ARTÍCULO ORIGINAL

Usefulness of direct antiglobulin test in neonatal screening

Utilidad de la prueba directa de Coombs en el tamiz neonatal

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Abstract

Background. Neonatal jaundice is a clinical event frequently present in newborns. The causes most frequently involved in hemolytic disease of newborn (HDN) are still the incompatibilities to the ABO/Rh blood system. Direct Coombs test (or direct antiglobulin test, DAT) allows identification of the presence of red blood cell antibodies (IgG isotype) coming from the maternal serum on the surface of the fetus erythrocytes. The purpose of this study is to show the results and specificity of DAT as screening in newborn infants.

Methods. We studied unselected neonates in a cross-sectional design. During the early hours of life, we determined ABO/Rh and DAT with poly- and monospecific reagents (anti-IgG and C3b/C3d).

Results. We included 5007 newborns; 181 cases (3.6%) were DAT positive. Newborns with A, B or AB blood groups showed an increased association of being DAT positive than group O (OR 2.3, 95% CI 1.7-3.1). DAT was positive in 3.5% of RhD positive infants and 1.9% of RhD negative infants. In six DAT-positive cases, 117 cases (64.6%) had anti-IgG bound to red cell membrane, complement in six cases (3.3%), and 52 newborns (28.8%) were polyspecific DAT positive and monospe-

Resumen

Introducción. La ictericia es un evento clínico frecuente que se presenta en los recién nacidos; las causas más frecuentes involucradas en la enfermedad hemolítica del recién nacido (EHRN) continúan siendo la incompatibilidad al sistema ABO y la isoinmunización a RhD. La prueba directa de Coombs (PDC) permite identificar la presencia de anticuerpos antieritrocitarios del isotipo IgG, provenientes del suero materno en la superficie de los eritrocitos del feto o neonato. Objetivo: presentar los resultados y especificidad de la prueba directa de Coombs (PDC) como prueba en el tamiz neonatal.

Métodos. Consecutivamente se incluyeron a recién nacidos no seleccionados. En las primeras horas de vida se determinó el grupo sanguíneo ABO/RhD y la PDC con suero poliespecífico y monoespecíficos (anti-IgG y C3b/C3d).

Resultados. Se incluyeron 5 007 recién nacidos, la PDC positiva se documentó en 181 neonatos (3.6%). Los casos del grupo sanguíneo A, B o AB, mostraron mayor riesgo de tener PDC positiva que los del grupo O (razón de momios 2.3, intervalo de confianza 95% 1.7-3.1). La PDC positiva se presentó en 3.5% de los neonatos RhD positivo y en 1.9% de los RhD negativo;

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cific DAT negative.

Conclusions. The high prevalence (3.6%) of DAT-positive cases in routine neonatal detection supports the indication to incorporate DAT into neonatal screening, regardless of the mother's blood group. The benefit of early intervention in neonatal jaundice remains to be established.

Key words: direct antiglobulin test, direct Coomb's test, hemolytic disease of newborn, neonatal hyperbilirubinemia, ABO/Rh blood group, neonatal screening.

72.9% de los neonatos con PDC positiva tuvieron titulaciones de 1:2, 1;4 y 1:8, con el 33.1, 19.9 y 19.9%, respectivamente. En los neonatos con PDC positiva se pudo establecer el isotipo anti-IgG en 117 casos (64.6%), complemento sólo en 6 casos (3.3%), la combinación de ambas en 6 casos (3.3%) y en 52 neonatos (28.8%), con PDC poliespecífico positivo, no se pudo identificar la especificidad de la reacción.

Conclusión. La prevalencia elevada (3.6 %) de PDC positiva en la detección neonatal rutinaria, apoya la indicación de incorporar dicha prueba al tamiz neonatal, independientemente del grupo ABO y Rh materno. Queda por establecer su beneficio en la detección temprana de la ictericia neonatal.

Palabras clave. Coombs directo, prueba directa de la antiglobulina humana, enfermedad hemolítica del recién nacido, hiperbilirrubinemia neonatal, grupo sanguíneo ABO/Rh, tamiz neonatal.

