Nucleated red blood cell count and Doppler ultrasound in low- and high-risk pregnancies

R. Axt-Fliedner[1], M. Wrobel[1], H. J. Hendrik[1], A. K. Ertan[1], D. Mink[1], J. König[2], W. Schmidt[1]

[1]Department of Obstetrics and Gynecology and [2]Department of Biostatistics and Epidemiology, University of the Saarland, Homburg/Saar (Germany)

Summary

Objective: The aim of this study was to evaluate the influence of circulatory deterioration in small-for-gestational-age fetuses (SGA) on the nucleated red blood cell count (NRBC).

Patients and methods: During a 12-month period 194 patients with a complete NRBC count post-partum were enrolled in the study. Using pulsed wave colour Doppler ultrasound we recorded blood flow velocity waveforms from both uterine arteries and from the umbilical artery and/or from the fetal aorta. Patients were assigned to seven groups according to the results of the Doppler examination. Mean outcome measures were birthweight, gestational age at delivery, NRBC count, incidence of preeclampsia or SGA and need of neonatal intensive care.

Results: Significantly higher nucleated red blood cell counts than in all other groups were found in cases with abnormal Doppler findings in both uterine arteries and the umbilical artery and/or fetal aorta ($p<0.001$). These newborns had significantly lower birth weights ($p<0.01$, $p<0.001$), lower gestational age ($p<0.001$), an increased likelihood of caesarean section for clinical signs of fetal distress ($p<0.001$) and had to be transfered more frequently to the neonatal intensive care unit ($p<0.01$, $p<0.001$).

Conclusion: Patients with abnormal Doppler velocimetry waveforms of the uterine arteries in the presence of an abnormal umbilical artery or fetal aorta Doppler findings have a high risk of prematurity, preeclampsia or delivering a small-for-gestational-age newborn. Fetal response to uteroplacental insufficiency may lead to elevated nucleated red blood cells in the fetal blood. This fact might help to discriminate the small-for-gestational-age fetus who is growth-retarded and suffers from chronic placental insufficiency from the small but healthy fetus.

Key words: Nucleated red blood cell count; Doppler ultrasound; Intrauterine hypoxemia; Intrauterine growth retardation.

Introduction

There is increasing evidence that in a majority of cases hypoxic-ischemic injury might not be related to labor, but may have occurred before the onset of labor [1-4]. Only in 10%-20% of cerebral palsy cases has an association with clinical or biochemical markers of fetal asphyxia been documented [3].

Nucleated red blood cells have been suggested as a marker for chronic intrauterine fetal hypoxemia [5-7]. The number of nucleated red blood cells in the circulating blood of healthy term newborns is variable. Generally up to 8 NRBCs per 100 white blood cells (WBCs) are found in the circulating blood of healthy term newborns [7].

Conditions proposed to be related to more than 8 nucleated red blood cells per 100 white blood cells include Rh sensitization, maternal diabetes mellitus, intrauterine growth restriction and prematurity [8-11].

A link between high counts of nucleated red blood cells and abnormal Doppler ultrasound patterns in the umbilicall artery has previously been noted and recently suggested by Baschat and co-workers [10, 11].

With this study we sought to determine whether abnormal Doppler ultrasound findings of the uterine and umbilical arteries as well as of the fetal aorta are related to an increase of nucleated red blood cell counts in the neonatal blood.

Patients and Methods

This prospective study was undertaken in the Department of Obstetrics and Prenatal Ultrasound in the University Hospital, Homburg/Saar, Germany. A total of 194 patients with a complete NRBC count and with a Doppler ultrasound assessment of both uterine arteries, the umbilical artery or fetal aorta within 5 days before delivery were enrolled in the study. The patients were recruited from the population of pregnant women referred to our ultrasound department for evaluation of fetal well-being in case of fetal growth retardation, oligohydramnios, maternal hypertension, preeclampsia, or pathologic fetal heart rate patterns. Exclusion criteria were multiple pregnancies, fetuses with chromosomal and structural anomalies, maternal cardiovascular pathology other than hypertension, pre-existing diabetes mellitus, chorioamnionitis, and maternal renal disease. The Doppler examinations were performed with an Acuson 128 XP/10 (Mountain View, California, USA) or with a Siemens-Sonoline-Elegra (Erlangen, Germany) ultrasound equipment. A 3.5 - MHz curved-array transducer was used to obtain the Doppler data. Flow velocity waveforms from both uterine arteries, the umbilical artery and the fetal aorta were measured. A high-pass filter of 100 Hz was used. For uterine artery Doppler the transducer was placed in the right or left lower part of the abdomen.

