ORIGINAL ARTICLE

Intrauterine infection and cord immunoglobulin M III. Serological analysis of infants with elevated cord serum immunoglobulin M*

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Summary: The presence of antibodies to rubella, cytomegalovirus and *Toxoplasma gondii* was determined at birth and at 6 months of age in a group of 147 infants with cord serum IgM levels ≥ 19.0 mg/dl and in 92 control infants. Maternal syphilis serology was determined in both groups as well. No significant differences in the prevalence or levels of antibodies to these pathogens were found between the two groups which might have led to the diagnosis of unsuspected intrauterine infection. Persistence of antibodies to 6 months of age was similar in the two groups, indicating that this is not a useful index of intrauterine infection.

Analysis of the results yielded the following data on the prevalence of antibodies to the pathogens studied: rubella virus, 90 and 75% seropositivity at birth and 6 months respectively; cytomegalovirus, 65 and 35%; and *Toxoplasma gondii*, 33% seropositivity at birth.

Résumé: *L'infection intra-utérine et l'immunoglobuline M du cordon. III. Analyse sérologique d'enfants dont l'IgM du cordon est élevée*

Chez un groupe de 147 enfants, on a établi la présence d'anticorps de la rubéole, du virus cytomégalique et du *Toxoplasma gondii*, à la naissance et à l'âge de 6 mois. Chez ces sujets, la concentration sérique du cordon en IgM était supérieure ou égale à 19.0 mg/dl. On s'est servi de 92 enfants comme témoins. On a également établi la sérologie syphilitique chez les mères des deux groupes. On n'a trouvé chez les deux groupes aucune différence notable dans la prévalence ou la concentration des anticorps de ces agents pathogènes et qui aurait pu mener à un diagnostic d'infection intra-utérine méconnue. La persistance des anticorps à l'âge de 6 mois était similaire dans les deux groupes, ce qui indique qu'il ne s'agit pas d'un index valable d'une infection intra-utérine.

L'analyse des résultats a fourni les renseignements suivants concernant la prédominance des anticorps aux agents pathogènes étudiés: virus rubéoleux — séropositivité de 90 et de 75% à la naissance et à 6 mois respectivement; virus cytomégalique, 65 et 35%; et *Toxoplasma gondii*, 33% séropositifs à la naissance.

We have previously reported the results of a study in which we selected for further evaluation 147 infants with elevated cord serum IgM values out of a total of 3474 infants screened to assess the possibility of using such values to detect infants with intrauterine infection.[1] Clinical evaluation of these infants led to the discovery of one infant with previously unsuspected congenital rubella infection. Comparison of the study group with an unselected control group of 92 infants with normal cord serum IgM values suggested that factors associated with increased risk of intrauterine infection were found more frequently in the study group; however, on clinical grounds both at birth and at 6 months of age no significant differences were noted between the two groups. We now report our analysis in the two groups of infants of levels of antibodies to the four major intrauterine pathogens — rubella virus, cytomegalovirus, *Toxoplasma gondii* and *Treponema pallidum*.

Materials and methods

Patient population

The study group comprised 147 infants with cord serum IgM levels ≥ 19.0 mg/dl, while the control group comprised 92 infants with cord serum IgM levels <19.0 mg/dl. The clinical characteristics of these subjects have been previously reported.[1] Serum for serological analysis was obtained from the cord blood between the second and third stages of labour and from a peripheral vein at 6 months of age and was stored at −70°C for variable periods of time until assayed.

Serological assays

Rubella virus: The standard rubella hemagglutination-inhibition (HAI) test was performed using the microtitre technique with commercial reagents (Flow Laboratories,

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Rockville, Md.).[2] Serum was tested at an initial dilution of 1:8, so that antibody levels of <1:8 were considered negative.

Cytomegalovirus: Complement-fixing (CF) antibody was determined using the standard microtechnique with commercial antigen derived from the AD 169 strain of cytomegalovirus (Flow Laboratories, Rockville, Md.).[3] Serum was tested at an initial dilution of 1:8, so that antibody levels of <1:8 were considered negative.

