REPERCUSSIONS OF ACIDOSIS ON POSTNATAL ERYTHROCYTE DEFORMABILITY IN TERM AND PRETERM NEWBORNS

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ABSTRACT

Erythrocyte deformability in newborns, a determining factor in neonatal blood hyperviscosity, is also often responsible for decreased blood flow in the microcirculation of several organs, such as the brain, kidneys, and digestive tract. In 70 neonates classified by gestational age and by the presence or absence of acidosis, we analyzed the filterability of erythrocytes in suspension through 5 polycarbonate membranes and its relation with gasometric determinations, Anion-GAP, plasma viscosity, plasma osmolality, erythrocyte volumes, and plasma lipids. Using a logistic regression analysis, controlling gestational age ($p = 0.17$), mean corpuscular volume (MCV) ($p = 0.63$), and mean corpuscular hemoglobin concentration (MCHC) ($p = 0.21$), the presence of acidosis ($p = 0.0049$, odds ratio: 3.60) is a risk factor for an increased rigidity index in newborns. Metabolic and respiratory acidosis were significantly related with lower erythrocyte deformability in the early neonatal period (below 5 days of age). Decreased plasma bicarbonate and increased Anion-GAP (even in compensated metabolic acidosis), as well as increased pCO_2 in respiratory acidosis, were significantly related with decreased erythrocyte filterability. In newborns under 32 weeks of gestational age the increase in erythrocyte rigidity index is more related to the low gestational age and increased MCV than to the presence of acidosis. These factors can produce changes in the microcirculation of these patients.

Keywords: Erythrocyte deformability; plasma viscosity; blood rheology; acidosis; asphyxia neonatorum; osmolality

Decreased erythrocyte deformability contributes to blood hyperviscosity, for which the potential risk during the neonatal period is 5%.[1,2] Acidosis and hypoxia, which influence erythrocyte rigidity, are especially frequent during this period.[3,4] Plasma viscosity has little repercussion on the development of the neonatal hyperviscosity syndrome in response to acidosis.[5] Erythrocyte deformability depends on three main factors:[6–8] cellular geometry (surface/volume ratio), intra-erythrocyte viscosity (reflected by mean corpuscular hemoglobin concentration (MCHC), and hemoglobin fluidity), and the viscoelastic properties of the membrane. However, many other influences modulate cell deformability: red blood cell (RBC), platelets and leukocytes,[1] acidosis and hypoxia,[4,9] the interaction between plasma proteins and the erythrocyte membrane,[10] different shear rates and cellular aggregability,[11] and metabolic factors (diabetes, hypertension, and hyperlipidemia).[6]

As anomalies in erythrocyte deformability can contribute to anomalies in the microcirculation of the brain, thus producing neurological deficits,[1,6,12] and these being more frequent in preterm infants and in newborns with acidosis, we designed the present study to search for differences between term and preterm infants with and without acidosis in erythrocyte deformability during the early postnatal period.

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METHODS AND MATERIALS

Patients

Seventy newborns were divided in the early neonatal period into four groups. Group 1 (G1) consisted of 15 term neonates with mean gestational age 39 +/− 1 weeks, with no clinical or laboratory abnormalities. Group 2 (G2) contained 12 term newborns (40 +/− 1 weeks of gestational age) with acidosis (pH below 7.36: 7.26 +/− 0.06). Group 3 (G3) comprised 18 preterm neonates (with a gestational age lower than 37 weeks: 33 +/− 2 weeks), and pH between 7.36 and 7.44 (7.38 +/− 0.03). Group 4 (G4) consisted of 25 preterm newborns (less than 37 weeks' gestational age: 31 +/− 3 weeks), and acidosis (pH below 7.36: 7.21 +/− 0.13). We excluded from the study those neonates with obstetric antecedents of maternal illness (e.g., diabetes, hypertension, hypotension, metabolic diseases, or prolonged rupture of the membranes) or neonatal diseases other than those caused by perinatal asphyxia (e.g., infection, heart abnormalities, polycythemia, or congenital malformations). Also excluded were neonates who previously to blood sampling had been subjected to plasma volume expansion, treatment with bicarbonate or parenteral nutrition.

