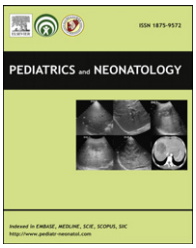
Pediatrics and Neonatology (2012) 53, 138–143

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



ORIGINAL ARTICLE

Association of ABO Incompatibility With Red Blood Cell Indices of Cord Blood Unit

Shu-Huey Chen [a,b,f], Marie Lin [c], Kuo-Liang Yang [b], Teng-Yi Lin [d], His-Hsiu Tsai [d], Shang-Hsien Yang [a,f], Yu-Hsun Chang [a], Yi-Feng Wu [e], Tso-Fu Wang [b,e,f,*]

[a] *Department of Pediatrics, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan*
[b] *Buddhist Tzu-Chi Stem Cells Center, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan*
[c] *Transfusion Medicine Research Laboratory, Mackay Memorial Hospital, Taipei, Taiwan*
[d] *Department of Laboratory Medicine, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan*
[e] *Department of Hematology and Oncology, Buddhist Tzu-Chi General Hospital, Hualien, Taiwan*
[f] *Department of Medicine, College of Medicine, Tzu-Chi University, Hualien, Taiwan*

Received Jun 13, 2011; received in revised form Aug 19, 2011; accepted Sep 9, 2011

Key Words
abo incompatibility;
cord blood unit;
hematocrit;
hemoglobin;
red blood cell

Background: Maternal–fetal ABO incompatibility is one of the causes of neonatal hyperbilirubinemia. We postulate that hemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) values for cord blood units (CBUs) are lower and erythroblast values higher for maternal–fetal ABO incompatible dyads than for compatible dyads.
Objective: We investigated the relationship between Hb, Hct, RBC, and erythroblast CBU values and maternal–fetal ABO blood type compatibility.
Methods: Mothers having blood group O who gave birth to infants with blood group A, B, or AB were classified as Group I. According to baby's blood group, the members of Group I were further divided into AO (baby group A, mother group O), BO (baby group B, mother group O), and ABO (baby group AB, mother group O) subgroups. Mothers having blood group A who gave birth to infants with blood group B or AB and mothers having blood group B who gave birth to infants with blood group A or AB were classified as Group II. All other maternal–fetal blood type pairs were considered ABO compatible and were classified as Group III. We compared mean Hb, Hct, RBC, and erythroblast values for the infants' CBUs among these three groups including the subgroups of Group I.
Results: Group I had lower mean Hb, Hct, and RBC values than Group II and Group III (both $p < 0.001$). Although the mean Hb, Hct, and RBC values for Group II were lower than for

* Corresponding author. Department of Hematology and Oncology, Buddhist Tzu-Chi General Hospital, Hualien, 707, Sec. 3, Chung-Yang Road, Hualien 970, Taiwan.
E-mail address: tfwang@tzuchi.com.tw (T.-F. Wang).

doi:10.1016/j.pedneo.2012.01.012

Group III, the difference was not statistically significant. Mean Hb and RBC for the AO group were higher and nucleated RBC (nRBC) ratios were lower than for the BO group; however, these differences were also not statistically significant. Interestingly, the mean Hct value of the BO group was significantly lower than that of the AO group ($p = 0.04$).
Conclusion: Group A or B neonates with a group O mother have lower mean Hb, Hct, and RBC values for CBUs than other neonates. The role of RBC indices in predicting neonatal hemolytic hyperbilirubinemia remains unclear and further studies are needed to identify the possible clinical association.

1. Introduction

Hemolytic disease of the newborns (HDN) due to maternal antibodies is a situation in which the lifespan of the neonate's red cells is shortened due to the activity of transplacental maternal antibodies. More than 99.5% of Taiwanese are D Rh positive and, therefore, HDN due to anti-D antibodies is rarely encountered.[1] In addition, hemolytic disease resulting from ABO incompatibility is clinically milder than anti-D disease. However, HDN and kernicterus do occasionally occur, and hydrops fetalis has been suggested as a result.[2,3] Thus, it is desirable to have a simple and reliable test that is able to predict the development of neonatal hyperbilirubinemia due to ABO incompatibility, after which preventive phototherapy can be used. Hemoglobin (Hb) levels, hematocrit (Hct) counts, reticulocyte counts, direct Coombs test results, bilirubin levels, and immunoglobulin G (IgG) titers that have been obtained from cord blood together with maternal anti-A/anti-B titers have been suggested as approaches for predicting the severity of hyperbilirubinemia in ABO HDN.[4–10]