Toxoplasma gondii: The indirect fluorescent antibody (FA) test using a fluorescein-conjugated rabbit anti-human-gamma-chain antibody was utilized to measure toxoplasma antibodies. The antigen was in the form of intact toxoplasma organisms fixed to small wells on teflon-coated glass slides (Cooke Engineering Company, Alexandria, Va.). Serum was tested at an initial dilution of 1:16, and fourfold rather than twofold dilutions were made to titrate positive sera. Unpublished studies in collaboration with Dr. J. Quinn (Department of Microbiology, Ontario Veterinary College, Guelph, Ont.) indicate a very close correlation between the indirect FA test and the Sabin-Feldman dye test.

Treponema pallidum: Routine serological tests for syphilis were performed on all women by the provincial laboratories at the time of confinement.

Results

Rubella virus

The distribution of rubella HAI antibody levels at birth and at 6 months of age is shown in Figs. 1A and 1B. The cord serum antibody levels reflect the pattern of rubella immunity in our community at the time of the study, with approximately 90% seropositivity. There was a normal distribution of antibody levels with a peak around 1:64 to 1:128. Serum samples obtained at 6 months of age still showed approximately 75% seropositivity but the titres had fallen considerably, the peak being around 1:16. There were two samples with high HAI titres, 1:256 and 1:128, the first from an infant whose serum converted from negative at birth, and the second from an infant with confirmed intrauterine rubella infection. There were no significant differences between the study and control groups in terms of antibody patterns at birth and 6 months of age.

Table I presents the paired analysis of cord serum antibody levels with the corresponding 6-months levels. One hundred and seven pairs from the study group and 43 pairs from the control group were available for assessment. Five categories were defined in an attempt to determine if there was a preponderance of infants with sustained antibody levels in the high IgM group. Categories A and B are infants whose serum at 6 months of age was negative or contained significantly less antibody than the cord serum. Categories C and D are those whose levels did not change significantly or increased. Category E represents those who were seronegative and converted to positive, and category F those who were seronegative on both occasions. Children with intrauterine infection might be expected to have a sustained antibody response,[4,5] and therefore should be found in excess in categories C and D. This is not borne out by our study.

Cytomegalovirus

No significant differences between study and control groups were seen in the distribution of cytomegalovirus CF antibody at birth and at 6 months of age as shown in Figs. 2A and 2B. Approximately 65% of the infants were seropositive, with titres normally distributed around values of 1:32 to 1:128. By 6 months of age only 35% were seropositive, with a mean titre of 1:16. The two samples with high titres (1:128) were from one infant whose serum converted to positive during the first six months of life and one infant in the high IgM group whose cord serum value was also 1:128. Analysis of changes in CF antibody

Table I—Analysis of differences in rubella hemagglutination-inhibition antibody levels at birth and at 6 months of age

	Study group (107)		Control group (43)	
Category	No.	%	No.	%
A. Positive to negative	15	13.9	9	20.9
B. Fourfold or greater decrease	69	63.9	28	65.1
C. No change (excluding F)	11	10.2	4	9.3
D. Fourfold or greater increase	0	0.0	0	0
E. Negative to positive	1	0.9	1	2.3
F. Persistently negative	11	10.2	1	2.3

Figures in parentheses indicate total number of paired cord and 6-months serum samples available for analysis.

