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Amanda J. H. Kim, MD,* Jamie B. Warren, MD, MPH*

Educational Gaps

1. Clinicians may not fully understand the history of how early cord clamping became a practice standard.
2. Clinicians should understand the factors that contribute to the amount of placental transfusion and concerns that have postponed the adoption of delayed cord clamping into standard practice.

Abstract

In the middle of the 20th century, practices regarding the timing of umbilical cord clamping changed from delaying cord clamping to clamping the umbilical cord soon after delivery of the infant. In the last several years, interest in reviving delayed cord clamping has led to an abundance of literature on the subject. On the basis of recent research, many professional organizations in the fields of obstetrics, midwifery, and pediatrics have started to recommend the use of delayed cord clamping for at least a subset of infants. In part 1 of this 2-part review, we present the history of the delayed cord clamping debate, discuss the rationale behind the use of delayed cord clamping from a physiologic standpoint, detail the factors that affect transfusion volume during a delay in cord clamping, and examine the concerns that exist regarding the use of delayed cord clamping. In part 2, we present the evidence surrounding timing of cord clamping for the preterm and term infant and maternal outcomes. Finally, we discuss alternatives to delayed cord clamping and present a summary of unanswered questions on the subject.

Objectives  After completing this article, readers should be able to:

1. Understand the reasons why early cord clamping was adopted into standard practice.
2. Describe the role cord clamping has in normal fetal-to-newborn transition.
3. Identify factors that contribute to the amount of placental transfusion.
4. Understand concerns that exist regarding implementation of routine delayed cord clamping.

The History of the Debate

"Another thing very injurious to the child is the tying and cutting of the navel string too soon, which should always be left till the child has not only repeatedly breathed but till all pulsation in the cord ceases. As otherwise the child is much weaker than it ought to be, a part of the blood being left in the placenta which ought to have been in the child and at the same time the placenta does not so naturally collapse, and withdraw itself from the sides of the uterus, and is not therefore removed with so much safety and certainty."—Erasmus Darwin (1731-1802) (1)

For centuries, this quotation represented the opinion of the medical community. Even Aristotle (384–322 BC) believed that it was harmful to tie and cut the umbilical cord too soon after birth. (2) Timing of cord clamping was studied early by the likes of Pierre Budin, who in his 1875 article entitled “When Should We Clamp the Umbilical Cord?” reported that the volume of blood retained in the placenta after early cord clamping (ECC) (and therefore denied to the infant) was 92 cm³. (3)(4) In 1899, the first surgical cord clamp was introduced to replace a tie with the objective of reducing infections; instructions were to apply the clamp after cord pulsation ceased. (2)(4) In these times, ECC occurred approximately

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1 minute after birth, whereas late clamping was considered as occurring more than 5 minutes after birth. (5)

In the middle of the 20th century, practices rapidly changed and cord clamping began to occur much earlier after birth. In retrospect, the reasons for this shift in practice are hard to determine, and we are left with only theories. Contributory factors likely included the following: (1) improvements in obstetric care with more women delivering in hospitals, (2) more obstetricians conducting deliveries, (3) an increasing number of surgical deliveries, and (4) increasing expertise and availability of neonatal resuscitation. (4)(5)(6) In fact, Virginia Apgar’s seminal 1953 article only included infants whose cords had been clamped early because the 60-second scores were determined after cord clamping. (4) Perhaps most important, in the 1960s, the package known as active management of the third stage of labor (AMTSL) was introduced with the overall goal of preventing maternal postpartum hemorrhage (PPH), a major cause of maternal mortality. (6) The AMTSL package included the following: (1) administration of a prophylactic uterotonic agent, (2) clamping and cutting the umbilical cord shortly after birth, and (3) controlled cord traction of the umbilical cord. (7) Although ECC was initially described as an option in AMTSL, ECC evolved into an unintended standard component of AMTSL that had no physiologic rationale. (6)(8) More recently, the components of AMTSL have been subject to renewed scrutiny; studies have confirmed that prophylactic uterotonic agents are beneficial in preventing PPH, while finding that controlled cord traction has little benefit and ECC has no benefit in preventing PPH. (8) Finally, a series of blood volume measurements performed in healthy term infants in the 1960s revealed that more than 90% of blood volume was transferred from placenta to child within the first few breaths after birth. Conclusions from these studies led to the redefinition of early vs late cord clamping, with ECC occurring within 15 seconds of birth and late clamping occurring at approximately 1 minute. (5)(9) Thus, over a period of several years and with relatively minimal scientific evidence of benefit, ECC became the norm, and delayed cord clamping (DCC) was “discarded from mainstream practice without careful study or regard to the physiologic processes at work.” (10)

Since the middle 20th century, when ECC became a standard practice, hints of the benefits of placental transfusion appear in the literature. One example of this is barker foal syndrome, described by 2 veterinarians in 1959 after their observations of births of thoroughbred foals. They noted that approximately 1% of foals born in captivity (under human supervision and with early umbilical cord clamping) developed a convulsive syndrome that was accompanied by a barking cough. (9)(11) At postmortem examination, the lungs of these foals were found to have hyaline membranes. Foals born in the wild, where umbilical cords commonly remained intact for up to 30 minutes, did not develop this syndrome. Residual placental blood volume in the foals born in captivity could be measured at up to 1,000 to 1,500 mL, whereas that in the foals born in the wild was usually as little as 50 mL. (11) The question was posed whether placental transfusion was of possible benefit.