Analytical Methods

The blood samples were obtained within 2 hr of life by venous puncture. In all neonates, 3 mL of blood were drawn; half of this volume was placed in tubes containing 10% ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and used to determine the rigidity index of erythrocytes. The other part of the blood volume was placed in a dry glass tube for serum measurements. In accordance with the guidelines of the International Committee for Standardization in Haematology,[13] the erythrocyte fraction was used to prepare an 8% suspension with cells that had been washed three times in an equal amount of phosphate buffered saline solution (PBS) (pH 7.4, osmolality 295 +/− 5 mOsm/kg). Filtration tests were always done within 3 hr of extraction, with blood stored at 25°C. The method was that described by Schmid-Schönbein et al[14] with slight modifications with a mean pore size of 5 μ. The times required to filter 1 mL of PBS and 1 mL 8% erythrocyte suspension in buffered saline were recorded with an electronic chronometer. Deformability, determined as the rigidity index (RI), was calculated with the expression $RI = [(ts - tt) / (tt \times Ht°)] \times 100$, where *ts* is the filtration time of 1 mL erythrocyte suspension, *tt* is the filtration time of 1 mL PBS, and $Ht°$ is the hematocrit of the erythrocyte suspension. Each sample was subjected to two measurements with different filters, and the final result was the mean of these two values. Serum measurements determined glucose, sodium, potassium, chlorine, calcium, urea, cholesterol, triglycerides, phospholipids, free fatty acids and high, low and very low density lipoprotein cholesterols (HDLc, LDLc, VLDLc).[15–19] Gasometric determinations [pO_2 (mmHg), pCO_2 (mmHg), pH, sO_2 (%) and bicarbonate (mEq/l)]were done in a blood gas analyzer (AVL-940). Anion-GAP (A-GAP) was calculated from the expression Anion-GAP (mEq/L) = Na - (Cl + Bicarbonate). The plasma viscosity (mPa.s) was determined using a Harkness capillary viscosimeter (Coulter, series 8052)[13] and the osmolality (mOsm/Kg) with a digital osmometer (Roebling). To determine the influence of gestational age, pH, CHCM, and MCV with increases of the rigidity index in newborns [RI > mean + 1 standard desviation *(SD)*] we perform a multiple regression analysis. The results for the different groups were compared with Student's *t* test. Within each group, correlation studies were done and the linear regression between RI and each of the other variables was calculated.

RESULTS

As shown in Table 1, erythrocyte rigidity index was lower in term than in preterm neonates without acidosis. Acidosis increased the RI in term and preterm neonates. We studied the influence of the red blood cell size (MCV), MCHC, gestational age, and acidosis (pH < 7.36) on the erythrocyte rigidity of newborns. Controlling in the logistic regression analysis the MCV ($p = 0.63$, NS), MCHC ($p = 0.21$, NS), and gestational age ($p = 0.17$, NS), the pres-

