Journal of Perinatology (2014) 34, 85–86

www.nature.com/jp

EDITORIAL

# Nucleated red blood cells and fetal hypoxia: a biologic marker whose 'timing' has come?

*Journal of Perinatology* (2014) **34,** 85–86; doi:10.1038/jp.2013.169

Nucleated red blood cells (nRBCs) are immature erythrocytes whose clinical significance in the term neonate is not clear. Low numbers of nRBCs can be identified in the peripheral blood of normal neonates up the fifth day of life. Significant elevations in circulating numbers of nRBCs in the neonatal circulation have long been associated with significant antenatal pathologic circumstances. Numerous clinical conditions have been shown to predispose to such hematologic change, such as maternal diabetes, fetal growth restriction and fetal anemia with fetal tissue hypoxia being a common factor.[1] Fetal hypoxemia is generally believed to be the underlying pathophysiology which leads to an elevation in erythropoietin (EPO) resulting in increased production and premature release of immature erythrocyte precursors into the circulation in an attempt to maximize tissue oxygen delivery.[2]

A number of investigators have reported an association between increased circulating nRBCs and intrauterine hypoxia-ischemia. This association was then utilized to attempt to 'time' when fetal neurologic injury occurred. However, the medical literature is conflicting as to the specific 'timing' of this biomarker. Some investigators suggest that the presence of nRBCs is more indicative of an acute process in the later/last stages of labor and delivery,[3] whereas others have noted that their presence indicates a subacute or chronic process that predates the immediate labor and delivery time frame by many hours or days and represent a preexisting asphyxic event.[4]

In this issue of the journal, Christensen and colleagues shed more light on this biologic process.[5] In a retrospective analysis of near-term/term neonates who received one or more doses of darbopoietin alpha, a synthetic EPO analog, nRBCs first appeared in the blood between 24 and 36 h after an administered dose. This 'emergence time' was not dose dependent. The authors note that the final emergence of nRBCs into the peripheral circulation following a fetal hypoxic process is a multistage process. First, there is an increase in plasma EPO which follows fetal hypoxia. It is theorized that hypoxia induces 'hypoxia inducible factor 2 alpha', a protein encoded by the EPAS1 gene. This accelerates transcription of the EPO gene in the fetal liver resulting in increased EPO levels in the blood. This process has been shown to take approximately 4–5 h.[6] Subsequently, a time period follows in which EPO now induces and results in an elevation in nRBCs seen in the circulation. In this study, the authors estimate an 'emergence time' or this second time period, by determining the length of time from EPO administration to the appearance of nRBCs in the blood.

The authors conclude that it takes at least 28–29 h from the onset of fetal hypoxia to see an elevation of nRBCs in the blood: 4–5 h from the onset of hypoxia to generate increased levels of EPO, then followed by at least 24 h for the nRBCs to emerge and appear in the peripheral blood.

Why is there such concern and interest in the appearance of nRBCs in the peripheral circulation of hypoxic neonates? As noted above, numerous investigators have attempted to time the onset of fetal hypoxia by examination of neonatal nRBC counts. This has great import for the obstetrician delivering an infant who happens to have a 'non-reassuring' fetal heart rate strip prior to delivery or some other issue associated with a potentially hostile intrauterine environment. If the obstetrician subsequently delivers an infant who develops a chronic neurologic problem, such as cerebral palsy, will he/she be accused of delivering substandard care which resulted in fetal brain injury? Some have stated that an elevated nRBC count at birth indeed represents the presence of an acute hypoxic process which occurred minutes to hours before delivery. It has been suggested that an increase in circulating nRBCs represents EPO-stimulated release of normoblasts from bone marrow stores, perhaps, a 'shaking loose' of nRBCs. Conversely, others have stated that the presence of an elevated nRBC count after birth indicates hypoxemia that likely occurred *in utero* at least a day before the time of delivery.

