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The effects of umblical cord clamping time on lymphocyte subgroups in term and late preterm infants

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Abstract

Aim: To evaluate the effect of umblical cord clamping time on lymphocyte subgroups in term and late preterm infants.

Material and Methods: Seventy-four infants between 34 and 41 weeks of gestation were included in the study. Of these, 37 were umbilical cord clamped immediately after birth and the remaining 37 were clamped after waiting one minute. Babies were divided into two groups as term and preterm. The prenatal, natal, postnatal characteristics of the infants were recorded. Hematologic and lymphocyte subgroups were investigated in cord blood and venous blood at day 7. Lymphocyte subgroups were evaluated using flow cytometry.

Results: With the delay of cord clamping, the leucocytes count and the percentage of CD3+T lymphocytes in cord blood of preterm infants decreased and this decrease continued at day 7. On the contrary, CD19+B lymphocyte levels in the cord blood of preterm infants increased, and this increase continued at day 7. Also, the percentage of CD4+T lymphocytes of preterm infants decreased with the delay of cord clamping at day 7. There was no difference between groups for the rate of sepsis development.

Conclusion: With the delay of cord clamping, the leucocytes count, the percentage of CD3+T, and CD4+T lymphocytes decreased, and the percentage of CD19+B lymphocytes increased in preterm infants. The delay in cord clamping time in term and preterm infants seems to have no impact on the rate of sepsis development. Larger series of studies are needed to assess the effect of these findings on the development of infection in late preterm infants who have delayed cord clamping.

Keywords: Cord clamping time, late preterm, lymphocyte subgroups, sepsis, term

Introduction

The placenta contains approximately 100 mL of blood. One-quarter of this blood crosses from the placenta to the baby in the first 15 seconds and half of it crosses in the first 1 minute. A 10% reduction or increase occurs in this blood volume with early or late clamping of the umbilical cord. Although the mean hemoglobin level does not change relatively at birth, a reduction or increase in the erythrocyte mass is reflected in the hemoglobin values with redistribution of the plasma volume after 48 hours (1). In a study conducted with term babies, the hematocrit level at birth was the same in babies whose umbilical cords were clamped immediately or late, whereas the hematocrit level at the 48th hour was found as 48% in the early-clamp group and 65% in the late-clamp group (2).

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Delaying cord clamping for at least 30 seconds in babies who do not need cardiac and respiratory resuscitation after birth was recommended in the neonatal resuscitation guideline of the American Heart Association, which was revised in 2015 (3). This application has no negative effect on survival, and it has positive effects including higher blood pressure, frequency of necrotizing enterocolitis, rarer and lower grade intraventricular hemorrhage, and a reduced need for blood transfusion after birth.

The immune system is composed of two parts. These are named the specific and nonspecific immune systems. The monocyte macrophage system and neutrophils phagocyte microbial pathogens in the nonspecific immune defense. Presentation of microbial antigens by the same monocyte macrophage system to T lymphocytes is the beginning of the specific immune response. The cells involved in the specific immune response include T and B lymphocytes (4). Although delayed cord clamping was shown to have an effect on erythrocytes, we found no data in the literature in relation to its effect on lymphocyte subgroups.

In this study, the effect of delayed cord clamping on complete blood count, lymphocyte subgroups, and infection was evaluated in late preterm and term babies.

Material and Methods

The study was conducted prospectively between February 2017 and March 2017 and 74 babies born by normal vaginal delivery or cesarean section with a gestational age above 34 weeks were included in the study. Approval was obtained according to World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subject from the Ethics Committee of the institution in which the study was conducted (October 7th, 2016, Decision Number: 16) and written consent was obtained from the families of all babies included in the study.

Babies of diabetic mothers who used insulin, babies of mothers with abruptio placentae and placental detachment, twin babies with a common placenta, babies who had anomalies incompatible with life, babies who were born in external centers and transported to our unit, babies who had severe intrauterine growth retardation, and pregnancies with a suspicion of twin-to-twin transfusion were excluded from the study. Babies who were born before the 37th gestational week from the first day of the last menstruation were considered preterm. Randomization was performed using a computer for babies who were planned to be included in the study. The babies included in the study were divided into four groups.