Revised manuscript accepted for publication March 24, 2000

Color Doppler imaging was used to identify the uterine artery at the crossing point with the external iliac artery. Pulsed Doppler was then used to obtain flow velocity waveforms. The examination was repeated on the opposite side. Doppler waveforms were obtained from a free-floating central part of the umbilial artery in the absence of body movement, fetal breathing or cardiac arrhythmia with the sample volume covering the whole vessel. The fetal aorta was localized in its abdominal part at the origin of the renal arteries by color Doppler imaging. For every vessel five consecutive waveforms of similar quality at an insonation angle of less than 55° were accepted for analysis. The ratio of mean peak systolic over end diastolic velocity (S/D ratio) was determined. Abnormal Doppler velocity waveforms were those above the mean ±2 SD for gestational age of our local reference ranges. In all patients, the Doppler examinations were performed in the late second or third trimester. Patients were divided with respect to circulatory deterioration into seven subgroups (Table 1). Because of the small study group we did not analyze unilateral or bilateral notching.

The estimated time of delivery was assessed by menstrual data and a vaginal ultrasound measurement of the crown-rump-length in the first 12 weeks of gestation. Small-for-gestational-age was defined as birth weight for gestational age below the 10th percentile according to local growth charts [12]. According to Davey and McGillivray, previously normotensive women with diastolic blood pressure ≥ 140/90 mmHg measured on two occasions six hours apart after 20 weeks of gestation and with proteinuria of at least 1 + on protein stick testing or a proteinuria > 0.3g/24h were considered preeclamptic [13]. Diabetes mellitus in pregnancy was defined according to White [14]. Histologic chorioamnionitis was diagnosed by the presence of acute inflammatory cell infiltration of both layers of the membranes. Prematurity was defined as delivery before 37 completed weeks of gestation. Outcome measures included nucleated red blood cell counts, birth weight, gestational age at delivery, mode of delivery (vaginal delivery, elective caesarean section and secondary caesarean section because of clinical signs of fetal distress; e.g. abnormal fetal heart rate patterns, meconium stained amniotic fluid), arterial pH, Apgar score, and admission to the neonatal intensive care unit.

Assessment of NRBC counts have previously been described in detail [15].

Distribution of nucleated red blood cells was non-normal. Results are presented as median and range. Statistical analysis included the Mann-Whitney *U*-Test, χ^2 analysis, regression analysis and analysis of variance. A p-value < 0.05 was considered to indicate a significant difference. All calculations were performed with the SPSS statistical software (SPSS Inc., Chicago, IL, USA).

Results

A total of 194 patients were included in the study. In 51 patients (26%) we found SGA fetuses and 21 patients (11%) developed preeclampsia. In 29 of the patients (15%) the pregnancy was complicated by both preeclampsia and SGA. Ninety-three patients (48%) had an uncomplicated course of pregnancy (Table 2). Fifty-four patients (28%, group 1) had normal Doppler velocity waveforms. Eighteen (9%, group 2) and respectively, five (3%, group 3) patients had unilateral or bilateral pathologic Doppler findings in the uterine arteries with normal results in the fetal vessels. Thirty-nine (20%, group 4) patients had abnormal Doppler results in only the umbilical artery or fetal aorta with normal Doppler results in the uterine arteries. Eighteen (9%, group 5) patients had abnormal Doppler results in only the umbilical artery or fetal aorta with an unilaterally pathological Doppler result in the uterine arteries. Group 6 includes 40 patients (21%) with pathologic Doppler findings in the umbilical artery or fetal aorta and in both uterine arteries. Group 7 includes 20 patients (10%) with pathologic Doppler flow velocity in all vessels examined (Table 1). Table 2 outlines the delivery outcome of the study groups.