A complete blood count (CBC) test is an absolute requirement before any CBU cryopreservation. CBC testing of CBUs is noninvasive, convenient, simple, and rapid. We hypothesized that ABO incompatibility influences Hb, Hct, red blood cell (RBC), and erythroblast values obtained from CBU. The veracity of this hypothesis could be tested using reference data and then used to help predict ABO incompatibility that is related to HDN. Most studies reported in the literature, when obtaining CBC samples, have used cord blood obtained directly from the umbilical veins at delivery rather than cord blood units. To our best knowledge, no similar study has been reported to date involving a sample of this great size. Using 3688 CBUs, we studied the impact of differences in the mothers' and fetuses' blood type combinations on Hb, Hct, RBC, and erythroblast values for CBUs.

2. Materials and Methods

2.1. Cord blood collection

Between September 2001 and November 2006, donated cord blood samples from healthy Taiwanese singleton neonates with a gestational age more than 36 weeks born to married mothers were collected by the Tzu Chi Cord Blood Bank. CBUs with a net weight of more than 90 g were accepted. CBUs where one parent carried thalassemia were not accepted, and collected CBUs were discarded if the baby was diagnosed as having glucose-6-phosphate dehydrogenase deficiency (G-6-PD) deficiency. Written informed consent was obtained from the mother donating the CBU before collection. All CBUs were collected *in utero* using a standard procedure. After delivery, the cord was sterilized and a 16-gauze needle was inserted into the umbilical vein. The cord blood was collected by gravity into a collecting bag containing 28 mL anticoagulant phosphate-citrate-dextrose. The bag was stored at 4°C to 10°C and sent to Tzu Chi Cord Blood Bank within 24 hours. Between 1 mL and 2 mL of the aspirated cord blood from cord blood bag was infused into an EDTA tube. Subsequently, the cord blood CBC, white blood cell differential count (WBC DC), Rh typing, and ABO typing were analyzed in the central laboratory of Hualien Buddhist Tzu Chi General Hospital by experienced technicians. Blood group typing was performed routinely using standard blood bank techniques.

2.2. Analysis of cord blood CBC and WBC DC

The CBC testing included RBC, Hb, Hct, WBC, and platelet counts, which were measured using a Sysmex XE2100™ automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Nucleated RBCs (nRBCs) were reported as the number of nucleated RBCs per 100 WBCs. According to the quality control chart and Westgard rules $1_{3S}/2_{2S}/R_{4S}$, the analyzer was calibrated twice daily using a commercial assayed control cell.[11]

2.3. Materials

Among the 5602 CBUs available, 1913 units lacked some data, and these were excluded. Furthermore, there was only one unit where the mother was AB blood group and baby was O group, therefore we excluded this CBU also. In total, 3688 healthy neonates were included in this study, and these were divided into three groups. Mothers having blood group O who had given birth to infants having blood group A, B, or AB were classified as Group I. Group I consisted of 555 CBUs (A, B, and AB; 305, 247, and 3, respectively) where the newborn's mother's blood group was O. Mothers having blood group A who had given birth to infants having blood group B or AB together with mothers having blood group B who gave birth to infants having blood group

A or AB were classified as Group II. Group II consisted of 326 newborns. Groups I and II were both considered to be maternal–fetal ABO-incompatible pairs. All other maternal–fetal blood group pairs were considered ABO-compatible and were classified as Group III. Group III consisted of 2807 newborns. According to the baby's blood group, either A, B, or AB, Group I was further divided to AO (mother O group with baby A), BO (mother O group with baby B), and ABO (mother O group with baby AB) subgroups and there were 305, 247, and 3 in the AO, BO, and AB subgroups, respectively. Maternal, neonatal, and cord blood data was then obtained from medical records for each CBU sample.

2.4. Statistical analysis

The gender of neonate and delivery route for the studied groups were compared by Chi-square test. The significance of differences for the CBU mean values for Hb, Hct, nRBC ratios, and RBC together with maternal age, gestational age, neonate's birth body weight, and cord blood weight across the three groups were evaluated by ANOVA. We used Student *t* test to evaluate the differences in mean Hb, Hct, nRBC ratios, and RBC across the AO and BO subgroups of Group I. A p value <0.05 was considered to be statistically significant.