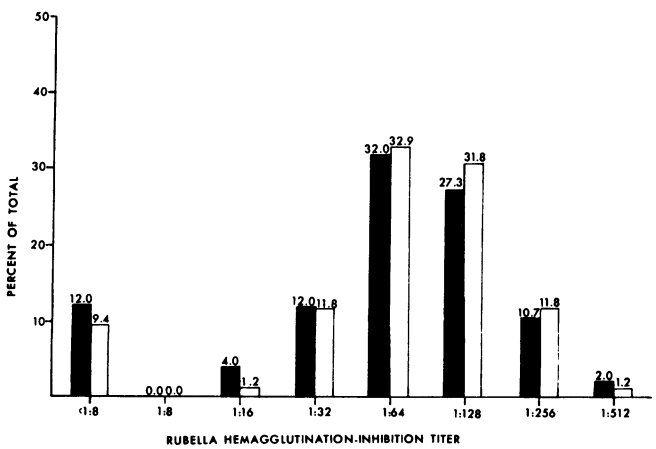

FIG. 1A—Distribution of rubella virus hemagglutination-inhibition antibody titres in cord serum. The black bars represent the study group and the white bars represent the control group. The figures above each bar indicate the percent of total serum samples with the antibody levels indicated.

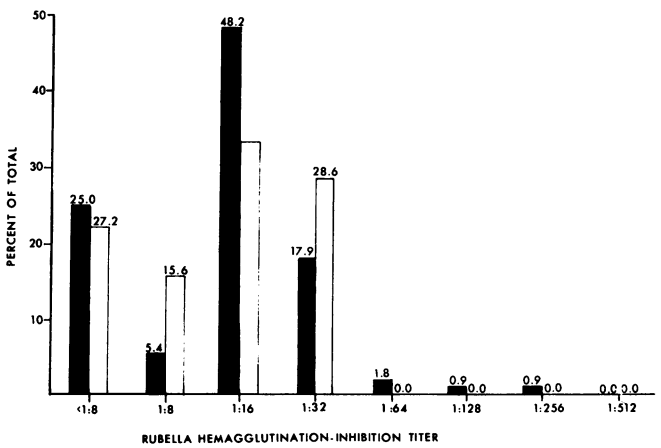

FIG. 1B—Distribution of rubella virus hemagglutination-inhibition antibody titres in serum obtained at 6 months of age.

levels between birth and 6 months of age, using the same categories as for the rubella HAI antibody level, is shown in Table II. Again, no preponderance of study infants was seen in the categories of sustained or increased antibody levels.

Toxoplasma gondii

Because of the complexity and cost of performing serial titration of the fluorescent antibody levels against toxoplasma, as well as the anticipated lower incidence of seropositivity, we screened all cord sera for antibodies but performed titrations only if the corresponding sample taken at six months was also positive. The results shown in Table III indicate that 40.8% of the study group were seropositive compared with 26.1% of the control group. At six months 21.3% of previously positive cases in the study group were still seropositive while the corresponding figure for the controls was 38.9%. Only one of 17 positive 6-months sera had a titre >1:64. This was a study infant whose antibody level remained at 1:2048 and whose cord serum IgM was 38.0 mg/dl. This infant, however, was clinically well at 6 months and also at 1 year of age. Analysis of paired cord and 6-months serum samples revealed that approximately 75% of both groups became seronegative by 6 months of age (Table IV). There was no difference between the two groups in the number with sustained antibody levels.

Table II—Analysis of differences in cytomegalovirus complement-fixation antibody levels at birth and at 6 months of age

Category	Study group (101)		Control group (40)	
	No.	%	No.	%
A. Positive to negative	35	34.7	9	22.5
B. Fourfold or greater decrease	14	13.9	7	17.5
C. No change (excluding F)	15	14.9	8	20.0
D. Fourfold or greater increase	1	1.0	0	0
E. Negative to positive	7	6.9	1	2.5
F. Persistently negative	29	28.7	15	37.5

Figures in parentheses indicate total number of paired cord and 6-months serum samples available for analysis.

Table III—Incidence of seropositivity in the *Toxoplasma gondii* fluorescent antibody test at birth and at 6 months of age

	Study group		Control group	
	No.	%	No.	%
Cord serum	60/147	40.8	24/92	26.1
6-months serum*	10/47	21.3	7/18	38.9

*Only those 6-months sera in which the corresponding cord serum was positive were assayed.