The highest prematurity rates were seen in groups 6 and 7 ($p<0.01$) compared to all other groups. Patients with bilateral pathologic Doppler results of the uterine arteries and fetal arteries were more likely to be delivered by caesarean section (80% vs. 44%, group 7 vs. group 1, $p<0.05$). In 70% of the patients in group 7 and in 67.5% of the patients in group 6 caesarean sections had to be performed because of clinical signs of fetal distress ($p<0.001$ vs. all other groups). The number of newborns who had to be transfered to the neonatal intensive care

Table 1. — *Doppler characteristics of the study groups.*

Study group	Umbilical artery or fetal aorta	Uterine artery	Number of patients
1	normal	normal	54
2	normal	unilateral pathologic	18
3	normal	bilateral pathologic	5
4	pathologic	normal	39
5	pathologic	unilateral pathologic	18
6	pathologic	bilateral pathologic	40
7	**both** pathologic	bilateral pathologic	20

Table 2. — *Delivery outcomes*

	Group 1 n=54	Group 2 n=18	Group 3 n=5	Group 4 n=39	Group 5 n=18	Group 6 n=40	Group 7 n=20
SGA	12 (22.2%)	2 (11.1%)	1 (20.0%)	11 (28.2%)	6 (33.3%)	13 (32.5%)	6 (30.0%)
Preeclampsia	3 (5.5%)	2 (11.1%)	1 (20.0%)	2 (5.1%)	3 (16.6%)	5 (12.5%)	5 (25.0%)
Both	0	0	0	0	2 (11.1%)	18 (45.0%)	9 (45.0%)
Preterm delivery	8 (14.8%)	2 (11.1%)	0 (0%)	5 (12.8%)	3 (16.6%)	19 (47.5%)**	19 (95%)**
Vaginal deliv.	30 (55.5%)	11 (61.1%)	4 (80.0%)	29 (74.3%)	10 (55.5%)	12 (30.0%)*	4 (20.0%)*
Caes. section	24 (44.5%)	7 (28.9%)	1 (20%)	10 (25.7%)	8 (44.5%)	28 (70.0%)**	16 (80.0%)*
Caes. section for fetal distress	2 (3.7%)	2 (8.3%)	1 (20.0%)	3 (7.6%)	5 (27.7%)	27 (67.5%)***	14 (70.0%)***
NICU admission	4 (7.4%)	3 (16.6%)	1 (20.0%)	8 (20.5%)	9 (50.0%)	34 (85.0%)**	18 (90.0%)***

$p<0.05$; ** $p<0.01$; *** $p<0.001$ vs group 1.

Abbreviations: SGA=small-for-gestational-age, Deliv.= delivery, Caes. section=caesarean section, NICU=neonatal intensive care unit.

Table 3. — *Fetal outcome of groups 1-7.*

	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	median	range	median	range	median	range	median	range	median	range	median	range	median	range
NRBC/100 WBC	5.1	0-20	5.5	1-13	6.2	1-13	5.4	0-37	8.9	1-31	27.2***	3-201	38.4***	7-201
Gest. age (weeks)	38.3	31.1-42.1	39.2	34.6-41.6	39.3	36.0-40.2	39.0	30.3-42.0	38.4	33.3-41.5	34.3***	26.3-41.5	32.6***	26.1-41.6
Birth weight (gm)	3423	1620-4300	3395	2480-4620	3109	2590-4300	3011	1360-4230	2935	1890-4600	2214**	550-4220	1848***	550-4260
apgar 1 min	8.4	4-10	7.6	4-10	8.6	8-10	8.7	6-10	7.9	4-10	7.3**	1-10	6.6**	1-10
apgar 5 min	9.3	6-10	8.5	6-10	9.2	9-10	9.6	7-10	9.1	4-10	8.5**	1-10	8.0**	1-10
apgar 10 min	9.8	8-10	9.0	7-10	10.0	9-10	9.5	8-10	9.4	6-10	8.6*	2-10	8.2**	3-10
pHart	7.29	7.10-7.40	7.29	7.13-7.40	7.27	7.18-7.32	7.28	7.14-7.45	7.29	7.00-7.41	7.26	6.88-7.36	7.25	6.88-7.35

*=p<0.05; **=p<0.01; ***=p<0.001 vs group 1.
Abbreviations: NRBC=nucleated red blood cell, WBC=white blood cell, Gest. age=gestational age, Gm=gram, pHart=arterial pH

unit was highest in patients with bilaterally abnormal Doppler velocity waveforms in the uterine arteries and pathologic Doppler results in the fetal vessels (group 6, p<0.01 and group 7, p<0.001) compared to the other groups.