3. Results

Totally, 3688 units of cord blood were eligible for analysis, and none of the mothers/infants were found to be DRh-positive blood type. There were 555, 326, and 2807 infants in Groups I, II, and III, respectively. In Group I, there were 305 infants in the AO subgroup and 247 infants in the BO subgroup. The mean, median, minimum, and maximum values for Hb, Hct, nRBC ratio, RBC, maternal age, gestational age, baby's birth body weight, and cord blood weight across the three groups are listed in Table 1. This shows that there was no statistical difference for gender (male/female ratio) or delivery route (vaginal delivery vs. cesarean section) across the groups. However, the means for Hb, Hct, and RBC values for Group I were significantly lower than those for Groups II and III (Hb: 10.6 g/dL, 10.8 g/dL, and 10.9 g/dL, respectively, $p < 0.001$; Ht: 35.1%, 35.7%, and 36.2%, $p < 0.001$; RBC: 3.01, $\times$ $10^6/\mu L$, 3.11 $\times$ $10^6/\mu L$, and 3.14$\times 10^6/\mu L$, $p < 0.001$). Although the mean Hb, Hct, and RBC values for Group II were lower than for Group III, these differences were not statistically significant. Furthermore, no significant differences were found among Groups I, II, and III when their nRBC ratios, maternal age, gestational age, infant's birth body weight, and cord blood weight were analyzed. These findings are presented in Table 2. In Group I, the mean Hct value of the BO subgroup was significantly lower than that of the AO group (34.7% for the BO group vs. 35.4% for the AO group, $p = 0.04$) (Table 3). Although there were no statistical differences in mean Hb, nRBC ratios, and RBC between the AO and BO subgroups, the mean Hb and RBC values for the AO group were higher and the nRBC ratios lower than for the BO group (Hb = 10.7 g/dL, 10.4 g/dL, respectively, $p = 0.06$; RBC = 3.04$\times 10^6/\mu L$, 2.98$\times 10^6/\mu L$, respectively,

Table 1 The mean, median, minimum, and maximum values for Hb, Hct, nucleated RBC (nRBC) ratio, RBC, maternal age, gestational age, and cord blood weight across Groups I, II, and III.

	Group													
	I					II				III				
	N	Mean	Median	Minimum	Maximum	*N*	Mean	Median	Minimum	Maximum	*N*	Mean	Median	Minimum	Maximum
Hb (g/dL)	555	10.6	10.5	7.3	14	326	10.8	10.8	6.8	14.9	2807	10.9	10.8	4.1	17.7
Hct (%)	555	35.1	35.0	24.1	48.1	326	35.7	35.7	22.3	49.7	2807	36.2	36.0	11.5	59.0
nRBC (/100 WBCs)	555	3.80	2.00	0.00	147.0	326	3.25	2.0	0.0	55.0	2807	3.19	2.00	0.00	147.0
RBC ($\times 10^6/\mu L$)	555	3.01	2.99	1.93	4.10	326	3.11	3.10	2.00	4.56	2807	3.14	3.11	0.76	5.05
Age of mother (y)	555	29.9	30.0	18.0	42.0	326	29.8	30.0	18.0	39.0	2807	29.9	30.0	16.0	73.0
Weight of cord blood unit (g)	555	141.1	138.0	94.0	233.2	326	141.8	139.5	96.5	226.2	2807	141.0	138.0	94.9	243.5
Gestational age (weeks)	555	38.8	39.0	36.0	42.0	326	38.9	39.0	36.0	42.0	2807	38.9	39.0	36.0	42.0
Baby birth body weight (g)	555	3257.5	3240.0	2280.0	4320.0	326	3248.1	3235.0	2276.0	4320.0	2807	3251.8	3225.0	2130.0	5000.0

Hb = hemoglobin; Hct = hematocrit; N = sample size; nRBC = nucleated red blood cells; WBC = white blood cells.

Table 2 Comparison of the mean Hb, Hct, RBC, nRBC ratio, maternal age, gestational age, cord blood volume, and cord blood weight across Groups I, II, and III.