Group 1: term babies whose cord clamping was performed immediately

Group 2: term babies whose cord clamping was performed late

Group 3: preterm babies whose cord clamping was performed immediately

Group 4: preterm babies whose cord clamping was performed late

In the immediate cord-clamping groups, the cord was clamped immediately after the baby was taken out of the uterus. In the delayed cord-clamping groups, the cord was clamped after waiting for one minute, keeping the baby at the level of the uterus or 30 cm below the uterus.

If the babies included in the study were hospitalized, they were evaluated during the hospitalization process. Babies who were hospitalized and discharged before the seventh day after birth were evaluated during the hospitalization process and on the postnatal seventh day after discharge. Babies who did not need hospitalization were evaluated immediately after birth and on the seventh day. All babies were evaluated at the 30th day in terms of neonatal infection, and possible problems were recorded. Blood samples (2 mL) were obtained from the cord and from the peripheral vein on the postnatal seventh day. Complete blood count and lymphocyte subgroups were examined in these blood samples.

Anti-CD3 FITC, anti-CD4 ECD, anti-CD8 PC7, anti-CD19 PC5, anti-CD16+56 PE, and anti-CD45 KrO antibodies were used in the specification of the lymphocyte subgroups using a flow cytometer device in the cord blood and peripheral blood obtained on the seventh day (Beckman Coulter). A 100-μL blood sample was obtained to determine lymphocyte subgroups; 10 μL from each monoclonal antibody was added onto this sample. After a 15-minute incubation, 0.5 mL lysis solution (Opti-lyse, Beckman Coulter) was added. After a waiting for ten minutes, 0.5 mL washing solution (Isoflow, Beckman Coulter) was added and left for 10 minutes. This prepared sample was examined using a Navios (Beckman Coulter) device.

In all subjects included in the study, prenatal characteristics including maternal age, gestational week, administration of antenatal steroid, presence of maternal infection, presence of multiple pregnancy and sex; perinatal characteristics including delivery mode, birth weight and APGAR scores in the 1st and 5th minutes, and status of development of sepsis in the postnatal period were evaluated.

The evaluation of sepsis was performed according to the Turkish Neonatal Association "Neonatal Infections Treatment and Follow-up Guideline-2014" guideline. A diagnosis of clinical sepsis was made when the causative agent could not be demonstrated and sepsis could not be excluded with clinical and laboratory findings. A confirmed diagnosis of sepsis was made when the causative agent was detected with culture (5).

Statistical Analysis

The SPSS 20.0 package program was used in the statistical analysis of the data. Qualitative data are expressed as number and percentage, and numeric data are expressed as mean and standard deviation (minimum-maximum, when necessary). The Chi-square test was used in the comparison of the qualitative data between groups. The Mann-Whitney U test was used in the comparison of the numerical values that did not show normal distribution between the two groups. GLM-Repeated Measure Analyses were used in the comparison of the changes in time in the numeric measurements performed at different times in the same individuals; in the model, cord blood and seven days were considered time-repeated measurements, status of clamp was considered 'subject' and status of term was considered 'covariate'. The correlation between these continuous measurements was examined using Spearman's correlation coefficient because numeric measurements did not provide for a normal distribution assumption. In all tests, a p value of <0.05 was considered statistically significant.

Results

Thirty-seven of 74 babies included in the study were term and 37 were preterm. Nineteen of the 37 term babies were included in group 1 and 18 were included in group 2, and 19 of the 37 preterm babies were included in group 3 and 18 were included in group 4. The family of one baby in group 1 and one baby in group 3 did not wish to continue the study, and these were excluded from the final analysis because their data and follow-up were deficient.