Fetal outcome parameters are presented in Table 3. NRBC counts were comparable in groups 1 to 5, with somewhat higher counts in group 5. There were no statistically significant differences from group 1 to group 5 with respect to the median birth weight, the gestational age, the Apgar scores and the arterial pH. However, significantly higher numbers of NRBC counts were obtained in the two groups with bilateral pathologic Doppler results in the uterine arteries and pathologic Doppler velocity waveforms of both the umbilical artery and/or fetal aorta (group 6 and group 7, p<0.001 vs. all other groups). In those two groups, a marked decrease of the median birth weight and median gestational age was observed (p<0.01, p<0.001 vs. all other groups). Apgar scores at 1 min, 5 min and 10 min were significantly lower in groups 6 and group 7 compared to the other groups (p<0.05, p<0.01). The results of cord blood gases did not show any significant differences between the groups, however there was a tendency towards lower arterial pH values in group 7.

A stepwise regression with NRBC count as a dependent variable and birth weight, gestational age and Doppler results of the uterine arteries, the umbilical artery and the fetal aorta revealed that birth weight (p<0.001), gestational age (p<0.001), uterine Doppler results (p>0.001) and Doppler results of the umbilical artery or fetal aorta (p<0.001) were significant independent determinants of the NRBC count. Furthermore, the number of nucleated red blood cells did correlate with the Apgar scores at 1 min, 5 min, and 10 min (p<0.01). Finally, we did not observe any correlation between arterial pH and NRBC count.

Discussion

It has become evident that long-term morbidity of the growth-retarded fetus is not mainly due to intrapartum hypoxic insults, but seems to be related to antenatal intrauterine hypoxemia [1-4]. Thus, the search for markers of antenatal chronic fetal hypoxemia is a relevant and important task.

Chronic hypoxemia leads to increased extramedullary hematopoiesis (e.g. in the liver) with release of nucleated erythrocytes into the fetal circulation which is supposed to be mediated by an increase of the erythropoietin concentration in the fetal plasma [16]. Nucleated red blood cells have been suggested a markes for chronic intrauterine fetal hypoxemia [5-7]. The number of NRBCs circulating in the blood of healthy term newborns is variable. Generally up to eight nucleated red blood cells per 100 white blood cells are found in healthy term newborns [7]. We have previously reported on comparable nucleated red blood cell counts in healthy term infants and our findings in the present study (groups 1 to group 5) are also in good accordance with those results [15].

Intrapartum fetal hypoxia has also been suggested to stimulate erythropoiesis [17]. Whether NRBC counts are a useful tool in determining the time of an intrapartum hypoxic insult remains a matter of debate [18, 19]. In an animal model, release of reticulocytes after hypoxia was not seen until the second or third day after the hypoxic stimulus [20]. Given this fact, elevated NRBC counts would be seen in the cord blood of the newborn only if the hypoxic insult occurred before the onset of labor. Therefore elevated NRBC counts after acute intrapartum hypoxia are not expected in the postpartum cord blood, but might result in elevated counts during the neonatal period. This is in accordance with our previous work which did not show any relation between mode or duration of delivery and nucleated red blood cells in uncomplicated term and post-term pregnancies [15].

However, others have observed elevated nucleated red blood cell counts after intrapartum hypoxia or within two hours after a brain-damaging ischemia or hypoxemia in infants who had developed cerebral palsy [19, 21]. One possible explanation for these discrepant findings might be that these fetuses had been exposed to a hypoxic stimulus before labor.