	Group I (N = 555)	Group II (N = 326)	Group III (N = 2807)	p value
Gender, M/F (%)*	289/266 (52.1/47.9)	146/180 (44.8/55.2)	1475/1332 (52.5/47.5)	0.38
Vaginal/CS (%)*	411/144 (74.1/25.9)	247/79 (75.8/24.2)	2029/778 (72.3/27.7)	0.25
RBC ($\times 10^6/\mu L$)	3.01	3.11	3.14	<0.001
Hb (g/dL)	10.6	10.8	10.9	<0.001
Hct (%)	35.1	35.7	36.2	<0.001
nRBC (/100 WBCs)	3.80	3.25	3.19	0.07
Age of mother (y)	29.9	29.8	29.8	0.92
Gestational age (weeks)	38.8	38.9	38.9	0.54
Weight of cord blood unit (g)	141.2	141.7	141	0.84
Baby birth body weight (g)	3257.5	3248.1	3251.8	0.92

Data are presented as the means and are compared by ANOVA test.
N = sample size; M = male; F = female; CS = cesarean section; Hb = hemoglobin; Hct = hematocrit; nRBC = nucleated red blood cells; WBC = white blood cells.
* Chi-square test.

p = 0.10; nRBC = 3.78, 3.84, respectively, p = 0.93) (Table 3).

4. Discussion

Many etiological factors are involved in the development of neonatal hyperbilirubinemia. For example, UDP-glucuronosyl transferase 1A1 (UGT1A1) is the key enzyme in bilirubin conjugation, and Huang et al's study on variation at nucleotide 211 of the *UGT1A1* gene shows that this is a risk factor for the development of neonatal hyperbilirubinemia among Taiwanese. The variant rate within the coding region of the *UGT1A1* gene in Taiwanese was found to be 29.3%.[12] In addition, ABO incompatibility is considered to be one of the most common causes of neonatal hyperbilirubinemia. The major anti-A and anti-B antibodies in blood group B and A individuals are immunoglobulin M (IgM); however, these antibodies in group O individuals are usually mostly IgG. The only immunoglobulin transferred from mother to fetus via the placenta is IgG. Lin et al reported that mothers and infants had the same anti-A and anti-B IgG titers when group O mothers and infants were compared.[13] ABO HDN is considered to occur relatively frequently and can be a significant cause of neonatal morbidity. In 14.3% of all pregnancies in Taiwanese, the mother is O and her neonate is either A or B.[14] However, ABO incompatibility-induced clinically severe hemolytic disease is comparatively rare. There are three main reasons for this. First, A and B blood type antigens are expressed at a low level on fetal RBCs. Second, IgG ABO antibodies are usually IgG2, which does not initiate RBC destruction. Thirdly, ABO antigens are present in many tissues, and any IgG antibodies crossing the placenta are likely to become bound to placental tissue.[15–18] As a result, this disease is usually mild; nevertheless, severe hemolysis may occasionally occur. Taiwanese neonatal jaundice has been found to be more severe than that found in Black and Caucasian populations.[9] Desjardins et al reported that among 1704 infants of blood group O mothers, infants with blood groups A or B had significantly lower cord blood Hb concentrations than did newborns with blood group O; they concluded that most ABO-incompatible infants showed some degree of hemolytic disease even though antibodies were not demonstrated by either the Coombs or eluate test.[19] In our study, Group I infants have a lower mean Hb, Hct, and RBC than the other two groups, and this supports Desjardins et al's findings. Chen and Ling demonstrated that a high IgG titer among group O mothers was associated with the development of ABO HDN in Taiwan.[10] However, in Taiwan, the cause of neonatal hyperbilirubinemia is quite complicated because close to 30% of the population have the *UGT1A1* gene mutation associated with hyperbilirubinemia.

Table 3 Comparison of the mean Hb, Hct, nRBC ratio, and RBC between the AO and BO subgroups within Group I.

	AO subgroup (N = 305)	BO subgroup (N = 247)	p value
RBC ($\times 10^6/\mu L$)	3.04	2.98	0.10
Hb (g/dL)	10.7	10.4	0.06
Hct (%)	35.4	34.7	0.04
nRBC (/100 WBCs)	3.78	3.84	0.93

Data are presented as means and compared by Student t test.
Hb = hemoglobin; Hct = hematocrit; N = sample size; nRBC = nucleated red blood cells; WBC = white blood cells.