Table 1. Characteristics belonging to the subjects

	Term Babies			Late Preterm Babies		
	Immediate clamping group n=19	Delayed clamping group n=18	p	Immediate clamping group n=19	Delayed clamping group n=18	p
Gestational week	39.1±0.8	38.7±1.1	0.18	35.6±0.7	35.0±0.7	0.08
	38-40	37-41		(34-36)	(34-36)	
Birth weight (g)	3572±536 2960-4750	3405±437 3625-4115	0.75	2635±482 1930-3760	2527±574 1600-3450	0.66
Normal vaginal delivery	8 (42.19)	7 (38.9)	1	0 (0)	1 (5.6)	1
Cesarean section	11 (57.9)	11 (61.1)		19 (100)	17 (94.4)	
Female	13 (68.4)	8 (44.4)	0.19	9 (47.4)	13 (72.2)	0.18
Male	6 (31.6)	10 (55.6)		10 (52.6)	5 (27.8)	
Apgar score in the 1st minute	8.5±0.8	8±1.3	0.28	7.9±0.4	7.4±1.4	0.25
	6-9	5-9		7-8	3-9	
Apgar score in the 5th minute	9.6±0.7	9.2±1	0.18	8.8±0.5	8.6±1.2	0.77
	8-10	7-10		8-9	5-10	

The values are expressed as mean±SD, minimum-maximum

Table 2. Distribution of the cord blood and seventh day values for the hematologic measurements and lymphocyte percentages by the main groups

		Time of clamping Mean±SD Min-Max			Gestational week Mean±SD Min-Max		
		Immediate clamping n:38	Delayed clamping n:36	p[b]	Term n:37	Preterm n:37	p[c]
Hemoglobin (g/dL)	Cord blood	17.9±2.3	16.8±3.2	0.001	17.6-±2.3	17.1±3.2	0.065
		13.8-21.3	7.5-23.5		12.2-23.0	7.5-23.5	
	7th day	17.3±2.4	17±3.2	0.937	16.7±3.1	17.6±2.4	0.270
		13.8-22.7[a]	7.8-21.7		7.8-21	13.8-22.7	
Hemato-crit (%)	Cord blood	52.2±6.3	49.9±8.9	0.122	52.3±6	50.2±9.2	0.615
		39.1-63.1	24.3-66.3		36.9-63.4[a]	24.3-66.3	
	7th day	49.6±6.7	49.8±8.1	0.892	49±7.9	50.4±7	0.461
		40.1-62.7[a]	31.2-63.5		31.2-63.5	38.8-62.9	
Leukocyte count ($10^3/\mu L$)	Cord blood	15.6±4.3	12±5.3	0.147	15.2±5.1	12.5±4.9	0.372
		7.9-25.8	1.6-27.4		7.7-27.4[a]	1.7-24.5	
	7th day	12.1±2.9	12.5±3.8	0.822	12.8±3.4	11.9±3.3	0.600
		7.4-18.2[a]	7.4-26.1		8.1-26.1	7.38-18.2	
CD3+T lympho-cyte	Cord blood	71±11.7	67.2±7.3	0.011	67.3±10	71±9.6	0.210
		47.3-95	52.2-84.4		47.3-84.4	52.2-95	
	7th day	79.6±6.7	75.2±5.7	0.007	75.1±5.6	79.9±6.7	0.003
		65.9-91.2[a]	60.6-87.0[a]		60.6-89.5[a]	66.7-91.2[a]	
CD4+T lympho-cyte	Cord blood	51.7±12.4	48±8.2	0.025	49.4±11.8	50.5±9.6	1.000
		21.8-74.3	30.9-65.9		21.8-69	30.9-74.3	
	7th day	60.2±7.1	56.3±6.2	0.015	57.3±6.8	59.2±7	0.341
		45-75[a]	37.5-65.5[a]		37.5-68[a]	45-75[a]	
CD8+T lympho-cyte	Cord blood	19.8±5.3	18.6±5.1	0.289	18.1±5.1	20.4±5.1	0.054
		9.1-32.6	9.9-31.4		9.1-31.4	9.9-32.6	
	7th day	20.2±5	19.1±4.1	0.608	18.3±4.8	21±4	0.023
		11.1-28.5	11.5-26.3		11.1-28.5	13.1-28.5	
CD19+B lymphocyte	Cord blood	15.8±5.2	19.9±6.8	0.006	18.7±4.4	16.8±7.8	0.055
		0.7-26.8	6.9-41.8		11.4-28.4	0.7-41.8	
	7th day	10.3±3.9	13.6±6.5	0.024	12.1±5.4	11.8±5.8	0.761
		2.1-19.4[a]	1.6-33.8[a]		3.9-33.8[a]	1.6-24.6[a]	
CD16/56+NK cell	Cord blood	11.4±9.1	10.8±6.3	0.475	12±8.7	10.2±6.8	0.499
		2.0-35.1	2.8-32.6		2-35.1	3.1-32.6	
	7th day	8.2±4.7	9.4±5.8	0.362	10.4±6	7.2±3.9	0.009
		1.6-22.6	0.6-27.5		1.6-27.5	0.6-18.9	

