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Nucleated Red Blood Cells and the Timing of Hypoxic Ischemic Encephalopathy

Jay P. Goldsmith, MD, Jonathan K. Muraskas, MD

"Nucleated red blood cells (nRBCs) are frequently seen in the blood of neonates. Often, medical-legal experts, in discussing the timing of a hypoxic-ischemic injury/encephalopathy, will point to the nucleated red blood cells seen in the placenta or identified in the first neonatal CBC to support their theory of timing of the injury."

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A 25-year-old primigravida mother is admitted at 40 5/7 weeks gestation for signs of early labor. Pregnancy had been unremarkable except for a decrease in amniotic fluid volume noted at the last ultrasound the day before induction. The mother presented with decreased variability on electronic fetal monitoring (category II), and after observation for several hours and failed attempts at intrauterine resuscitation, a non-emergent cesarean section was performed. The newborn was depressed at birth with low Apgar scores and a cord pH of 7.05 with a base deficit of -15. He showed early signs of encephalopathy and was treated with therapeutic hypothermia for 72 hours. An MRI at four days of age showed restricted diffusion noted diffusely in the subcortical white matter consistent with hypoxic-ischemic encephalopathy (HIE). A follow-up evaluation of the child revealed global developmental delay and mild quadriparetic cerebral palsy. A malpractice suit is filed against the obstetrician and hospital for delay in performing the cesarean section after the mother's admission to the hospital. The defense argues that the injury occurred prior to the mother's hospital admission and points to the persistent non-reactive fetal monitoring strip after admission and the nucleated red blood cells seen in the placenta and noted on the first neonatal CBC to support their argument.

Nucleated red blood cells (nRBCs), often called erythroblasts or normoblasts, are red blood cells that contain a cell nucleus. NRBCs are normally found in the bone marrow of humans of all ages but can also be seen in the blood of fetuses and newborn infants. After infancy, the nucleus is normally ejected from the cell as a normal part of cellular differentiation *before* the cell is released into the bloodstream. Fetal NRBCs are produced in the bone marrow and liver and can be released into the circulation in response to many acute and chronic intrauterine stimuli. Such stimuli may cause an increase in erythropoietin (EPO), increasing RBC production over time. However, an acute stimulus may also cause a release of nRBCs into the circulation from the storage pools in the bone marrow and liver. Stimuli include acute, subacute, and chronic hypoxia, fetal anemia, prematurity, intrauterine infection, maternal diabetes mellitus, and intrauterine growth restriction, among others. Teleologically, one can think of a hypoxic stimulus as causing a need for more oxygen-carrying capacity, and the body responds by putting more young red cells (i.e., nRBCs) into circulation to help in this condition.

"NRBCs can be reported in the neonate as the number of nRBCs per 100 white blood cells (WBCs) or as the absolute number of nRBCs per unit volume (expressed as the number of nRBCs per mm (3)). Many automated white blood cell counters report all nucleated cells as WBCs, and then the nRBCs must be subtracted to get the actual (corrected) WBC count."

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Under normal conditions, nRBCs are not found in the placenta or fetal circulation after the first trimester. However, in practice, the stresses of labor may cause a few preformed nRBCs to be released into circulation. However, the finding of an increased number of nRBCs in the circulation (i.e.,> 5/100 WBCs or >1000 total) usually indicates an acute or chronic condition. With prolonged stress, the increase in erythropoietin will stimulate an even greater number of nRBCs into the placental and neonatal circulations, and in the neonate, these cells will take longer to return to normal val-