Table 1. Differences Between Groups

	Term Neonates			*Preterm Neonates*		
	Control (n = 25)	*Acidosis (n = 25)*	*p*	*Control (n = 18)*	*Acidosis (n = 25)*	*p*
Rigidity index	20.8 +/− 7.1	42 +/− 28	*	30 +/− 11.6	50.6 +/− 25	**
P. Viscosity (mPa.s)	0.96 +/− 0.09	0.92 +/− 0.08	NS	0.89 +/− 0.11	0.90 +/− 0.12	NS
Osmol. (mOsm/Kg)	286.8 +/− 7	285.5 +/− 6	NS	290.7 +/− 9	287.2 +/− 9	NS
MCV (fl)	105.7 +/− 5.5	105.5 +/− 4.7	NS	107.2 +/− 8	113.8 +/− 7	*
MCH (pg)	36.2 +/− 1.63	35.7 +/− 1.44	NS	36.1 +/− 2.6	37 +/− 2.9	NS
MCHC (g/dL)	34.2 +/− 1.5	33.5 +/− 1.1	NS	33.9 +/− 1.7	33.1 +/− 1.5	NS
pCO_2 (mmHg)	35 +/− 3.1	37.8 +/− 5.7	NS	31.4 +/− 3.6	47.1 +/− 13	***
pH	7.41 +/− 0.03	7.26 +/− 0.06	***	7.38 +/− 0.03	7.21 +/− 0.13	***
COH_3 (mEq/L)	22.6 +/− 1.52	17.17 +/− 3.04	**	19.4 +/− 2	18.9 +/− 3	NS
Anion-GAP (mEq/L)	11.54 +/− 4.5	16.31 +/− 5.6	NS	10.6 +/− 4.2	11.3 +/− 7.1	NS

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Table 2. Influence of Acidosis, Gestational Age, MCHC, and MCV on the Erythrocyte Rigididty in Newborns

	p	*Odds Ratio*
Acidosis (pH < 7.36)	0.0049	3.60
Preterm neonates	0.17	1.60
MCHC (g/dL)	0.21	1.57
MCV (fl)	0.63	1.22

Logistic Regression Analysis (n = 70): $p < 0.0001$.

ence of acidosis ($p = 0.0049$, odds ratio: 3.60) is risk factor for an increased RI (Table 2).

Plasma lipid concentrations, plasma viscosity, and plasma osmolality were not related with erythrocyte filterability in any of the four groups studied.

Term Neonates

In term neonates ($n = 27$), acidosis ($p = 0.043$, odds ratio = 3.8) is a risk factor for increased erythrocyte rigidity, the filterability not being influenced by the MCV ($p = 0.95$, NS) nor by the MCHC ($p = 0.37$, NS).

We observed a significant relation between RI and A-GAP in term neonates ($r = 0.68$, $p < 0.001$). This relation is defined by the ecuation RI = $-11.7 + 3.17 \times$ A-GAP. In the term group with acidosis the RI also correlates with the pCO_2 ($r = -0.67$, $p < 0.05$) and with bicarbonate ($r = -0.75$, $p < 0.01$).

Preterm Neonates

In preterm neonates ($n = 43$), the RI correlates significantly with MCV ($r = 0.41$, $p < 0.01$), perinatal asphyxia (pH: $r = -0.37$, $p < 0.05$), pCO_2: $r = 0.53$, $p < 0.001$) and gestational age ($r = -0.45$, $p < 0.01$) (Fig. 1).

In our study, preterm infants with high erythrocyte rigidity (RI > mean + 1 SD) had a lower gestational age (30.5 +/− 2.5 weeks vs. 33 +/− 2.5 weeks, $t = 2.99$, $p = 0.0046$), higher MCV (115.6 +/− 8.2 fl vs. 109.5 +/− 7.5 fl, $t = 2.99$, $p = 0.028$), with no significative differences in pCO_2 (38 +/− 0.6 mmHg vs. 47 +/- 17 mmHg, $t = 1.7$, p:NS) and pH (7.24 +/− 0.15 vs. 7.29 +/− 0.12, $t = 1.26$, p:NS) than preterm newborns with normal erythrocyte filterability (RI < mean + 1 SD).

We questioned if the lower erythrocyte deformability of preterm newborns was due mainly to the lower gestational age, to the higher MCV or to the severity of perinatal asphyxia (higher pCO_2 and lower pH) (Table 1). Studying the influence of all these factors (logistic regression analysis) we observe that the greater erythrocyte rigidity is influenced mainly by the lower gestational age ($p = 0.04$) and the MCV ($p = 0.04$) than by perinatal asphyxia (pCO_2: $p = 0.08$; pH : $p = 0.09$), and little by MCHC ($p = 0.62$, NS).