Introduction

Jaundice is a frequent clinical event with a variable intensity affecting ~15% of the newborn population.[1] Blood group incompatibility and hemolytic disease of newborns (HDN) is due to anti-erythrocyte antibodies, with an incidence of 4-50% of all newborns who are evaluated for hyperbilirubinemia.[2,3] The most frequent causes involved in HDN continue to be ABO[4] system incompatibility and RhD isoimmunization.[5-7] Gradually, involvement of antibodies directed against other antigens of the Rh system such as Rhc,[8] RhC,[8] or RhE,[9] or other erythrocyte systems such as Kell,[10] Diego or Duffy has become apparent.[7] In our hospital environment, anti-D is the anti-erythrocyte antibody most frequently involved in HDN, but other antibodies are involved in 5-15% of the cases.[5]

In the case of the ABO system, >50% of mothers with O blood type presents clinically significant concentrations of anti-A and anti-B antibodies of IgG isotype. Approximately 15-18% of pregnancies are incompatible with the ABO system and are at risk for hemolytic disease.[11] In the case of RhD antigen, <1% to 5% of the obstetric population is RhD negative, with isoimmunization rate up to 15% in obstetric hospitals.[12] Overall strategy of the study of HDN includes the determination of the direct test of human antiglobulin or direct Coombs test [also known as direct antiglobulin test (DAT)] either with the classic technique of agglutination in either tube[13] or in gel.[14] This test allows us to identify the presence of anti-erythrocyte antibodies of IgG isotype, originating in maternal serum on the surface of erythrocytes of the fetus or newborn.[15,16] In cases of ABO incompatibility, >80% of the DAT is negative, but when performing the technique of leaching or eluating, reactivity of the test results increases proportionately.[4]

In some obstetric centers it is recommended to determine ABO and Rh blood type as well as the DAT from cord blood or from the infant during the first hours of life.[16,17] Variants of this practice include routine screening of all newborns or those with a family history or maternal relative with HDN, only women who are RhD negative, when the mother has type O blood, or in cases with jaundice during the first hours of life.[18] This leads to a wide prevalence of positive DAT in the neonatal population (1-9%).[18,19] Health care officials in Mexico[20] recommend routine investigations of ABO/RhD group and

DAT in the newborn of an RhD negative mother with suspicion of isoimmunization risk. However, universal neonatal screening for determination of ABO/Rh group and DAT is not recommended by obstetricians and neonatologists, citing various reasons such as the lack of clinical studies regarding its frequency, clinical impact or cost-benefit assessment.

With this reference marker, the objective of this study is to report the occurrence of the reactivity of the DAT included in neonatal screening, as well as additional information about the presence of IgG or complement fraction 3 associated with the erythrocyte membrane.

Patients and methods

We included all newborns consecutively from 2005-2007 at the Hospital Médica Sur, independent of their gestational age, birth weight or neonatal morbidity. It is an institutional practice to determine ABO and Rh blood type. Results of the determination of the DAT were reported to the patient's physician for evaluation and monitoring. Performance of the DAT was achieved using whole blood samples obtained by a puncture of the umbilical vessels at the end of the placental or simultaneously with the collection of blood samples for neonatal screening. We collected 200-μL samples of whole blood in test tubes with neonatal EDTAK3 as anticoagulant. We determined the ABO blood group by hemagglutination with test tube technique. For the direct phase, commercial reagents were used that contain monoclonal antibodies with anti-A, anti-B and anti-AB specificity (Gamma Biologicals, Licón). Because fetal erythrocytes contain fewer antigenic sites on the erythrocyte surface, we were not able to make the identification of the group A2.[21] The same blood sample was tested to identify the RhD antigen using the hemagglutination test tube technique, using a mixture of polyclonal and monoclonal anti-D antibodies (Gamma-clone Gamma Biologicals, Licon), with a control of Rh (Rh Control Gamma, Gamma Biologicals, Licon). When in the phase of rapid saline we documented the presence of agglutination, and the newborn was defined as RhD+. In the case of the absence of agglutination, the mixture of erythrocytes problem with the anti-D was incubated at 37°C, and if the agglutination was absent or <2+, it was carried to the human antiglobulin phase with a mixture of murine monoclonal antibodies anti-IgG-C3d (Gamma-clone Gamma Biologicals, Immucor Gamma, Houston, TX). The positive results were evaluated later using the reactive murine monoclonal of anti-human IgG antiglobulin (Gamma-clone Gamma Biologicals, Immucor Gamma) and human antiglobulin anti-C3b + C3d (Gamma-clone Gamma Biologicals, Immucor Gamma). The absence of agglutination defined the condition of RhD negative; the presence of agglutination at any of the stages defined the condition of RhD positive. In the absence of antiglobulin phase reactivity, the consumption of antihuman globulin (AHG) reagent with sensitized cells was performed (Coombs Control Cells Strong, Check Cells, Immucor Gamma). In cases of DAT positive, titrations were given with progressive geometric dilutions (1:2, 1:4, 1:8, successively) of AHG with isotonic saline solution with previously washed neonatal erythrocytes with their respective positive and negative controls. DAT titer was defined to the last test tube where agglutination reaction was observed.[21] A positive result of the poly-specific AHG infers that the neonatal erythrocytes are sensitized against an anti-erythrocyte antibody, complement fraction 3 or both without specifying which one. The intensity of agglutination was defined as 4+ when cellular clumping was observed to be solid, unique and with clear supernatant, 3+ with agglutination in one large clump and several small ones, 2+ with agglutination of several medium clumps and slightly red supernatant, 1+ when small clumps are observed, erythrocyte-free and red supernatant, and ± when there was very fine-grained suspension of erythrocytes observed with red supernatant and, finally, negative when there was no macroscopic agglutination or hemolytic supernatant observed.