A previous study with a small series of patients performed by Bernstein and co-workers compared nucleated red blood cell counts obtained post-partum in small-for-gestational-age fetuses with end-diastolic velocity present (EDV) and those with absent/reversed end-diastolic velocity (ARED) [10]. The authors found a significant increase in NRBCs in those fetuses with ARED-flow. More recently Baschat *et al.* found increased NRBC counts in IUGR fetuses to be associated with abnormal arterial and venous Doppler flow results [11]. The

authors found higher numbers of NRBCs in those fetuses with centralization compared to a group with only high resistance indices in the umbilical artery and they found the highest nucleated red blood cell counts in fetuses presenting abnormal flow velocity waveforms in the inferior vena cava or in the ductus venosus.

Our results showing a relation between the number of NRBCs in neonatal blood and the degree of circulatory disorders are in accordance with those previous studies. However, we have not yet examined cases of absent or reverse diastolic velocity or cases with abnormal venous blood flow. On the other hand, our results show a relation between Doppler findings in the uterine arteries and the NRBC count.

Of note are the differences between the study by Baschat *et al.* and our results concerning fetal oxygenation and Apgar scores. They reported on significant lower arterial pH values in the group with abnormal venous flow velocity waveforms compared to the group of fetuses with centralization or elevated pulsatility indexes only [10]. Centralization of fetal blood flow or abnormal venous blood flow is known to be related to lower arterial pH values in the neonatal blood [22]. A possible explanation might be that in our study we did not include either fetuses with centralization or with abnormal venous blood flow. However it is surprising that Baschat and co-workers did not find differences in the Apgar scores among the groups as we did. They of reported of only two out of 36 fetuses with abnormal venous blood flow with Apgar scores < 7 after 5 min; one would expect lower Apgar scores in a compromised fetus with a severe circulatory disorder. We observed lower gestational age at delivery and lower weight with poorer fetal outcome in the two groups with pathologic Doppler results of the uterine arteries and umbilical artery and/or fetal aorta. Those newborns had to be transfered to the neonatal intensive care unit more often than infants in groups 1 to group 5. Also the preterm delivery rate was higher in the last two groups (groups 6 and 7). These findings are in good accordance with other Doppler studies pointing out an important role of Doppler ultrasound in assessing the fetus at risk [22, 23].

It would be interesting to know whether there are associations between nucleated red blood cell counts and histopathologic findings of the placenta in cases of abnormal uterine and fetal Doppler values. This study is currently in progress.

In conclusion, we have shown an association between NRBC counts in the cord blood of newborns with pathologic Doppler patterns of the uterine arteries and the umbilical artery and/or the fetal aorta.

In our opinion the results presented underline the importance of Doppler examination of both uterine arteries together with the fetal vessels in antenatal monitoring of the fetus. On the basis of these results we speculate that the nucleated red blood cell count of the newborn in combination with the Doppler results might be helpful in differentiating between the healthy small-for-gestational-age newborn and the small-for-gestational-age newborn who suffered from placental insufficiency.