ues. The timing of nRBC release into the circulation has been very controversial, possibly because older studies did not separate acute and chronic stimuli and lumped all increased nRBC findings into one group. More recent literature indicates that nRBCs already in the storage pool can be seen in circulation within 1 to several hours after an acute hypoxic stimulus. (2) With continued or prolonged hypoxic stress, the number of nRBCs will continue to increase, stimulated by erythropoietin (EPO) production. Thus, in most circumstances the number of nRBCs and their perpetuation in the neonatal circulation will reflect the chronicity and severity of the condition which triggered their production. In the most extreme circumstances (e.g., chronic fetal-maternal transfusion or severe intrauterine growth restriction), which may last weeks or months, the number of nRBCs will be in the 100s/100WBCs and erythropoiesis may be found in atypical visceral organs. The concept of the emergence time of nRBCs was studied by Christensen et al. (3). He divided the emergence time into 2 phases: the generation time for erythropoietin and the subsequent emergence of nRBCs. Thirty-one neonates without other stressors and no nRBCs in their CBCs were given darbepoetin. nRBCs first appeared in the blood 24-36 hours after the darbepoetin dose. The researchers concluded from previous fetal studies that following fetal hypoxia, it takes 4-5 hours to see an elevation of plasma EPO, and once there is an elevation of EPO, it takes another 24 to 36 hours to see nRBCs in the circulation. Thus for a EPO mediated rise in nRBCs from hypoxia, it takes at least 28-29 hours, suggesting that an elevated nRBC count at birth resulted from a hypoxic event 28-29 hours before birth. However, in this model, the release of nRBCs from the storage pool is not addressed. In another study of 152 infants with moderate to severe HIE, a *normal* nRBC count after birth was associated with a brief acute, profound event requiring emergent delivery and was modestly predictive of a better prognosis. (4)

"The disappearance of nRBCs from the neonatal circulation may also give clues to the timing of the hypoxic stimulus. Several studies have concluded that the nRBC count's fall rate will mirror the rate of rise. Thus, a modest rise of nRBCs from the storage pool in an acute, profound hypoxic event will fall back to normal in hours or less than one day."

The disappearance of nRBCs from the neonatal circulation may also give clues to the timing of the hypoxic stimulus. Several studies have concluded that the nRBC count's fall rate will mirror the rate of rise. Thus, a modest rise of nRBCs from the storage pool in an acute, profound hypoxic event will fall back to normal in hours or less than one day. On the other hand, a chronic hypoxic event over many days will stimulate EPO production of new nRBCs and will take several days to return to normal. Persistent elevation of nRBCs over many days may also suggest a cause other than hypoxia for the elevated count, such as maternal diabetes mellitus or congenital infection. (2)

In summary, there appears to be a biphasic response of nRBCs to multiple stimuli, including hypoxia-ischemia. Thus, to a medical-legal probability, more likely than not (not to a scientific certainty), an acute, profound hypoxic event of 30 minutes or less will result in a modest increase of nRBCs in the fetal/neonatal circulation from the preformed cells already in the storage pool in less than one to several hours after the event or no response at all (not enough time to mobilize cells in the storage pool). The rise in nRBCs will usually be <20 cells/100 WBCs or less than 2000 absolute count and will usually resolve in less than a day. A more prolonged hypoxic-ischemic event will stimulate EPO, resulting in a much higher normoblastemia (usually greater than 50 cells and often into the hundreds), lasting longer in the neonatal circulation (days). Although the research on this topic is ongoing, this biphasic response may help time the hypoxic-ischemic injury. No one biomarker or combination of biomarkers can identify the exact time and duration of a hypoxic-ischemic insult. Similar to nRBCs, other laboratory values, such as liver function tests and creatinines, follow similar patterns to support or refute allegations of acute intrapartum asphyxia.

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In the case cited above, the defense argued that the MRI at four days of age in a baby that was cooled allowed a window of opportunity for the injury to occur within the last ten days or up to 6 days prior to birth. This was supported by the decrease in amniotic fluid, the non-reactive fetal monitoring strip on hospital admission and the high number of nRBCs (80) seen in the first neonatal CBC. The plaintiff claimed that an earlier cesarean section would have ameliorated any injury and that the *Neonatal Encephalopathy and Neurologic Outcome* monograph, published by the American College of Obstetrics and Gynecology and the American Academy of Pediatrics (2014), stated that "there are no proven biomarkers that are diagnostic for neonatal HIE or the timing of a potential brain injurious event…" (5). The case was settled on the weekend prior to the start of the trial.

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Jay P. Goldsmith, MD
Professor of Pediatrics
Tulane University
New Orleans, Louisiana
Email: goldsmith.jay@gmail.com

Corresponding Author

Jonathan Muraskas MD
Professor of Pediatrics and Neonatal-Perinatal Medicine
Professor of OB/Gyne and Maternal Fetal Medicine
Director of Neonatal-Perinatal Research
Loyola University Stritch School of Medicine
2160 S 1st Avenue
Maywood,Illinois 60153
Office 708 216-1067
Fax 708 216-5602
E mail: jmurask@lumc.edu