In group A and B individuals, naturally occurring anti-B and anti-A antibodies are mainly IgM, which cannot cross the placenta. By contrast, in the group O individuals, anti-A and anti-B antibodies are predominantly IgG, which can cross the placenta. Dufour and Monoghan reported that 37.9% of group O mothers having blood group A or B infants showed laboratory evidence of ABO HDN, whereas only 0.8% of mothers of group A with infants having blood group B or AB and mothers of group B with infants having blood group A or AB showed laboratory evidence of HDN.[20] In our study, the values for Hb, Hct, and RBC in Group I were significantly lower than those for Groups II and III (Hb: 10.6 g/dL, 10.8 g/dL, and 10.9 g/dL, respectively, p < 0.001; Ht: 35.1%,

35.7%, and 36.2%, $p < 0.001$; RBC: 3.01 $\times$ $10^6/\mu L$, 3.11 $\times$ $10^6/\mu L$ and 3.14 $\times$ $10^6/\mu L$, $p < 0.001$). Furthermore, Group II had lower Hb, Hct, and RBC values than Group III (Hb 10.8 g/dL, 10.9 g/dL, respectively; Hct 35.7%, 36.2; RBC 3.11 $\times$ $10^6/\mu L$, 3.14 $\times$ $10^6/\mu L$), but these differences did not reach statistical significance. Thus, our results were consistent with Dufour and Monoghan's findings.

nRBCs are immature RBCs. Many pathological conditions, including hemolytic disease and bleeding, can cause an increase in the number of nRBCs. Hanlon-Lundberg and Kirby evaluated 1661 neonates and reported that cord blood nRBC counts were lower in infants with ABO compatibility than in infants with ABO incompatibility and that group B infants who were borne by group O mothers had the highest nRBC counts.[21] However, the differences in nRBC ratios in our three groups did not reach statistical significance; furthermore, there was also no significant difference between the AO and BO subgroups for the nRBC ratios. Their study obtained the CBC samples directly from the umbilical vein. Our study obtained the CBC samples from a cord blood bag that contained 28 mL of anticoagulant, and this might have caused a dilution effect. This difference in CBC sample collection may explain the different results.

HDN in Group B infants due to blood group antibodies was claimed to be more severe than HDN hemolytic disease in Group A infants in some studies.[4,22,23] However, some other studies have reported no difference in severity between AO and BO incompatibility.[9,11] In our study, mean Hb and RBC were 10.7 g/dL, 3.04 $\times$ $10^6/\mu L$, and 10.4 g/dL, 2.98 $\times$ $10^6/\mu L$ in AO and BO group, respectively. Although the mean Hb and RBC values for the BO group were lower than those for the AO group, the difference did not reach statistical significance. Furthermore, although the nRBC ratio in the BO group was higher than in AO group, again, the difference was not statistically significant (3.78 and 3.84 in AO and BO groups, respectively). Interestingly, the Hct for the AO group was higher than for the BO group (35.4% and 34.7% in AO and BO subgroups, respectively) and this difference was statistically significant ($p = 0.04$).

CBU Hb, Hct, and RBC values are part of the routine data collected by all cord blood banks. We have established Taiwanese cord blood CBC normal reference values and the mean values for Hb, Hct, RBC, and nRBC are 11.2 g/dL, 36.9 (%), 3.22 $\times$ $10^6/\mu L$, and 3.4/100 WBC), respectively.[24] We can probably use these RBC indices as a reference for predicting ABO HDN. In this study, there was no personal identification data available because of confidentiality regulations of the public cord blood bank, and therefore, a clinical analysis of the incidence of hyperbilirubinemia was impossible. Nonetheless, it seems likely that CBU Hb, Hct, reticulocyte count, direct Coombs test, eluate test, and bilirubin result may help with predicting the severity of hyperbilirubinemia in ABO HDN.[4–10,13,25] However, these laboratory results do not provide a reliable early diagnosis for ABO HDN because neonatal hyperbilirubinemia involves multiple etiologies. These etiologies include *UGT1A1* gene variations, maturity of the neonatal liver, conditions that increase bilirubin production, conditions that increase enterohepatic circulation and breast feeding failure. Further study needs to focus on the association between CBU laboratory values and clinical outcome in order to clarify the clinical role of the CBU RBC indices.