References

[1] Jaisle F.: "Zur Ätiologie der Zerebralparese". *Z. Geburtsh. Neonatol.*, 1996, *200*, 169.
[2] Blair E., Stanley F. J.: "When can cerebral palsy be prevented? The generation of causal hypotheses by multivariate analysis of a case-controlled study". *Pediatr. Perinat. Epidemiol.*, 1993, *7*, 272.
[3] Palmer L., Blair E., Patterson B., Burton P.: "Antenatal antecedents of moderate and severe cerebral palsy". *Pediatr. Epidemiol.*, 1995, *95*, 171.
[4] Dürig P., Schneider H.: "Diagnostik und Möglichkeiten der Prävention der geburtsassoziierten Asphyxie als Ursache der hypoxisch ischämischen Enzephalopathie". *Gynäkologe*, 1999, *31*, 680.
[5] Phelan J. P., Ahnn M. O., Korst L. M., Martin G. I.: "Nucleated red blood cells: A marker for fetal asphyxia?". *Am. J. Obstet. Gynecol.*, 1995, *173*, 1380.
[6] Korst L. M., Phelan J. P., Ahn M. O., Martin G. I.: "Nucleated red blood cells: An update on the marker for fetal asphyxia". *Am. J. Obstet. Gynecol.*, 1996, *175*, 843.
[7] Hanlon-Lundberg K. M., Kirby R. S., Gandhi S., Broekhuizen F. F.: "Nucleated red blood cells in cord blood of singleton term neonates". *Am. J. Obstet. Gynecol.*, 1997, *176*, 1149.
[8] Philip A. G. S., Tito A. M.: "Increased nucleated red blood cell counts in small for gestational age infants with very low birth weight". *Am. J. Dis. Child.*, 1989, *143*, 164.
[9] Green D. W., Mimouni F.: "Nucleated erythrocytes in healthy infants and in infants of diabetic mothers". *J. Pediatr.*, 1989, *116*, 129.
[10] Bernstein P. S., Minior V. K., Divon M. Y.: "Neonatal nucleated red blood cell counts in small-for-gestational age fetuses with abnormal umbilical artery Doppler studies". *Am. J. Obstet. Gynecol.*, 1997, *177*, 1079.
[11] Baschat A. A., Gembruch U., Reiss I., Gortner L., Harmann C. R., Weiner C. P.: "Neonatal nucleated red blood cell counts in growth-restricted fetuses. Relationship to arterial and venous Doppler studies". *Am. J. Obstet. Gynecol.*, 1999, *181*, 190.
[12] Hohenauer L.: "Intrauterine Wachstumskurven für den Deutschen Sprachraum". *Z. Geburtsh. Perinatol.*, 1980, *184*, 167.
[13] Davey D. A., MacGillivray I.: "The classification and definition of the hypertensive disorders of pregnancy". *Am. J. Obstet. Gynecol.*, 1989, *158*, 892.
[14] White P.: "Diabetes mellitus in pregnancy". *Clin. Perinatol.*, 1974, *1*, 331.
[15] Axt R., Ertan K., Hendrik J., Wrobel M., Mink D., Schmidt W.: "Nucleated red blood cells in cord blood of singleton term and post-term neonates". *J. Perinat. Med.*, 1999, *27*, 382.
[16] Snijders R. J. M., Abbas A., Melby O., Ireland R. M., Nicolaides K. H.: "Fetal plasma erythropoetin concentration in severe growth retardation". *Am. J. Obstet. Gynecol.*, 1993, *168*, 615.
[17] Maier R. F., Böhme K., Dudenhausen J. W., Obladen M.: "Umbilical venous erythropoietin und umbilical arterial pH in relation to different markers of fetal hypoxia". *Obstet. Gynecol.*, 1993, *81*, 575.
[18] Naeye R. L., Localio A. R.: "Determining the time before birth when ischemia and hypoxemia initiated cerebral palsy". *Obstet. Gynecol.*, 1995, *86*, 713.
[19] Leikin E., Verma U., Klein S., Tejani N.: "Relationship between neonatal nucleated red blood cell counts and hypoxic-ischemic injury". *Obstet. Gynecol.*, 1996, *87*, 439.
[20] Georgieff M. K., Schmidt R. L., Mills M. M., Radmer W. J., Widness J. A.: "Fetal iron and cytochrome c status after intrauterine hypoxemia and erythropoietin administration". *Am. J. Physiol., 262*, R 485.
[21] Thilaganathan B., Athanasiou S., Ozmen S., Creighton S., Watson N. R., Nicolaides K. H.: "Umbilical cord blood erythroblast count as an index of intrauterine hypoxia". *Arch. Dis. Child.*, 1994, *70*, F192-F194.
[22] Hecher K., Snijders R., Campbell S., Nicolaides K.: "Fetal venous, intracardiac and arterial blood flow measurements in intrauterine growth retardation: relationship with fetal blood gases". *Am. J. Obstet. Gynecol.*, 1995, *173*, 10.
[23] Joern H., Rath W.: "Comparison of Doppler sonographic examinations of the umbilical and uterine arteries in high-risk pregnancies". *Fetal Diagn. Ther.*, 1998, *13*, 150.

Address reprint requests to:
ROLAND AXT-FLIEDNER, M.D.
Department of Obstetrics and Gynecology
University of the Saarland
66421 Homburg/Saar (Germany)
e-mail: raxtfliedner@hotmail.com