Table IV—Analysis of differences in *Toxoplasma gondii* fluorescent antibody levels at birth and at 6 months of age

Category	Study group (47)		Control group (18)	
	No.	%	No.	%
A. Positive to negative	37	78.7	13	72.2
B. Fourfold or greater decrease	9	19.1	4	22.2
C. No change (excluding F)	1	2.1	1	5.6
D. Fourfold or greater increase	0	0	0	0
E. Negative to positive	ND	—	ND	—
F. Persistently negative	ND	—	ND	—

Figures in parentheses indicate total number of paired cord and 6-months serum samples available for analysis.
ND = not done

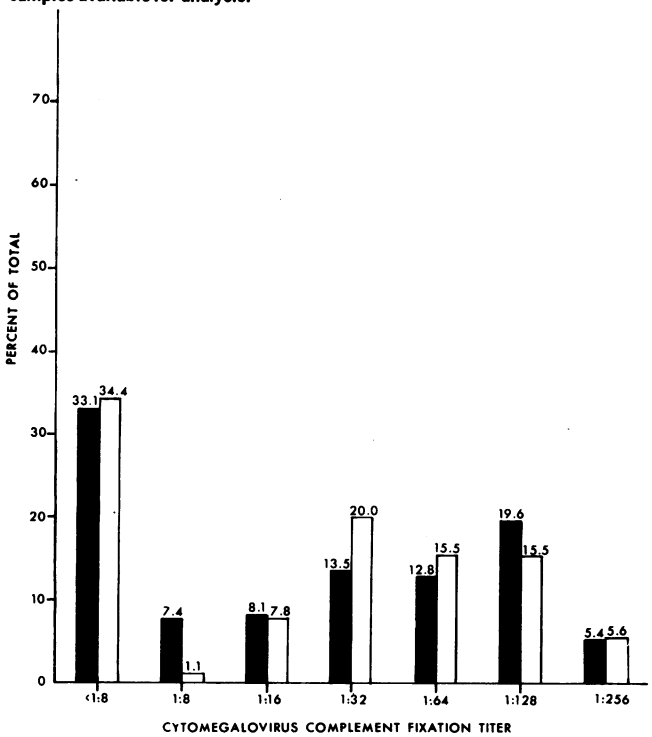

FIG. 2A—Distribution of cytomegalovirus complement-fixation titres in cord serum.

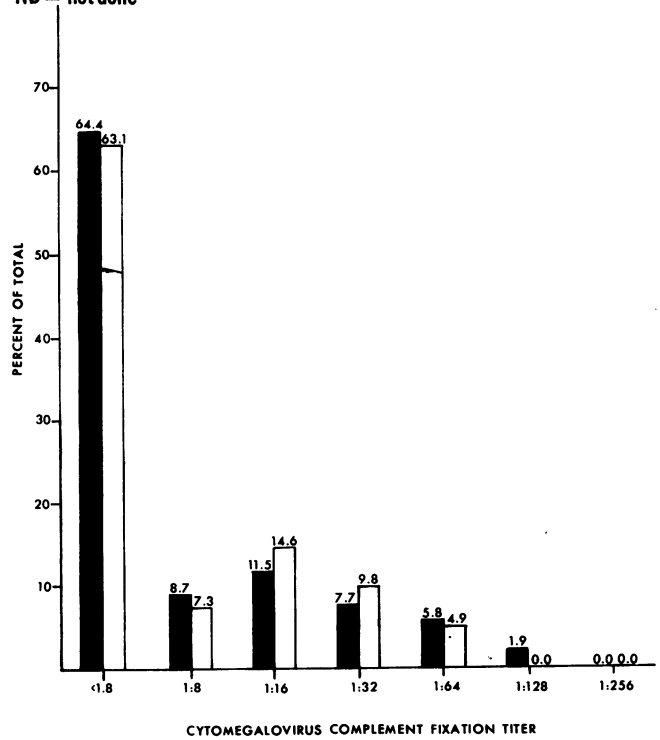

FIG. 2B—Distribution of cytomegalovirus complement-fixation titres in serum obtained at 6 months of age.