References

1. Lin-Chu M. The Rhesus blood group in Taiwan. *J Formosan Med Assoc* 1984;**83**:796–9.
2. Chen W, Shih JS. Etiological factors and clinical aspects of Chinese neonatal hyperbilirubinemia. *Acta Paediatr Sin* 1981; **22**:141–9.
3. Weng YH, Chiu YW. Spectrum and outcome analysis of marked neonatal hyperbilirubinemia with blood group incompatibility. *Chang Gung Med J* 2009;**32**:400–9.
4. Clifford JH, Mathews P, Reiquam CW, Palmer HD. Screening for hemolytic disease of the newborn by cord blood Coomb testing: analysis of a five-year experience. *Clin Pediatrics* 1968;**7**:465–9.
5. Alter AA, Feldman F, Twersky J, et al. Direct antiglobulin test in ABO hemolytic disease of the newborn. *Obstet Gynec* 1969; **33**:846–51.
6. Orzalesi M, Gloria F, Lucarelli P, Bottini E. ABO system incompatibility: relationship between direct Coombs test positivity and neonatal jaundice. *Pediatrics* 1973;**51**:288–9.
7. Risemberg HM, Mazzi E, Macdonald MG, Peralta M, Heldrich F. Correlation of cord blood bilirubin levels with hyperbilirubinaemia in ABO incompatibility. *Arch Dis Child* 1977;**52**: 219–22.
8. Peevy KJ, Wiseman HJ. ABO hemolytic disease of the newborn: evaluation of management and identification of racial and antigenic factors. *Pediatrics* 1978;**61**:475–8.
9. Chen W, Wu WF, Shih JS. ABO incompatibility in Chinese newborn infants. *Acta Paed Sin* 1980;**21**:158–66.
10. Chen JY, Ling UP. Prediction of the development of neonatal hyperbilirubinemia in ABO incompatibility. *Chin Med J (Taipei)* 1994;**53**:13–8.
11. Westgard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 1981;**27**:493–501.
12. Huang CS, Chang PF, Huang MJ, Chen ES, Hung KL, Tsou KI. Relationship between bilirubin UDP-glucuronosyl transferase 1A1 gene and neonatal hyperbilirubinemia. *Pediatr Res* 2002; **52**:601–5.
13. Lin-Chu M, Broadberry RE, Chang FC, Yang TF, Huang FY. Blood group antibodies and hemolytic disease of the newborn. *J Formosan Med Assoc* 1986;**85**:799–806.
14. Chen SH, Lin KS, Chen CL. ABO incompatibility and neonatal hyperbilirubinemia. *Acta Paed Sin* 1985;**84**:1233–9.
15. Sherer DM, Abramowicz JS, Ryan RM, Sheils LA, Blumberg N, Woods Jr JR. Severe fetal hydrops resulting from ABO incompatibility. *Obstet Gynecol* 1991;**78**:897–9.
16. Stiller RJ, Herzlinger R, Siegel S, Whetham JC. Fetal ascites associated with ABO incompatibility: case report and review of the literature. *Am J Obstet Gynecol* 1996;**175**:1371–2.
17. Polley MJ, Adinolfi M, Mollison PL. Serological characteristics of anti-A related to type of antibody protein (7 $S\gamma$ or 19 $S\gamma$). *Vox Sanguinis* 1963;**8**:385–409.
18. Brouwers HA, Overbeeke MA, Gemke RJ, Mass CJ, van Leeuwen EF, Engelfriet CP. Sensitive methods for determining subclasses of IgG anti-A and anti-B in sera of blood-group-O women with a blood-group-A or -B child. *Br J Haematol* 1987; **66**:267–70.
19. Desjardins L, Blajchman MA, Chintu C, Gent M, Zipursky A. The spectrum of ABO hemolytic disease of the newborn infants. *J Pediatr* 1979;**95**:447–9.
20. Dufour DR, Monoghan WP. ABO hemolytic disease of the newborn: a retrospective analysis of 254 cases. *Am J Clin Pathol* 1980;**73**:369–73.

21. Hanlon-Lundberg KM, Kirby RS. Association of ABO incompatibility with elevation of nucleated red blood cell counts in term neonates. *Am J Obstet Gynecol* 2000;**183**:1532—6.
22. Farrell AGW. ABO incompatibility and haemolytic disease of the newborn. *S Afr Med J* 1970;**44**:211.
23. Sisson TRC. Phototherapy in ABO incompatibility. *J Pediatr* 1972;**80**:1063—4.
24. Chang YH, Yang SH, Wang TF, Lin TY, Yang KL, Chen SH. Complete blood count reference values of cord blood in Taiwan and their influence by gender and delivery route on them. *Pediatr Neonatol* 2011;**52**:155—60.
25. Procianoy RS, Giacomini CB, Farina DM, et al. Early diagnosis of ABO haemolytic disease of the newborn. *Eur J Pediatr* 1987; **146**:390—3.