Avidity of IgG for Rubella: An Evaluation of the Need for Implementation at the Materno-Infantil Presidente Vargas Hospital in Porto Alegre, Rio Grande do Sul, Brazil

Reis, M.M.[1,2], Tessaro, M.M.[1], Cruz e Silva, J.[3], Giordano, S. A.[1] and d'Azevedo, P.A.[2]

[1]Pathology Service of the Hospital Materno Infantil Presidente Vargas (HMIPV), [2]Department of Microbiology and Parasitology of the Fundação Faculdade Federal de Ciências Médicas de Porto Alegre (FFFCMPA), [3]Faculty of Pharmacy of the Universidade Luterana do Brasil (ULBRA), Porto Alegre, RS, Brazil

Rubella serum assays performed in the laboratory of the Materno-Infantil Presidente Vargas Hospital (HMIPV) from 1998 to 2002 were reviewed to determine if IgG avidity assays should be implemented. IgG was determined using the Enzyme Linked Fluorescent Assay, ELFA, VIDAS® system, bioMérieux or the Microparticle Enzyme Immunoassay, MEIA, Axsym® system, Abbott, and IgM was determined using the ELFA, VIDAS® system, bioMérieux, a capture format assay. Specific IgG was assayed in 2,863 samples, with positive results for 84% of the patients, for the most part with high levels of antibodies. IgM was assayed in 2,851 samples, being positive in 14 (0.49%) and inconclusive in 25 (0.88%). Serology for toxoplasmosis was also positive or inconclusive in 5 patients. After a cost-effectiveness analysis, it was decided not to implement avidity assays, considering that the HMIPV is a public institution, with limited funding. Difficulties concerning the integration of the Clinical Pathology Service with the Clinical Staff of the institution were also considered.

Key Words: Rubella virus infection, congenital infection, avidity of IgG for rubella, serology for rubella virus.

Primary infection by the rubella virus is benign and is usually subclinical, but even if the mother has no symptoms the fetus may be severely affected, especially if infection occurs during the first 12 weeks of pregnancy [1-5]. The risk of teratogenicity if the mother is infected during the first eight weeks of gestation is nearly 100% [6]. The dissemination of the virus to the fetus probably begins with placental infection during maternal viremia. Possible mechanisms of cytopathogenicity include induction of apoptosis by the virus and inhibition of cell division [7-11]. When the fetus is infected after the 18th week of gestation, organogenesis is already complete, making the presence of anomalies much less probable [6].

The rubella virus establishes a chronic infection in the fetus, and its elimination may take years [12]. Manifestations of congenital infection by the rubella virus may be *transient* with spontaneous regression, including neonatal thrombocytopenic purpura, hemolytic anemia, and hepatitis; *permanent*: deafness, congenital heart disease, cataract, glaucoma, pigmentary retinopathy, and mental retardation; and *tardive*: diabetes mellitus, hypo- and hyperthyroidism, and panencephalitis [13-16].

Serum assays have been used to evaluate immunity to the virus, determining the need to vaccinate women at reproductive age or to diagnose infection. The diagnosis of infection is serologic and is based on the presence of specific IgG and IgM antibodies [12,17].

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Address for correspondence: Dr.Pedro Alves d'Azevedo, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Sarmento Leite street 245/211 – Porto Alegre, RS, Zip code: 90050-170, Brazil. Phone: 55 51 32248822. Fax: 55 51 32269756. E-mail: pedroaze@fffcmpa.tche.br

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More recently, assays of the avidity of IgG, i.e. the strength of IgG binding to a multivalent antigen of the virus, have been used to distinguish recent from old infections in individuals with high IgG levels [18,19]. At the onset of the immune response (acute phase), the IgG generated by the antigenic stimulus has low avidity, i.e. binds with less avidity to the antigen, but as time goes by in the convalescence, after the first 2-4 months of infection, avidity increases.

Symptomatic postnatal primary infection is serologically diagnosed through *seroconversion*, negative IgG reaction during the exanthem but positive reaction after two weeks, presence of IgM, and low avidity of IgG. An isolated sample with positive specific IgG and IgM levels and low avidity of IgG in the mother must raise a suspicion of asymptomatic primary infection [12].