The results of each blood group and the DAT reaction are presented as frequencies and proportions. The intensity of the association between positive DAT and the ABO/RhD groups was established using odds ratio (OR) with confidence interval of 95% (95% CI).

Results

We evaluated the ABO/Rh blood type and DAT in 5007 newborns. Frequency distribution according to their status as RhD positive and RhD negative was 4642 (92.7%) and 365 newborns (7.3%), respectively. Frequency distribution in the determination of the ABO blood grouping was 55.2% (2767 cases) for group O, 32.1% (1605 cases) for group A, 10.4% (521 cases) for group B and 2.3% (114 cases) for blood group AB (Table 1). We identified the presence of serologic reactivity for the DAT in 181 newborns (frequency 3.6%).

In considering the results of the DAT compared to ABO blood group and RhD group (Table 2), occurrence of the 181 newborns with positive direct Coombs according to frequency was 80 cases (44.2%) for newborns with blood group A, 64 cases (35.4%) for blood group O, followed by infants with blood group B with 36 cases (19.9%) and, finally, cases of group AB with one case (0.5%).

When compared with newborns of blood group O, the possibility of having a positive DAT was higher in newborns of blood group B (OR 3.1, 95% CI 2.0-4.8); followed by cases of blood group A (OR 2.2, 95% CI 1.6-3.0). When considering the infants with blood group A, B or AB, the association with the DAT resulted to be similar (OR 2.3, 95% CI 1.7-3.1).

The 3.5% of newborns with RhD positive blood group and 1.9% of newborns that were RhD negative had DAT positive with no statistical association (OR 2.0, 95% CI 0.9-4.2).

In infants with DAT positive we were able to establish the anti-IgG isotype with the reactive monospecific in 117 cases (64.6%), complement C3b and C3d in only 6 cases (3.3%), the combination of both circumstances in six cases (3.3%), and in 52 cases (28.8%), with poly-specific DAT positive, we could not identify the specificity of the reaction.

The occurrence of anti-IgG only occurred in 54.7%, 70% and 72.2% of the infants with blood type O, A and B, respectively. Failure to identify the specificity occurred in 35.9%, 27.6% and 19.4% for the same blood groups. No statistically significant association was found (data not shown). The frequency of complement fraction 3, alone or in combination, was found in two cases in a similar manner for each blood type. Anti-IgG was the most frequent specificity in newborns with RhD positive (66.1%) but with similar distribution to those observed in newborns according to their ABO group.

Infants with a degree of DAT positive ^{3}1:16 showed the greater occurrence in newborns of blood type O, followed by type B and type A; 32.8%, 16.87% and 7.5%, respectively (Table 3).