In 2003, the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics jointly published a monograph entitled 'Neonatal Encephalopathy and Cerebral Palsy: Defining the Pathogenesis and Pathophysiology'.[7] This publication concluded that most cases of neonatal encephalopathy and static motor disorder have their pathogenesis during a time frame before labor and delivery, and not during the labor and delivery process. Some of the conclusions in that publication have been hotly debated by physicians and plaintiff and defense attorneys alike. Nonetheless, at that time, the authors concluded that 'nRBCs counts to determine the timing of neurologic injury should be considered investigational.' Currently, the data provided by Christensen *et al* provide biologic evidence of the time frame in which EPO results in elevated nRBCs—a process which takes at least 24 h. With Christensen's observations, we are moving from an investigational era to one with human biologic data to assist our clinical observations and support conclusions on the timing of perinatal brain injury.

There are some limitations in the study noted by the authors. Importantly, in the study, the administration of EPO was a one-time acute stimulus simulating an acute event rather than a chronic hypoxic process which can occur *in utero*, for example, chronic placental abruption and fetal-to-maternal hemorrhage, and so on. The authors conclude that if an elevated nRBC count is found at birth, the implication is that a hypoxic process commenced at least 28–29 h previously. This is a sound conclusion that adds to the body of literature concerning the biology of nRBCs.

Elevated nRBC counts should be not be relied upon as the sole determinant of the etiology of subsequent neurologic impairment following fetal hypoxia. That inquiry requires a detailed analysis of (1) the hostility (or lack thereof) of the *in utero* environment before and at the time of delivery, (2) clinical correlation with the neonatal course, specifically neonatal neurologic examinations over time with laboratory data noting any evidence of multi-system derangement, medical imaging (ultrasound and magnetic resonance imaging), (3) the subsequent neurologic status and (4) pathologic examination of the placenta to help identify both acute and chronic processes which affect placental functioning.

The obstetrician present at the time of delivery may or may not be in a position to alter the outcome of pregnancy by virtue of their presence at delivery. In addition to newer experimental information coming to light concerning the role of biochemical mediators such as cytokines in the pathogenesis of fetal/neonatal

brain injury, future laboratory studies may help us grasp a better understanding of the timing of neonatal brain injury.

For now, the simple nRBC count obtained after birth can provide useful and meaningful information; however, let us not overinterpret or rely solely on this one result. Brain injury may follow fetal hypoxia—but when? The elevated nRBC count after birth can help, but is only one part of the clinical data set. Critical analysis of the 'big picture' of maternal history, placental findings, neonatal physical examination, laboratory and radiologic findings still constitute the 'gold standard'.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

AD Bedrick
*Department of Pediatrics, University of Arizona Medical Center, Tucson, AZ, USA*
*E-mail: abedrick@peds.arizona.edu*

## REFERENCES

1 Constantino B, Cogionis B. Nucleated RBCs–significant in the peripheral blood film. *Lab Med* 2000; **31**: 223–229.
2 Teramo K, Widness J. Increased fetal plasma amniotic fluid erythropoietin concentrations: markers of intrauterine hypoxia. *Neonatology* 2009; **95**: 105–116.
3 Phelan J, Kirkendall C, Korst LM, Martin G. Nucleated red blood cell and platelet counts in asphyxiated neonates sufficient to result in permanent neurologic impairment. *J Matern Fetal Neonatal Med* 2007; **20**(5): 377–380.
4 Perrone S, Bracci R, Buonocore G. New biomarkers of fetal-neonatal hypoxic stress. *Acta Pediatr Suppl* 2002; **438**: 135–138.
5 Christensen R, Lambert D, Richards D. Estimating the nucleated red blood cell 'emergence' time in neonates. *J Perinatol* 2013 (this issue).
6 Widness J, Teramo K, Clemons G, Garcia J, Cavalieri R, Piasecki G *et al.* Temporal response of immunoreactive erythropoietin to acute hypoxemia in fetal sheep. *Pediatr Res* 1986; **20**: 15–19.
7 Task force on neonatal encephalopathy and cerebral palsy. Staff, American College of Obstetricians and Gynecologists with American Academy of Pediatrics. Neonatal encephalopathy and cerebral palsy: defining the pathogenesis and physiology. The American College of Obstetricians and Gynecologists. Washington DC, 2003.