[a]p<0.05 comparison of cord blood and seventh day (Wilcoxon test)
[b]Comparison of the immediate-clamping group and delayed clamping groups (Mann-Whitney U test)
[c]Comparison of term and preterm groups (Mann-Whitney U test)

Table 3. Distribution of the cord blood and seventh day values for the hematological measurements and lymphocyte percentages by subgroups

		Subgroups Mean±SD Min-Max					
		Group 1 Immediat+Term n:19	Group 2 Delayed+Term n:18	Group 3 Immediate+Preterm n:19	Group 4 Delayed+Term n:18	p^e	p^f p^g
Hemoglobin (g/dL)	Cord blood	17.8±2.0	17.3±2.5	17.8±2.5	16.2±3.6		0.077
		14.8-21.3	12.2-21.0	13.8-21.0	7.5-23.5	0.002	0.174
	7th day	17.3±2.3	16.1±3.7	17.2±2.5	17.8±2.3		
		14.5-20.7	7.8-21.1	13.8-22.7	13.8-21.7	0.746	
Hematocrit (%)	Cord blood	52.9±5.3	51.5±6.7	52.1±7.3	42.9±10.5		0.047
		44.3-62.3	36.9-63.4	39.1-63.1	24.3-66.3	0.362	0.410
	7th day	49.8±6.6	48.2±8.9	49.3±6.9	51.3±7		
		40.7-61.1	31.2-63.5	40.1-62.7[a]	38.8-62.9	0.538	
Leukocyte count ($10^3/\mu L$)	Cord blood	16.4±4.5	13.9±5.3	14.8±3.9	10±4.5		0.041
		11.2-25.8	7.7-27.3[d]	7.9-24.5	1.6-17.5[c]	0.370	0.718
	7th day	12.4±2.2	13.1±4.3	11.7±3.5	11.9±3.1		
		8.4-17.9[a]	8.1-26.1	7.41-18.1[a]	7.38-18.1	0.681	
CD3+T lenfosit	Cord blood	66.2±12.3	68.5±7	75.7±9.1	65.9±7.4		0.079
		47.3-78.8	55.2-84.4	56.2-95[b]	52.2-81.5[c]	0.004	0.049
	7th day	76.7±5.9	73.5±4.9	82.4±6.20	76.9±6		
		65.9-89.5[a]	60.6-82.0[a]	66.9-91.0[ab]	66.7-87.0[ac]	0.001	
CD4+T lymphocyte	Cord blood	49.3±13.9	49.3±9.3	54±10.4	46.6±6.8		0.089
		21.8-69	32.1-65.9	33.4-74.3	30.9-58.6	0.073	0.585
	7th day	58.1±6.2	56.4±7.3	62.2±7.4	56.1±4.9		
		6.2-68.0[a]	37.5-65.5[a]	45.0-75.0[a]	46.4-64.9[ac]	0.026	
CD8+T lymphocyte	Cord blood	17.7±4.6	18.4±5.5	21.8±5.1	18.7±4.6		0.898
		9.1-25.0	10.5-31.4	13.5-32.6	9.9-26.7	0.110	0.119
	7th day	18.9±5.2	17.6±4.2	21.3±4.5	20.5±3.4		
		11.1-28.5	11.5-23.7	13.9-28.5	13.1-26.3	0.141	
CD19+B lymphocyte	Cord blood	18.1±3.9	19.2±4.8	13.3±5.2	20.4±8.3		0.002
		12.2-24.0	11.4-28.4	0.7-26.8[b]	6.9-41.8[c]	0.001	0.282
	7th day	10.8±3.2	13.3±6.8	9.7±4.5	13.9±6.4		
		5.8-15.9[a]	13.9-33.8[a]	2.1-19.4[a]	1.6-24.6[ac]	0.121	
CD16/56+NK cell	Cord blood	13.7±10.9	10±5.1	9±6.1	11.5±7.4		0.925
		2.0-35.1	2.8-25.0	3.1-22.1	3.3-22.1	0.582	0.128
	7th day	9.8±5.7	10.8±6.2	6.4±2.4	7.8±4.9		
		1.6-22.6	2.4-27.5	3.3-11.5	0.6-18.9	0.056	