Table 1. Distribution of the results in accordance with ABO group and RhD condition

RhD group	Blood group ABO			
	O	A	B	AB
5007	2767 (55.2%)	1605 (32.1%)	521 (10.4%)	114 (2.3%)
Positive	2577	1460	498	107
4642 (92.7%)	(55.5%)	(31.5%)	(10.7%)	(2.3%)
Negative	190	145	23	7
365 (7.3%)	(52.1%)	(39.7%)	(6.3%)	(1.9%)

Data represent the number of cases and percentage between parentheses.

This association resulted to be statistically significant when compared to infants of blood type O against those of blood type A or B, with OR of 3.4 (95% CI 1.5-7.8). Infants with a degree of DAT positive [3]1:16 showed similar occurrence in the infants of Rh positive blood group in contrast to RhD negative, with 18.4 and 14.0, with no statistically significant difference, with OR of 1.6 (95% CI 0.6-11.6); 72.9% of newborns with DAT positive was located within the ratios of 1:2, 1:4 and 1:8 (33.1%, 19.9% and 19.9%, respectively) (Figure 1).

Discussion

Of the 30 blood group systems identified,[22] ~60 antigens are capable of inducing HDN (Table 4). This disorder is principally associated with the antigen D of the Rh system and the ABO system incompatibility. In the indigenous population of Me-

Table 2. Specificity of the reactivity of direct Coombs test (DCT) in accordance with ABO/Rh group

	ABO blood group				RhD group	
Positive result of DCT	**O**	**A**	**B**	**AB**	**P**	
Polyspecific: 181 (3.6%)	64 (35.4%)	80 (44.2%)	36 (19.9%)	1 (0.5%)	1	
Monospecific						
Anti-IgG only: 117 (64.6%)	35 (54.7%)	56 (70.0%)	26 (72.2%)	——	115 (66.1%)	3 (42.9%)
Anti-C3b/C3d only: 6 (3.3%)	3 (4.7%)	1 (1.2%)	1 (2.8%)	1	5 (2.9%)	——
Anti-IgG and anti-C3b/C3d } combined: 6 (3.3%)	3 (4.7%)	1 (1.2%)	2 (5.6%)	——	6 (3.4%)	——
Not identified: 52 (28.8%)*	23 (35.9%)	22 (27.6%)	7 (19.4%)	——	48 (27.6%)	4 (57.1%)

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Risk for DCT positive in neonates A vs. group O (odds ratio, OR 2.2, 95% confidence interval, CI 1.6-3.0).
Risk for DCT positive in neonates B vs. group O (OR 3.1, 95% CI 2.0-4.8).
Risk for DCT positive in neonates A, B or AB vs. group O (OR 2.3, 95% CI 1.7-3.1).
Risk for DCT positive in neonates Rh positive vs. negative (OR 2.0, 95% CI 0.9-4.2).
Risk for DCT not identified vs. monospecific positive in neonates of blood groups A, B and AB vs. blood group O (OR 0.6, 95% CI 0.3-1.1).
Risk for DCT not identified vs. monospecific positive in Rh+ vs. Rh- neonates (OR 0.3, 95% CI 0.1-1.3).
Data represent number of cases and the percentage between parentheses.
*Positive reaction of polyspecific DCT but without reactivity with anti-IgG or anti-C3b/C3d.

Table 3. Results of titers of DCT according to blood group ABO and according to Rh

	Neonatal blood group ABO*		
Dilution (n =180)†	**A (n = 80)**	**B (n = 36)**	**O (n = 64)**
≥1:16 (33/18.2%)	6 (7.5%)	4 (16.7%)	21 (32.8%)
≤1:8 (148/81.8%)	74 (92.5%)	30 (83.3%)	43 (67.2%)

	Neonatal blood group RhD**	
Dilution (*n* = 181)	**Negative (*n* = 7)**	**Positive (*n* =164)**
≥ 1:16 (33/18.2%)	1 (14.0%)	32 (18.4%)
≤ 1:8 (148/81.8%)	6 (86.0%)	142 (81.6%)

*Group O vs. group A or group B (OR 3.4, 95% CI 1.5-7.8).
**RhD+ group *vs* RhD- (OR 1.6, 95% CI 0.6-11.6).
†Case with AB group not included.
Data represent the number of cases and the percentage between parentheses.