Treponema pallidum

There was only one mother in the study group who had a positive VDRL (1:2). The KRP test was negative and the VDRL of the infant was negative. Serology was not done in one mother in each group.

Discussion

We have reported previously that routine cord blood screening for intrauterine infection by assay of IgM levels was not a helpful case-finding procedure.[1] The very low incidence of clinically apparent intrauterine infections and the even lower yield of inapparent infections was thought to be related to the relatively high socioeconomic conditions prevalent in our study population. The present report sought to determine whether we could increase the number of clinically inapparent intrauterine infections detected by analysis of changes in levels of antibody to specific pathogens. However, there were no significant differences between the control and high-IgM groups in terms of sustained levels of antibody to rubella virus, cytomegalovirus or *Toxoplasma gondii* at 6 months of age. In a serial study of 688 infants with no history of maternal rubella Vesikari[6] found that passively acquired rubella HAI antibodies disappear by 7 months of age. It has also been reported that the presence of rubella HAI antibody after 6 months of age is indicative of (intrauterine) infection.[5,7] In confirmation of the report of Cloonan, Hawkes and Stevens[8] our data demonstrate clearly that this is not the case, for 75% of both control and study groups were still seropositive at 6 months of age, the figure at birth being 90%. The rate of decline of cytomegalovirus CF antibody levels was somewhat greater than the rate for rubella HAI antibody: seropositivity decreased from 65% at birth to 35% at 6 months of age. Similar data are not available for toxoplasma antibodies since only infants who were seropositive at birth were examined for toxoplasma antibodies at 6 months of age. Of these 21.3 and 38.9% of the study and control groups, respectively, still had antibody at 6 months. It is therefore clear that the presence of antibodies to these agents at 6 months of age is not helpful in confirming the diagnosis of intrauterine infection.

In an attempt to look briefly at patterns of fall of antibody levels all subjects with similar levels of antibody in any one test at birth and at 6 months of age were analysed with respect to the pattern in the other two assays. In the great majority of cases antibody levels in the other two assays fell significantly from birth to 6 months. There was no difference in the patterns between the controls and the study group. Our findings in this regard are similar to those of others who have shown that patterns of decline of passively acquired antibody vary according to the antigen involved as well as with the individual.[9]

There was one rubella seroconversion in each group, and seven cytomegalovirus seroconversions in the study group and only one in the control group. The significance of this latter difference, which represents an increase in the seroconversion rate of nearly threefold, is difficult to assess because of the small numbers involved.

When one examines the subjects with very high antibody levels at 6 months of age there appears to be a preponderance among the high IgM group. These subjects include one infant with congenital rubella, one normal infant with high *Toxoplasma gondii* antibodies at birth as well as at 6 months of age, two seroconvertors and a few otherwise normal infants.

The antibody levels at birth and 6 months were individually analysed in the 17 infants with cord IgM levels $\geqslant$ 30 mg/dl. No additional information was obtained to incriminate any individual pathogen in each case.

It was our original intention to look for specific IgM antibodies to each of the three pathogens studied because this is a more specific test of recent infection and has been advocated as an adjunct to the specific diagnosis of intrauterine infection.[10-12] The only technique that is practical when one has many serum samples to analyse but small volumes available is the specific IgM fluorescent antibody technique. After extensive testing of sera from cases of acquired and congenital rubella we were unable to demonstrate reproducibly IgM antibody with the fluorescent antibody test even when physical separation techniques indicated that antibody was present in the 19S fraction of the serum. Less extensive testing of sera from patients with acquired cytomegalovirus and *Toxoplasma gondii* infection yielded similar unreliable results. We were therefore unable to perform any specific tests for IgM antibody in this study.

In conclusion, analysis of paired cord and 6-months serum samples from normal infants and infants with high cord blood IgM levels failed to reveal any differences that could have led to the identification of cases of intrauterine infection otherwise inapparent. There were not even any significant differences indicative of a basic biologic difference between the two groups. A failure to lose all passively acquired antibodies by 6 months of age is therefore not an indication of intrauterine infection as has been previously suggested.[5,7]

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