In immune, as well as in vaccinated females, IgG is present, avidity is high, and IgM is absent. The IgG level is important to indicate susceptibility to reinfection, common in females with levels below 10 IIU/mL [12]. Reinfections, for the most part asymptomatic, pose a low risk to the fetus, but they may develop with the presence of IgM, particularly in the reinfection of vaccinated females [20-22]. Reinfection is suspected in a specific sample when the specific IgG is positive and the avidity of IgG is high, accompanied or not by IgM [12]. The differential diagnosis between asymptomatic primary infection and reinfection is extremely important, yet difficult, because under both conditions antibody titers are increased and IgM may be present. Avidity of IgG may be useful to distinguish between the two conditions, being low in primary infection and high in reinfection [23,24].

Specific IgG determination is performed through enzymatic techniques with chromogenic (such as the Enzyme–Linked Immunosorbent Assay; ELISA), fluorescent (MEIA, ELFA), or chemiluminescent substrates [12,17]. The results, expressed in IU/mL, are compared to standards provided by the World Health Organization – International Laboratory for Standardization in Biology (Copenhagen, Denmark). Specific IgM is determined preferentially using a capture assay format, which dispenses with the serum treatment stage to eliminate the rheumatoid factor and maternal IgG, and through anti-VR conjugates, F(ab')2 fraction [25,26]. The determination of avidity of IgG complements the serologic diagnosis in the IgG and IgM-positive mother.

In order to determine the need for implementing avidity of IgG in the Hospital Materno-Infantil Presidente Vargas, we reviewed the results of rubella serum assays for specific IgG and IgM determination performed in the laboratory from 1998 to 2002.

Material and Methods

Samples from the Prenatal, Pediatric or Pregnant/ Parturient and hospitalized children wards were referred to the laboratory to be serologically tested for rubella. The blood samples were obtained through venous puncture using the Vacutainer System in a tube with separating gel. In the Immunology Section, each sample was centrifuged and the serum processed by the ELFA method, VIDAS® system, bioMérieux or MEIA, Axsym® system, Abbott for IgG, and ELFA, VIDAS® system, and bioMérieux for IgM, following the recommendations of the respective manufacturers. We suggested repeating the test two weeks later in all cases of indeterminate results, both for IgG and IgM.

Reference ranges for IgG in the ELFA were as follows: *negative*: below 10 IU/mL, *inconclusive*: between 10 and 15 IU/mL, and *positive*: equal to or above 15 IU/mL. For the MEIA they were: *negative*: below 5 IU/mL, *inconclusive*: between 5 and 10 IU/ mL, and positive*:* equal to or above 10 IU/mL. For IgM, reference ranges for ELFA were: *negative*: below 0.80, *inconclusive*: between 0.8 and 1.2, and *positive*: equal to or above 1.2.

Results

Of the 2,863 patients tested for specific IgG, 2,406 (84%) were positive, with levels ranging between 15 and 50 IU/mL in 18.4%, between 50 and 300 IU/mL in 63%, and >300 IU/mL in 18.2% of patients.

Table 1. Data on patients with positive IgM for rubella

Patients	Age	IgG	IgM	Date
P1	14 months	119	14.62	02.26.99
P2	1 year	154.3	4.99	06.19.02
P3	16 months	>400	1.24	12.03.98
P4	9 years	89.5	1.81	10.31.01
P5	19 years	67.5	2.25	02.27.02
		65.3	0.84	05.22.02
P6	19 years	146.0	2.0	11.17.99
P7	21 years	>400	1.64	09.19.00
P8*	17 years	32.3	1.23	03.06.01
P9	25 years	>400	1.43	05.03.00
P10	31 years	138.0	1.27	04.29.99
P11	26 years	>400	1.27	04.03.00
		>400	0.91	06.19.00
P12	U	128.2	6.94	05.16.02
P13**	4 months	N	2.20	09.10.00
P14	4 months	N	1.94	02.23.99

P=positive, N=negative, I=inconclusive, U=unknown. *Toxo IgG=31.7(P), IgM=1.1 (P). **Toxo IgG=N, IgM=0.63 (I).

Inconclusive results were obtained in 37 patients (1.3%), but none of these returned to be tested again. In 420 patients (14.7%), the results were negative.

Of the 2,851 patients tested for specific IgM, 2,812 (98.6%) were negative, 25 (0.88%) were inconclusive, and 14 (0.49%) were positive. The results of patients with positive and inconclusive IgM are shown in Tables 1 and 2, respectively.