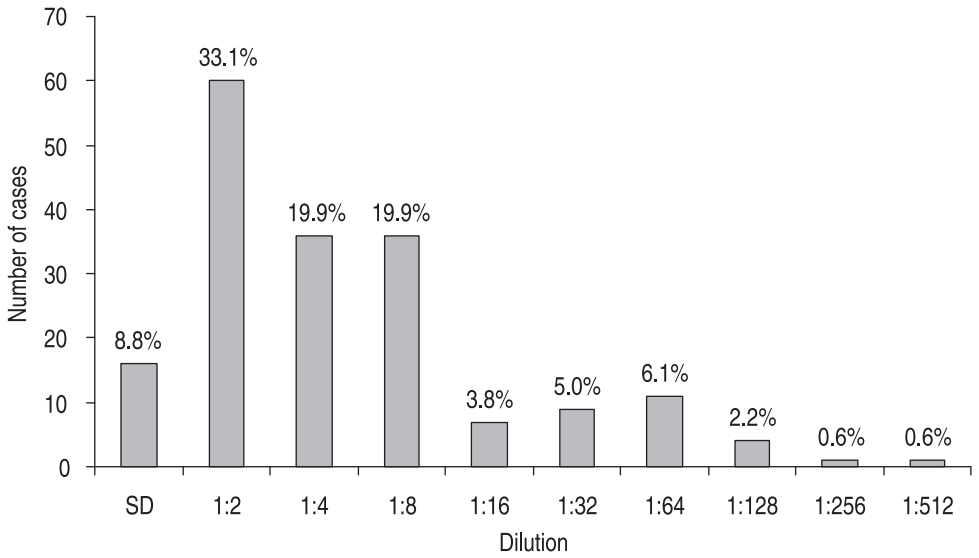

Figure 1. Distribution of frequencies of dilutions in positive direct Coombs test.

soamerica, blood type O and RhD positive is observed in >95% of subjects studied.[23] In general terms, for the Mexican population the O blood type is found in frequencies >55% followed by type A (20-35%), type B with 10-15% and AB in <5%. RhD positive is present in >97% of the population, whereas RhD negative is found in between 0 and 3%.[11] In our area, the frequency of incompatibility between the mother and child is between 0.302 and 0.168 for ABO, from 0.044 to 0.036 for RhD, as well as 0.013 and 0.006 for double incompatibility, with a probability of 0.0309 for estimated maternal isoimmunization.[11] In the current report it is documented that the most frequent ABO group in our population was O positive followed by A and B positive, with the same order of frequency as that reported in the literature.[4,18]

The positiveness of DAT was also observed in RhD negative newborns. This is relevant as it has been discussed in some centers that this group of infants should be excluded from this determination because the result of the DAT is often negative.[4,15] The possible reasons for a newborn with RhD negative may be due to a positive DAT and may have included ABO incompatibility (for example, newborn A is RhD negative whose mother is non-isoimmunized type O RhD negative or whose mother is RhD positive), that the mother is isoimmunized, and that she may have one or more IgG antibodies different from the anti-D.[7,12]

The presence of C3b and C3d on the erythrocytes of the cases with positive DAT reflects, on one hand, the neonatal origin of the complement and, on the other hand, the incomplete activation of the complement system without causing cellular lysis, secondary to antigen-antibody interaction anti-erythrocyte. In 52 newborns who initially were DAT positive, we could not identify the specificity of the reaction. The possible causes involve the elevated sensitivity of the poly-specific serum, with low specificity to identify other immunoglobulins (IgM or IgA) or proteins that are not immunoglobulins such as Wharton jelly, drugs or the inherent limitations of the technique.[21]

The prevalence of positive DAT in the neonatal population varies widely from 1 to 9%[18,19] and depends on the intention of the assessment. ABO incompatibility occurs in between 6.9 and 11% of all live births and ~13% present DAT positive. Newborns with ABO incompatibility and DAT positive are five times more likely to develop a hemolytic dis-

ease. Newborns who are DAT positive, regardless of the degree, have hemoglobin or hematocrit values much lower at birth as well as a higher occurrence of hyperbilirubinemia compared with those who are DAT negative.[18]

DAT is useful in early prediction of jaundice or hyperbilirubinemia, especially when compared with infants who are DAT negative even in the presence of ABO incompatibility.[24] Up to 23% of infants with positive DAT require phototherapy.[25] Of the infants receiving phototherapy, 15.1% show a DAT positive.[26]

