 weeks	2	-	P	> 0.8/ > 3 months	Category with AI compulsory

AI ≥ 0.8 – primary infection older than 3 months; AI = 0.2 - 0.8 – equivocal; AI < 0.2 – primary infection in the last 3 months

Table 5. CMV and Toxoplasmosis serology for newborns (Medical Analysis Laboratory, Cantacuzino Institute)

Newborns	No. of cases	CMV		Toxo		Conclusions
		IgM	IgG	IgM	IgG	
Prematurity, fetal injury	1	P	P	P	P	Double fetal infection
No apparent injuries	1	-	-	N	P	IgG transfer or subsequent evolution
3.5 months, prematurity, brain injury	1	-	-	N	P	Fetal toxo infection? Fetal infection with another agent?

CMV IgG antibodies positive, 5 cases with AI of 0.2-0.8 equivocal, presented a medium risk. The other 5 cases with AI > 0.8 still presented a minimal risk due to the presence of specific CMV IgM antibodies.

The group of 8 cases with 7-25 weeks of pregnancy, with undetermined CMV IgM antibodies and positive CMV IgG antibodies, including 3 cases with indeterminate AI and 5 cases with AI > 0.8, presented a minimal risk.

In the group of 3 cases 8-25 weeks of pregnancy, only CMV IgM antibody detection was performed, with negative result. Given the unknown CMV IgG antibody values, it is difficult to assess the degree of risk for these cases. There is, in theory, only a risk for pregnant women over 12 weeks of pregnancy. This highlights the importance of determining both specific antibodies classes and AI for IgG, if any.

In the group of 2 cases of 8-12 weeks of pregnancy with tested CMV IgG antibodies only, with positive results and AI > 0.8, the risk is minimal or even null. However, in this category with positive CMV IgG antibodies, AI is mandatory.

A newborn case with prematurity and symptomatic fetal impairment showed positive IgM and IgG serology for CMV and Toxoplasma infection, denoting a double fetal infection (Table 5). The presence of IgM antibodies along with symptomatology made testing of IgG AI unnecessary.

Rubella

In the case of pregnant women infected with rubella virus, eight women at different ages of pregnancy were tested (Table 6). A 13-week pregnant woman showed negative anti-Rubella IgM antibodies, positive anti-Rubella IgG antibodies with AI>60%. Because it is considered that a high avidity index excludes infection in the last 4 to 6 weeks, and the lack of specific anti-Rubella IgM antibodies showed the absence of acute infection, the risk of fetal infection is minimal or even null.

A group of 4 pregnant women with 8-25 weeks of pregnancy showed positive Rubella IgM and IgG antibodies, AI IgG > 60%. This serological aspect suggested the lack of acute infection, but the persistence of Rubella IgM antibodies certified some fetal risk.

In the two pregnant women of 18 to 32 weeks of pregnancy with indeterminate Rubella IgM antibodies, and positive Rubella IgG antibodies, AI>60% denoted no acute infection with a minimal fetal risk.

The case of pregnant women aged 24 weeks with negative Rubella IgM and IgG antibodies indicated the lack of infection up to this age.

Syphilis

A group of 7 pregnant women aged 14 to 18 weeks (Table 7), with no clinical-epidemiological data, showed IgM negative and IgG positive antitreponemal antibodies.

It was probably an old infection and the fetal risk was minimal. A 16-week pregnant woman had positive IgM and IgG antitreponemal antibodies, indicating a maximum fetal risk. Another pregnant woman with a 17-week pregnancy, indeterminate IgM and positive IgG antitreponemal antibodies showed a medium fetal risk.

Since antitreponemal IgM may be close to conversion, it was necessary to repeat the IgM test for several months in a row.

As with other infections, the presence of IgGs and an old treponemic infection minimize

Table 6. Rubella IgM, IgG serology and IgG AI (Viral Respiratory Infections Laboratory, Cantacuzino Institute)

Age of pregnancy	No. of cases	IgM	IgG	AI	Conclusions
13 weeks	1	N	P	> 60% / lack of acute infection	Risk ↓
8 – 25 weeks	4	P	P	> 60% / lack of acute infection	Risk ↓
18 - 32 weeks	2	E	P	> 60 % / lack of acute infection	Risk ↓
24 weeks	1	N	N	lack of infection	-

AI = Avidity Index; < 40% low avidity; 40-60% equivocal; > 60% high avidity (excludes infection in the last 4-6 weeks)

Table 7. Syphilis IgM, IgG serology (Sexually Transmitted Infections Laboratory, Cantacuzino Institute)

Age of pregnancy	No. of cases	IgM	IgG (FTA-Abs)	Conclusions
14 – 18 weeks (without serological specification)	7	N	P	Minimal risk
16 weeks	1	P	P	Risk ↑↑↑
17 weeks	1	I	P	Risk ↑↑ Repeat IgM

Table 8. Syphilis serology in newborns and infants (Sexually Transmitted Infections Laboratory, Cantacuzino Institute)

Congenital asymptomatic syphilis?	No. of cases	IgM	IgG (FTA-Abs)	Conclusions
2 days – 13 months (without mother serology)	6	N	P	Minimal risk
1 day (without mother serology)	2	P	P	Risk ↑↑↑
1 day (without mother serology)	1	N	P	No infection

fetal transmission significantly. A total of 9 newborns and infants aged 1 day to 13 months were also tested, with no maternal serology (Table 8).