[a]p<0.05 comparisons of the cord blood and 7th day (Wilcoxon test)
[b]p<0.017 between group 1 and group 2 (Mann-Whitney U test)
[c]p<0.017 between group 3 and group 4 (Mann-Whitney U test)
[d]p<0.017 between group 1 and group 2 (Mann-Whitney U test)
[e]Comparison of 4 groups (Kruskal-Wallis test)
[f]repeated comparison by time
[g]comparison by time and clamping interaction (In GLM-Repeated measure analyses; cord blood and seventh day were considered repeated measurement, status of clamping was considered "subject" and status of term was considered "covariate")

The gestational weeks, birth weights, delivery modes, sexes, and Apgar scores of the babies included in the study are shown in Table 1; no statistically significant difference was found between the groups.

The distribution of the cord blood and seventh day values of the hematologic measurements and lymphocyte percentages by the main groups (clamping time and gestational week) is given in Table 2. According to these results, the increase in CD3+T and CD8+T lymphocyte percentages and the decrease in CD16/56+NK lymphocyte percentages in the blood obtained on the seventh day between the term and preterm babies were found to be statistically significant. A significant reduction was found in CD3+T and CD4+T lymphocyte percentages both in the cord blood and on the seventh day between the immediate and delayed clamping groups, whereas a significant increase was found in the CD19+B lymphocyte percentages. No significant difference was found between the immediate and delayed clamping groups in terms of CD8+T and CD16/56+NK lymphocyte percentages.

In dual comparisons, the leukocyte counts and CD3+T, CD4+T and CD19+B lymphocyte percentages in the cord blood and on the seventh day showed difference between the immediate and delayed cord- clamping groups. Accordingly, the leucocyte counts and CD3+T lymphocyte percentages in the preterm babies whose cords were clamped late were found to be lower compared with those whose cords were clamped immediately, and their CD3+T lymphocyte percentages were found to be higher. The CD3+T and CD4+T percentages in the blood obtained on the seventh day in preterm babies whose cords were clamped late were found to be lower compared with those whose cords were clamped immediately, and the CD19+T lymphocyte percentages were found to be higher. According to the results evaluated when a multiple variance analysis model was applied in repeated measurements (the model in which the cord blood and seventh day were considered time-repeated measurements, the status of clamping was considered the main factor-'subject' and the gestational week was considered the 'covariate' factor), the hematocrit level, leukocyte count, and CD3+T and CD19+B lymphocyte percentages showed differences by time. A correlation of the CD3+T levels with gestational week and clamping time was found at the same time. In preterm babies whose cords were clamped late, the hematocrit levels, leukocyte counts, and CD3+T and CD4+T percentages were found to be significantly decreased, whereas the CD19+T lymphocyte percentages were found to be significantly increased (Table 3).