Combining the influence of these factors by means of a discriminant analysis, the lower gestational age ($p = 0.024$) and the greater MCV ($p = 0.09$: NS) are the two most important risk factors for erythrocyte rigidity in preterm newborns.

DISCUSSION

In contrast with earlier findings in adults,[20] we found that in term and preterm neonates with and without acidosis, differences in the filterability of washed erythrocytes in suspension were in fact associated with differences in gestational age,[4,21] and

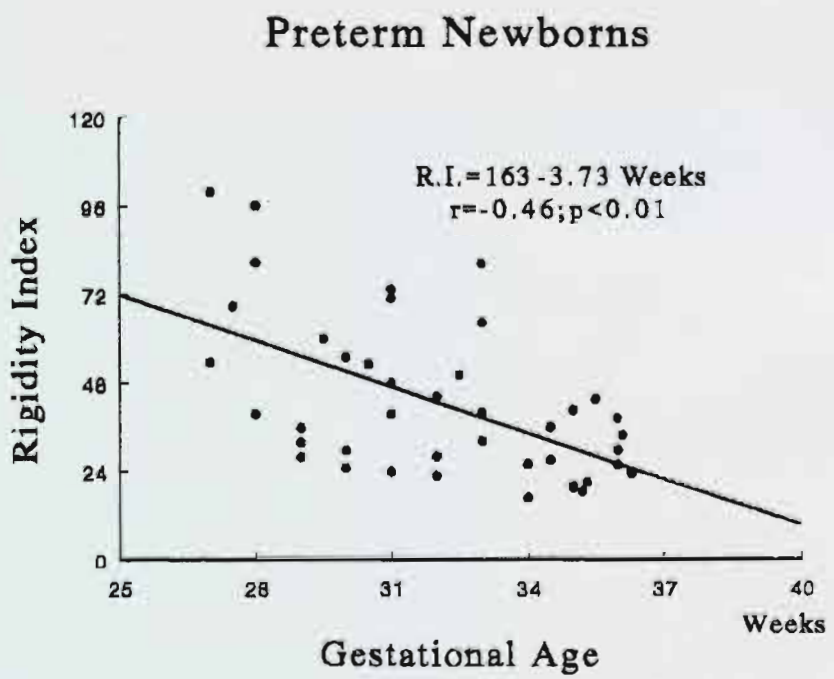

Figure 1. Correlation between gestational age and rigidity index in preterm newborns.

with the presence of metabolic or respiratory acidosis (Table 1).[1,4,21,22]

Neonatal erythrocyte metabolism presents several peculiarities, including decreased ATP stability,[23] diminished ATPase activity,[6,24] decreased phosphate uptake,[25] and weakened competence of the membrane cation pump.[26] All these factors contribute to the decreased erythrocyte filterability found in neonates in comparison with adults.[4,27]

Hypoxia and acidosis enhance cellular oxidative processes (autoxidation and superoxide formation), which produce an increase in hemoglobin-bound ATP,[28,29] increases in hemoglobin oxidation[9] and crosslinking reactions between membrane proteins and hemoglobin,[9,30-33] which increase the membrane rigidity.[9,30-34]

The increased erythrocyte rigidity in newborns with acidosis ($p = 0.0049$, Odds ratio: 3.60), cannot be attributed to the neonatal red blood cell size (MCV, $p = 0.63$) nor to the mean corpuscular hemoglobin concentration (MCHC, $p = 0.21$). These findings suggest that changes in the elasticity of the red blood cell membrane might be the most important factor for the decrease in filterability in newborns with acidosis.