In two samples (P13 e P14), from two 4-month-old females, IgM was positive and IgG was negative. We did not have obstetric (mother's serum) or clinical data regarding these patients, who also did not return to be tested again. Patient 13 had an inconclusive IgM for toxoplasma.

Among the results of inconclusive IgM (Table 2), two samples were from children. Patient 2 had positive results for toxoplasmosis, with extremely high levels of IgM, observed in acute infection with *T. gondii*. Patient 6 also presented results indicative of recent toxoplasmosis, probably acquired within the last two years, with high levels of IgG and low levels of IgM. Patient 8 presented inconclusive levels of IgM for toxoplasmosis.

Discussion

Vaccination campaigns have considerably reduced the incidence of congenital infection with the rubella virus [27]. In our sample 81.2% of the patients, mostly adult females at reproductive age, were found to have high levels of specific IgG. The 37 patients with inconclusive IgG did not return to be tested again as recommended, possibly because they were IgM negative.

The IgG avidity test is indicated as an aid in the diagnosis of symptomatic primary infection and to make the differential serological diagnosis between asymptomatic primary infection and reinfection with the rubella virus, especially in pregnant women.

Table 2. Data on patients with inconclusive IgM for rubella

Patients	Age	IgG	IgM	Date
P1	2 years	>400	1.10	01.13.00
P2*	2 years	203.4	0.89	03.19.01
P3	17 years	133.0	1.12	10.23.00
P4	36 years	317.0	1.12	04.10.00
P5	16 years	107.8	1.04	05.06.02
P6**	24 years	375.0	1.01	09.05.00
P7	15 years	31.1	1.0	11.22.00
P8***	27 years	16.9	0.98	12.04.00
P9	30 years	149.0	0.94	04.29.99
P10	24 years	347.0	0.93	02.08.00
P11	21 years	46.0	0.90	01.21.00
P12	20 years	173.0	0.89	09.28.00
P13	26 years	165.0	0.86	09.01.00
P14	16 years	110.0	0.84	06.07.99
P15	42 years	>400	0.82	03.29.01
P16	23 years	237.0	0.81	04.08.99
P17	U	32.0	0.86	11.11.98
P18	U	257.0	1.17	02.09.99
19	U	451.0	1.01	11.27.02
P20	U	85.1	0.84	03.05.02
P21	U	>400	0.83	01.17.02
P22	U	52.4	1.17	03.23.01
P23	U	283.2	0.82	11.23.00
P24	U	92.0	0.87	06.13.00
P25	17 years	N	0.81	12.22.00

P=positive, N=negative, I=inconclusive, U=unknown.* Toxo IgG=>300 (R), IgM=15.89 (P). ** Toxo IgG=>300 (R), IgM=0.97 (P). *** Toxo IgG=22.7 (R), IgM=0.56 (I.)

In the original group, we found 7 patients who could have benefited from an IgG avidity test. In 2 patients, the second sample (P5 at 2 months and 25 days, and P11 at 2 months and 13 days) showed a slight reduction of specific IgM levels. In the group of patients with inconclusive IgM, none returned to be tested again.

The highest IgM levels occur between 4 and 35 days in the ELISA, becoming negative within 2-3 months, rarely within one year [28-30]. IgM persists for much longer in the capture assays, following primary infection; in addition, these assays are more sensitive and also detect IgM when there is reinfection [12].

Of concern, even in such a small sample, is the finding of positive or inconclusive serologic results for toxoplasmosis, obtained in five patients, with serum tests required for both agents.

During the four years, only 21 to 30 inconclusive or positive IgM samples were detected (out of 2,851), which could have benefited from IgG avidity assays. However, the Hospital Materno-Infantil Presidente Vargas is a public institution, in which investment

priorities have to be determined on a cost-effectiveness basis. Vaccination prevents the occurrence of congenital infection, even in campaigns with a risk of inadvertently vaccinating a pregnant woman, there being no scientific evidence of congenital rubella caused by the vaccine.

For the above-mentioned reasons, it has been decided not to implement IgG avidity assays for the rubella virus in this hospital. The decision of implementing a new technique must also consider if the diagnostic resources available are properly used by the clinical staff of the institution. Without optimal integration, as is the case in the HMIPV, a school hospital, the introduction of a new test might generate false expectations and more insecurity among physicians and patients already bewildered with the amazing technological progress in the field of diagnosis.

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