Only one case of sepsis developed in the delayed-clamping term baby group, whereas sepsis developed in seven babies each in the immediate and delayed-clamping preterm baby groups. No statistically significant difference was found between the groups in terms of sepsis rates ($p>0.05$). No growth occurred in the blood cultures in any of the babies with sepsis. All babies with sepsis were followed up with a diagnosis of clinical sepsis. Among the lymphocyte subgroups in the preterm babies who developed sepsis, only the cord blood CD8+T lymphocyte percentage was found to be higher compared with the group who did not develop sepsis ($p=0.04$). None of the babies included in the study died.

Discussion

In this study, we aimed to investigate the effect of delayed cord clamping, which is one of the methods for increasing placental transfusion, on lymphocyte subgroups in term and preterm babies. Although there are numerous studies related to delayed cord clamping in the literature, we found no data related to the effect of delayed cord clamping on lymphocyte subgroups (6-12). There are also very few data related to lymphocyte subgroups in preterm babies (13, 14).

Delayed cord clamping saves time for the transfer of blood from the placenta to the baby (15). The amount of blood transferred varies according to the level at which the baby is kept and the time delay for cord compression (16, 17). The time delay is in a wide spectrum from 30 seconds to the time when the cord pulse stops. The World Health Organization recommends a delay of 1-3 minutes before clamping the cord (18). In this study, the cord was clamped after waiting for one minute in the groups in which delayed cord clamping was applied.

In many studies in which the effects of delayed cord clamping on hemoglobin and hematocrit levels were compared, the levels were found to be higher in the early period (6-10, 12). In the study conducted by Olveira et al. (11) with term babies, the hemoglobin and hematocrit levels in the cord blood of babies in whom cord clamping was delayed for 60 seconds were found to be similar to babies whose cords were clamped immediately. In our study, no difference was found between the hemoglobin values measured in the cord blood and on the seventh day in the term and preterm babies whose cords were clamped immediately and late, whereas a significant reduction was found in the hematocrit levels in the preterm babies in whom delayed cord clamp-

ing was applied. The reason for the incompatibility in the study results may be related to differences in the times of cord clamping and the times of sampling.

In the only study related to the effect of placental transfusion on leukocytes in the literature, Kılışdağ et al. (19) reported that the leukocyte count decreased in preterm babies in whom cord milking was applied. In this study, the neutrophil counts measured on the first, third, and seventh days in the group in whom cord milking was applied before cord clamping were found to be lower compared with the immediate cord-clamping group. In our study, the cord blood leukocyte counts were also found to be lower in the preterm baby group in whom delayed cord clamping was applied. However, this reduction was not found in the blood sample obtained on the seventh day. In our study, placental transfusion was provided by delayed cord clamping rather than using cord milking.

It is assumed that the immune system is underdeveloped in babies born before the 30th gestational week, but little is known about the immunologic steps and maturation of lymphocyte subgroups (13). There are studies related to lymphocyte percentages, ratios, and counts in babies diagnosed as having sepsis, as well as studies in which the relationship between cord clamping time and late-onset sepsis has been evaluated (10, 14, 20-27). In a portion of the studies conducted with preterm babies, it was found that delayed cord clamping caused a reduction in the frequency of late-onset sepsis, yet other studies found no reduction in the frequency of late-onset sepsis in babies in whom delayed cord clamping was applied (10, 28-30). Although we found a difference between the lymphocyte subgroup values in the cord blood and blood samples obtained on the seventh day in term and preterm babies in whom immediate and delayed cord clamping was applied, no significant difference was found between the groups in terms of the frequency of sepsis development.