To explain our findings, we considered the possible influence of erythrocyte membrane viscoelasticity in each of our four groups. Viscoelasticity depends on the fluidity, lipid, and protein composition of the membrane, and the interaction of its components with the intra- and extracellular environment. Colin et al[35] found no differences between preterm fetuses, neonates, and adults in lipid composition or erythrocyte membrane fluidity. Moreover, these authors failed to find a relationship between erythrocyte filterability and membrane fluidity or lipid composition in any of the groups they studied. Because the erythrocyte is unable to synthesize membrane lipids "de novo," depending instead on exchanges with plasma lipids, we examined the possible influence of total cholesterol, HDLc, LDLc, phospholipids, triglycerides, and free fatty acids on filterability. Our results showed no connection between erythrocyte deformability and plasma lipid concentration.

In term neonates, small decreases in blood bicarbonate concentration and increases in A-GAP were associated with decreased erythrocyte deformability ($r = 0.69$, $p < 0.001$).[4,36] This association is of interest clinically, as in compensated metabolic acidosis, microcirculatory blood flow[27] and tissular oxygen diffusion[37] can be affected despite the maintenance of normal pH values. Pardi et al,[38] recording Doppler velocimetry of the umbilical artery in fetuses with intrauterine growth retardation, found that while no fetus with normal velocimetry had hypoxia or acidosis, 42.8% of the fetuses with abnormal velocimetry (decreased distolic flow) had moderate lactic acidosis, hypoxia and/or low pH values.

In term newborns these findings could be related to an increase in lactic acid (local changes in osmolality, agregability, and trans-membrane ionic exchange)[1,39,40] and to a lower resistance of fetal erythrocytes to oxidative stress in situations of acidosis,[1,4,6,7,32,41] which would imply a greater RI. Preterm neonates do not show an increase of the A-Gap in response to the acidotic stress. This situation could be related to a greater placental transfer of bicarbonate from the mother to the fetus in preterm neonates.[42]

The increased erythrocyte rigidity in term newborns with acidosis is not related with the neonatal red blood cell size (MCV, $p = 0.97$) nor with increases in the intraerythrocyte viscosity, which depends upon the mean corpuscular hemoglobin concentration (MCHC, $p = 0.37$).

According to our tests with five pore size filters, erythrocytes from preterm neonates were less deformable than cells from term infants, the RI being related to corpuscular volumes in preterm neonates. These findings are similar to those published by Colin et al,[35] who studied preterm infants together with term babies, and are also compatible with those of Gross et al.[4]

In preterm newborns with perinatal asphyxia the erythrocyte filterability is decreased (Table 1), the descent of pH being a risk factor for increased erythrocyte rigidity.[4] We believe that as in term newborns, the erythrocyte changes caused by situations of oxidative stress (hypoxia, acidosis, and hypercapnia)[1,4,6,9,34] can affect the erythrocyte deformability in preterm infants.

The greater influence of gestational age and MCV than the pH on the erythrocyte rigidity in preterm newborns is probably due to the lower gestational age of the preterm newborns with acidosis. This suggests that in newborns with a gestational age under 32 weeks of gestation (30.6 +/− 2.5 weeks) the immaturity of the fetal erythrocyte and its enzymatic mechanisms can be of a greater influence on the erythrocyte deformability than the oxidative stress caused by acidosis (Fig. 1).

We can conclude that respiratory and metabolic acidosis are risk factors for increased erytrocyte rigidity in newborns.[43] This decreased deformability is probably due to changes in membrane elasticity or changes in intra-erythrocytic viscosity not dependent on the MCHC, being little affected by the size of the erythrocyte (MCV). An exception are the great premature newborns, in whom the lower gestational age and the MCV seem to affect more the erythrocyte filterability than the cellular changes caused by acidosis.

Our findings suggest that acidosis produces important changes in erythrocyte deformability that could affect the normal development of the fetus[38] and contribute to produce complications in newborns with acidosis,[1,4,6,44,45] which are especially frequent in preterm newborns.

In the future, the advances in studies of tissue perfusion and metabolism of the newborn will contribute to a better understanding of the influence of blood rheology and acidosis on changes in tissue blood flow and its relation to the complications of newborns with acidosis.

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