In the low-risk postpartum programs, DAT is also useful to identify infants at an increased risk of early readmission to hospital for jaundice.[17] In 4% of the cases it is related to problems of blood group incompatibility.[3] The goal is for newborns at risk of clinically relevant jaundice to benefit, as well as in other conditions, from the measurements or programs to identify risk or early detection of various adverse clinical conditions, such as hyperbilirubinemia encephalopathy, prolonged hospitalization, intense hemolytic anemia or others.[27,28] Inclusion of DAT in the neonatal screening allows early intervention with prophylactic phototherapy[2,19] or the prediction of hyperbilirubinemia rebound following suspension of phototherapy.[1,3] The establishment of this type of programs constitutes recommended interventions for the benefit and protection to the newborn, the family and the healthcare system.

For near-term gestation newborns, the American Association of Pediatrics (AAP)[17] recommends, as a secondary prevention measure, the determination of ABO and Rh blood groups in all pregnant women. In cases where the maternal ABO/Rh group are unknown or whether the mother is RhD nega-

Table 4. Characteristics of anti-erythrocyte antibodies associated with hemolytic disease in newborns

Erythrocyte system	Antibody produced	Isotype present	Hemolytic disease
ABO	Anti-A	Both, principally IgM	Frequent, light to intense
	Anti-B	IgG frequent	
MNS	MN	Principally IgM, rarely IgG	Very rare
	SsU	Principally IgG, rarely IgM	Rare but severe
P1	P1	Principally IgM, rarely IgG	Not reported
Rh	Anti-D	All principally IgG, rarely IgM	Very frequent
Anti-C, c, E, e		Intense	
Lutheran	Lua	Principally IgG, ocasionally IgM	Moderate, rare
	Lub	Principally IgG, rarely IgM	Moderate, rare
Kell	Kell	Principally IgG, rarely IgM	Moderate to severe, frequent
Duffy	Fya	Both principally IgG	Both moderate to severe anemia
	Fyb	IgM rarely	Occasionally
Kidd	Kidd	Principally IgG, rarely IgM	Moderate, less frequent
Diego	Diego	Principally IgG, rarely IgM	Moderate, less frequent
Cartwright	Yt	Principally IgG, rarely IgM	Not reported
Scianna	Scianna	Principally IgG, rarely IgM	Very rare
Dombrock	Dombrock	Principally IgG, rarely IgM	Very rare
Colton	Colton	Principally IgG, rarely IgM	Moderate to severe
LW	LW	Principally IgG, rarely IgM	None to moderate
RhAg	Anti-Rh	Principally IgG, rarely IgM	None to moderate (Rh null)

Without actual report of those associated with hemolytic disease of newborns in the following systems: Lewis (Lea, Leb), Xg (Xg), Ch/Rg (Chido-Rodgers), Hh (Hh), Kx, Gerbich, Cromer, Knops, Indian, OK, Raph, John Milton Hagen, I (I, i), Globoside (P, Pk)[5-8,13,23]

tive or is type O RhD positive, the AAP established the need for determining the ABO and RhD group as well as the DAT in all newborns.[28]

In conclusion, the prevalence of direct antiglobulin tests is high in the open neonatal population (3.6%) used as a neonatal screening test, whereas in nearly a third of the cases of DAT positive may not have membrane-bound IgG or C3. This information should be evaluated in terms of potential usefulness for possible early intervention or secondary prevention, as well as being assessed for its cost effectiveness.