The role of regulatory B cells in neonatal sepsis is not known. In a study conducted by Sofatzis et al. (23), it was found that CD3+T lymphocyte and CD4+T lymphocyte counts in the first week of life were significantly increased in babies with sepsis compared with healthy babies. In the study by Aygün et al. (26), the CD4+T lymphocyte ratio and absolute count were found to be lower in newborns who had culture-confirmed sepsis compared with those without culture-confirmed sepsis. In a study by Bussel et al. (24) with preterm babies, the CD3+T lymphocyte and CD8+T lymphocyte percentages in the first eight weeks of life were found to be lower compared with term babies, whereas the CD19+ B lymphocyte percentages were higher. In a study conducted by Bochennek et al. (14), a reduction in NK cell functionality was found in preterm babies who had late-onset sepsis. In our study, the cord blood CD3+T percentages were lower in preterm babies in whom delayed cord clamping was applied compared with those whose cords were clamped immediately, whereas the CD19+T lymphocyte percentages were higher. The CD3+T and CD4+T lymphocyte percentages in the blood samples obtained on the seventh day in the preterm babies in whom delayed cord clamping was applied were found to be lower compared with those whose cords were clamped immediately, whereas the CD19+T lymphocyte percentages were found to be higher.

In conclusion, it was found that delayed cord clamping in preterm babies caused a reduction in the hematocrit level, leukocyte count, and CD3+T and CD4+T lymphocyte percentages, and an increase in the CD19+B lymphocyte percentage. Delayed cord clamping was found to have no effect on the frequency of sepsis in term and preterm babies. Studies with larger series should be conducted to evaluate the effect of these data on the development of infections.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Çukurova University School of Medicine (7.10.2016/16).

Informed Consent: Written informed consent was obtained from patients' parents.

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References

1. Diab Y, Luchtman-Jones L. Hematologic and oncologic problems in the fetus and neonate. In: Martin RS, Fanaroff AA, Walsh MC, (eds). Fanaroff and Martin's neonatal-