A group of 6 cases aged between 2 days and 13 months was negative for antitreponemal IgM and positive for antitreponemal IgG. These are probably IgG transfer antibodies and the lack of IgM indicates that the risk of infection was minimal or null. In these cases, IgG antibodies may persist for several months and are continuously decreasing. Two newborns aged one day showed positive antitreponemal IgM and IgG antibodies. These newborns were intrauterine infected with or without specific symptomatology at birth. A newborn of one day showed negative IgM and positive IgG antitreponemal antibodies. It is a case of passive intrauterine passage of specific IgG antibodies and therefore is free of infection.

Table 9. Toxoplasmosis IgM, IgG serology and IgG Avidity Index (Medical Analysis Laboratory, Cantacuzino Institute)

Age of pregnancy	No. of cases	IgM	IgG	AI / Primary Infection	Conclusions
Pregnancy preparing	1	N	P	> 0.3 / > 4 months	Ideal
6 – 13 weeks	13	N	P	> 0.3 / > 4 months	Risk ↑
Pregnancy preparing	2	P	P	< 0.2 / < 4 months	Treatment
10 – 25 weeks	5	P	P	1 pregnant women /E 4 pregnant women no AI	Risk ↑↑↑
8 – 17 weeks	2	E	P	> 0.3 / > 4 months	Risk ↑
10 – 17 weeks	4	-	P	> 0.3 > 4 months	Risk ↑
11 weeks	1	P	E	E	Risk ↑↑ / Pregnancy Intrerruption?

AI < 0.2 low avidity; 0.2 ≤ AI < 0.3 echivocal; ≥ 0.3 high avidity

Toxoplasmosis

A number of 28 women before conception and pregnant women in different ages of pregnancy have were tested (Table 9). An ante-pregnancy tested woman had a negative anti-Toxoplasma IgM antibodies, and positive anti-Toxoplasma IgG antibodies result with an AI > 0.3, which indicates a primary infection more than 4 months ago. This is the ideal situation in which the infection is old, the future mother is immune, and the fetal risk is minimal.

Another 2 women in the same situation tested positive for anti-Toxoplasma IgM and IgG antibodies, with AI < 0.2, suggesting an infection produced earlier than 4 months with a risk for eventual pregnancy, and therefore specific treatment for toxoplasmosis should be instituted immediately.

A group of 13 women of 6 to 13 weeks pregnancy age had negative anti-Toxoplasma IgM, positive anti-Toxoplasma IgG antibodies with AI > 0.3. This group presented a minimal risk because the infection was older than 4 months.

A group of 5 pregnant women with pregnancy of 10 to 25 weeks showed positive anti-Toxoplasma IgM and IgG antibodies, one woman with AI indeterminate and the other four with AI not performed. The fetal risk for this group is maximal. A group of 6 pregnant women with pregnancy of 8 to 17 weeks of which two pregnant women had equivocal anti-Toxoplasma IgM antibodies and positive anti-Toxoplasma IgG antibodies with AI > 0.3 and the other 4 positive anti-Toxoplasma IgG antibodies with AI > 0.3, without anti-Toxoplasma IgM tested, due to infection older than 4 months, presented a minimal fetal risk.

An 11-week pregnant woman with positive anti-Toxoplasma IgM antibodies, indeterminate anti-Toxoplasma IgG antibodies, indeterminate AI, has a high fetal risk and may be candidate for pregnancy interruption.

CONCLUSIONS

The analysis of the results on differential testing for syphilis, CMV, toxoplasmosis, rubella, 119 samples from women preparing for pregnancy or women at different ages of pregnancy, as well as from newborns, reveals the importance of an early diagnosis of these infections, before or during pregnancy, taking into account that more recent studies showed that fetal transplacental infection can occur much earlier than expected, beginning with weeks 9-10.

For more accurate dating of the evolutive stage of the infection, the specific IgM detection by ELISA is absolutely mandatory, taking into account the early synthesis of IgM and their short half-life (5 days).

In most cases, IgM-specific antibody testing provides early pre- and post-natal diagnosis with the possibility of timely treatment, with major importance especially in asymptomatic cases where the lesions do not appear or have not reached a certain level of gravity.

The stable disappearance of specific IgM, following treatment, is a microbial sterilization indicator.

Although the ELISA IgM detection test has high sensitivity and specificity, it is not always absolute, also producing apparently false negative reactions (very recent infection in the mother, intrauterine HIV infection) or false positives (e.g. anti-nuclear antibodies in toxoplasmosis).

The association of this test with the concomitant determination of specific IgG, and IgG avidity ensures even more diagnostic accuracy. In some cases, the PCR test is also needed.

In addition, it is relatively simple to perform IgM, IgG, IgA ELISA detection tests and the benefit-cost ratio is favorable to the latter.

Conflict of interests: None to declare.

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