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References

1. Burke BL, Robbins JM, Bird TM, Hobbs CA, Nesmith C, Tilford JM. Trends in hospitalizations for neonatal jaundice and kernicterus in the United States, 1988-2005. Pediatrics 2009;123:524-532.
2. Yaseen H, Khalaf M, Rashid N. Does prophylactic phototherapy prevent hyperbilirubinaemia in neonates with ABO incompatibility and positive Coombs' test? J Perinatol 2005;25:590-594.
3. Jonguitud AA, Martínez PB, Bravo A. Admisión de neonatos con ictericia al servicio de pediatría de un hospital general. Rev Mex Pediatr 2003;70:171-175.
4. Cid VJ, Eelies-Fibia E. Estudio inmunohematológico de la enfermedad hemolítica ABO. An Esp Pediatr 2000;53:249-252.
5. Baptista GHA, Rosenfeld MF, Pérez PJ, Quintanar GE. Anticuerpos irregulares antieritrocitarios fuera del sistema ABO en el período neonatal. Bol Med Hosp Infant Mex 1991;48:814-820.
6. Miquel E, Cavelier B, Bonneau JC, Rouger P. Foetomaternal erythrocyte incompatibilities: from immunohaematologic surveillance of pregnant women to haemolytic disease of the newborn. Transfus Clin Biol 2005;12:45-55.
7. Lobo GA, Nardozza LM, Camano L. Non-anti-D antibodies in red-cell alloimmunization. Int J Gynaecol Obstet 2006;94:139-140.
8. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Semin Fetal Neonatal Med 2008;13:207-214.
9. Joy SD, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-E alloimmunization. Obstet Gynecol 2005;105:24-28.
10. Santiago JC, Ramos-Corpas D, Oyonarte S, Montoya F. Current clinical management of anti-Kell alloimmunization in pregnancy. Eur J Obstet Gynecol Reprod Biol 2008;136:151-154.
11. Peon HL, Pacheco CMG, Zavala RM, Madueno LA, Garcia GA. Blood group frequencies and ABO and RhD incompatibilities in La Paz, Baja California Sur, México. Salud Pública Mex 2002;44:406-412.
12. Baptista GHA, Trueba GR, Santamaría HC. Grupos Sanguíneos de Importancia Clínica, fuera de los Sistemas ABO y Rh. México: Editorial Prado; 2006. pp. 145-159.
13. Dittmar K, Procter JL, Cipolone K, Njoroge JM, Miller J, Stroncek DF. Comparison of DATs using traditional tube agglutination to gel column and affinity column procedures. Transfusion 2001;41:1258-1262.
14. Novaretti MC, Jens E, Pagliarini T, Bonifacio SL, Dorlhiac-Llacer PE, Chamone DA. Comparison of conventional tube test technique and gel micro column assay for direct antiglobulin test: a large study. J Clin Lab Anal 2004;18:255-258.
15. Meberg A, Johansen KB. Screening for neonatal hyperbilirubinaemia and ABO alloimmunization at the time of testing for phenylketonuria and congenital hypothyreosis. Acta Paediatr J 1998;87:1269-1274.
16. Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
17. Committee on Fetus and Newborn. Levels of neonatal care. Pediatrics 2004;114:1341-1347.
18. Dinesh D. Review of positive direct antiglobulin tests found on cord blood sampling. J Paediatr Child Health 2005;41:504-507.
19. Cianciarullo MA, Jurfest CMA, Coasta VCF. Prevalence of immunohematologic tests at birth and the incidence of hemolytic disease in the newborn. Rev Assoc Med Bras 2003;49:45-53.
20. Norma Oficial Mexicana NOM-007-SSA2-1993. Atención de la mujer durante el embarazo, parto y puerperio y del recién nacido. Criterios y procedimientos para la prestación del servicio. México: DOF; 1994.
21. Leger RM. The positive direct antiglobulin test and immune-mediated hemolysis. in technical manual. 16th ed. Bethesda, MD: AABB; 2008. pp. 499-523.
22. Daniels G, Castilho L, Flegel WA, Fletcher A, Garratty G, Levene C, et al. International Society of Blood Transfusion Committee on Terminology for Red Blood Cell Surface Antigens: Macao report. Vox Sang 2009;96:153-156.

23. Lisker R. In: Estructura de la Población Mexicana: Aspectos Médicos y Antropológicos. México: Salvat; 1981.
24. Herschel M, Karrison T, Wen M, Caldarelli L, Baron B. Isoimmunization is unlikely to be the cause of hemolysis in ABO-incompatible but direct antiglobulin test-negative neonates. Pediatrics 2002; 110:127-130.
25. Kaplan M, Kaplan E, Hammerman C, Algur N, Bromiker R, Schimmel MS, et al. Post-phototherapy neonatal bilirubin rebound: a potential cause of significant hyperbilirubinaemia. Arch Dis Child 2006;91:31-34.
26. Adeniji AA, Fuller I, Dale T, Lindow SW. Should we continue screening rhesus D positive women for the development of atypical antibodies in late pregnancy? J Matern Fetal Neonatal Med 2007;20:59-61.