perinatal medicine. 10th edition. Philadelphia: Elsevier, 2015.p.1294-1343.
2. Linderkamp O, Nelle M, Kraus M, Zilow EP. The effects of early and late cord-clamping on blood viscosity and other hemorheological parameters in full-term neonates. Acta Paediatrica 1992; 81: 745-50. [CrossRef]
3. Wyckoff MH, Aziz K, Escobedo MB, et al. Part 13: Neonatal Resuscitation: 2015 American heart association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2015; 132: 543-60. [CrossRef]
4. Benjamin JT, Mezu-Ndubuısı O, Maheshwarı A. The immun system. In: Martin RS, Fanaroff AA, Walsh MC, (eds). Fanaroff and Martin's neonatal-perinatal medicine. 10th edition. Philadelphia: Elsevier, 2015.p. 696-720.
5. Satar M, Arısoy A. Türk Neonatoloji Derneği Yenidoğan Enfeksiyonları Tedavi ve İzlem Rehberi 2014.p.6-11 Erişim:http://www.neonatology.org.tr/wp-content/uploads/2016/12/enfeksiyon.pdf.
6. McDonald S, Middleton P, Dowswell T, Morris S. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. Cochrane Database Syst Rev 2013; 7: 74. [CrossRef]
7. Emamed MO, Van Rheenen P, Brabin BJ. The early effects of delayed cord clamping in term infants born to Libyan mothers. Trop Doct 2004; 34: 218-22. [CrossRef]
8. Shirvani F, Radfar M, Hashemieh M, Soltanzadeh MH, Khaledi H, Mogadam MA. Effect of timing of umbilical cord clamp on newborns' iron status and its relation to delivery type. Arch Iran Med 2010; 13: 420-5.
9. Ceriani Cernadas JM, Carroli G, Pellegrini L, et al. The effect of timing of cord clamping on neonatal venous hematocrit values and clinical outcome at term: a randomized, controlled trial. Pediatrics 2006; 117: 779-86. [CrossRef]
10. Dicky O, Ehlinger V, Guyard-Boileau B, Assouline C, Arnaud C, Casper C. Delayed umbilical cord clamping in preterm infants born before 37 weeks of gestation: A prospective observational study. Arch Pediatr 2017; 24: 118-25. [CrossRef]
11. Oliveira FDE CI, Yard KF, Martins MC, et al. Timing of clamping and factors associated with iron stores in full-term newborns. Rev Saude Publica 2014; 48: 10-8. [CrossRef]
12. Raju TN. Timing of umbilical cordclamping after birth for optimizing placental transfusion. Curr Opin Pediatr 2013; 25: 180-7. [CrossRef]
13. Huenecke S, Fryns E, Wittekindt B, et al. Percentiles of lymphocyte subsets in preterm ınfants according to gestational age compared to children and adolescents. Scand J Immunol 2016; 84: 291-8. [CrossRef]
14. Bochennek K, Fryns E, Wittekindt B, et al. Immune cell subsets at birth may help to predict risk of late-onset sepsis and necrotizing enterocolitis in preterm infants. Early Hum Dev 2016; 93: 9-16. [CrossRef]
15. Airey RJ, Farrar D, Duley L. Alternative positions for the baby at birth before clamping the umbilical cord. Cochrane Database Syst Rev 2010; 10: 55.
16. Kc A, Målqvist M, Rana N, Ranneberg LJ, Andersson O. Effect of timing of umbilical cord clamping on anaemia at 8 and 12 months and later neurodevelopment in late pre-term and term infants. BMC Pediatr 2016; 16: 35-6. [CrossRef]
17. Duley L, Batey N. Optimal timing of umbilical cord clamping for term and preterm babies. Early Hum Dev 2013; 89: 905-8. [CrossRef]
18. WHO Guidelines Approved by the Guidelines Review Committee. Guidelines on basic Newborn Resuscitation. Geneva, World Health Organization, 2012.
19. Kilicdag H, Gulcan H, Hanta D, et al. Is umbilical cord milking always an advantage? J Matern Fetal Neonatal Med 2016; 29: 615-8. [CrossRef]
20. Melville JM, Moss TJ. The immune consequences of preterm birth. Front Neurosci 2013; 7: 79-80. [CrossRef]
21. Strunk T, Temming P, Gembruch U, Reiss I, Bucsky P, Schultz C. Differential maturation of the innate immune response in human fetuses. Pediatr Res 2004; 56: 219-26. [CrossRef]
22. Pan X, Ji Z, Xue J. Percentage of peripheral CD19+CD Regulatory B cells in neonatal sepsis patients and ıts functional implication. Med Sci Monit 2016; 22: 2374-8. [CrossRef]
23. Sofatzis J, Driva I, Mexi Bourna P, Liossis G, Costalos C. Lymphocyte subpopulations in neonatal sepsis. Prenatal and Neonatal Medicine 2012; 3: 353-7.
24. Bussel JB, Cunningham-Rundles S, La Gama EF, Shellabarger M. Analysis of lymphocyte proliferative response subpopulations in very low birth weight infants and during the first 8 weeks of life. Pediatric Res 1988; 23: 457-62. [CrossRef]
25. Juretić E, Juretić A, Uzarević B, Petrovecki M. Alterations in lymphocyte phenotype of infected preterm newborns. Biol Neonate 2001; 80: 223–7. [CrossRef]
26. Aygün AD, Çıtak Kurt AN, Gödekmerdan A, et al. Neonates with culture proven sepsis have lower amounts and percentage of CD45RA+ T cells. Inflammation 2008; 31: 222-6. [CrossRef]
27. Hotoura E, Giapros V, Kostoula A, Spyrou P, Andronikou S. Pre-inflammatory mediators and lymphocyte subpopulations in preterm neonates with sepsis. Inflammation 2012; 35: 1094-101. [CrossRef]
28. Mercer JS, Vohr BR, McGrath MM, Padbury JF, Wallach M, Oh W. Delayed cord clamping in very preterm infants reduces the incidence of intraventricular hemorrhage and late-onset sepsis: a randomized, controlled trial. Pediatrics 2006; 117: 1235-42. [CrossRef]
29. Menget A, Mougey C, Thiriez G, Riethmuller D. Advantage of delayed umbilical cord clamping in newborn infant. Arch Pediatr 2013; 20: 1022-7. [CrossRef]
30. Dong XY, Sun XF, Li MM, Yu ZB, Han SP. Influence of delayed cordclamping on preterm infants with a gestational age of <32 weeks. Zhongguo Dang Dai Er Ke Za Zhi 2016; 18: 635-8.