During the past several years, interest in DCC has become reinvigorated. Ironically, those interested in reviving DCC as a practice are now burdened with presenting the rigorously gathered evidence of its benefit. The recent evidence has led to recommendations for the use of DCC by many professional organizations in the fields of obstetrics, midwifery, and pediatrics (Table). However, despite these recommendations, widespread adoption of these policies has been difficult. In this 2-part report, we present an updated review of the evidence and the recommendations. We also highlight possible future avenues of exploration. Changes in practice and implementation of policy are left to the discretion of the reader.

**Back to Basics: The Physiology of Fetal-to-Newborn Transition**

To truly understand the differences between DCC and ECC, we need to go back to the basics and review the physiology of transition from intrauterine to extrauterine life. The clamping and cutting of the umbilical cord are not necessities for this transition to occur; in fact, if we do not cut the umbilical cord, it will dry up and fall off within a few days after birth. (2) In other words, a physiologic closure of the umbilical vein and arteries occurs without the intervention of clamping the cord. The precise mechanism of closure of the umbilical vein and arteries is debated, but it is believed that the arteries constrict first, with arterial flow rapidly diminishing during the first 20 to 25 seconds after birth and becoming negligible by 40 to 45 seconds. This prevents the loss of blood from the infant back into the placenta. (19) The umbilical vein remains open, allowing flow for up to 3 minutes to facilitate transfusion of blood from placenta to the infant. (19)(20) After 3 minutes, flow is insignificant, and placental circulation absolutely ceases by 5 minutes in 95% of infants. (19)(21) Several definitions of ECC vs DCC exist. To simplify things, one could say that ECC is the application of a clamp across the umbilical cord while there is still a significant circulation occurring through the umbilical vessels, and DCC is sometime later, after physiologic closure has begun or has been completed. (2)
During fetal life, only approximately 10% of cardiac output is sent to the lungs, whereas more than 50% of cardiac output is sent to the placenta, the fetal organ of respiration. (10) After birth, the neonatal lungs need to take over as the organ of respiration. To facilitate this transition, a neonate must breathe. The initiation of pulmonary ventilation will stimulate a decrease in pulmonary vascular resistance and lead to the increase in pulmonary blood flow until approximately 50% of neonatal cardiac output is sent to the lungs. (5)(10) If the cord is not clamped and placental transfusion occurs during this transition, many experts believe that the transfused blood smoothly supplies the blood volume needed to fill the pulmonary vessels. (2)(5) Because most healthy infants are able to breathe at birth, increasing pulmonary blood flow and subsequently increasing pulmonary venous return, they are able to handle this transition despite early clamping of the cord. (6)

We do not tend to think of the consequences that early clamping of the cord could have because most healthy newborns tolerate ECC. Cord clamping blocks the umbilical arteries and vein simultaneously. Blocking of the umbilical arteries causes a marked increase in left ventricular afterload because of the removal of the low-resistance placental circulation; with this pressure increase, the foramen ovale is forced shut. (5) Equally important is the cessation of umbilical venous flow that had previously been supplying 50% of the right ventricular filling volume. This leads to a subsequent decrease in right ventricular output by 50% and a proportionate decrease in flow to the left heart via the foramen ovale. (5) Pulmonary ventilation is necessary for a smooth transition because the increased pulmonary blood flow will lead to increased pulmonary venous return and a continued supply of blood to the left side of the heart that will then be able to overcome its newly increased afterload.

In the absence of initiation of pulmonary ventilation or if insufficient blood is present to fill the pulmonary vasculature, ECC can lead to important dangerous consequences. Inadequate ventilation prevents the normal postnatal decrease in pulmonary vascular resistance, increase in pulmonary blood flow, and return of oxygenated blood to the left side of the heart. In this situation, ECC increases left ventricular afterload and stops the delivery of oxygenated blood from the placenta to both the right- and left-sided circulations. Altogether, this leads to a significant decrease in left ventricular output, which may manifest as shock. (2)(5)(6) If insufficient blood is present to supply cardiac output to the lungs, blood may be shunted from other important areas and lead to hypoperfusion injury to sites such as the brain and gut. (20) As a consequence of clinical signs of shock and hypoperfusion, interventions

Table. **Recommendations From Professional Organizations**

<table>
<thead>
<tr>
<th>Organization</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>World Health Organization (2014)</td>
<td>Late cord clamping (approximately 1–3 minutes after birth) is recommended for all births; early cord clamping (&lt;=1 minute after birth) is not recommended unless the neonate needs to be moved immediately for resuscitation. (12)</td>
</tr>
<tr>
<td>American College of Obstetricians and Gynecologists (2012)</td>
<td>Evidence supports delayed cord clamping of 30–60 seconds after birth in preterm infants; insufficient evidence exists to support or refute benefits of delayed cord clamping for term infants, particularly in resource-rich areas. (13)</td>
</tr>
<tr>
<td>International Liaison Committee on Resuscitation/Neonatal Resuscitation Program (2010)</td>
<td>Delay in umbilical cord clamping for at least 1 minute is recommended for newborn infants not requiring resuscitation; there is insufficient evidence to support or refute a recommendation to delay cord clamping in infants requiring resuscitation. (14)</td>
</tr>
<tr>
<td>Society of Obstetricians and Gynaecologists of Canada (2009)</td>
<td>When possible, delaying cord clamping by at least 60 seconds is preferred in preterm infants (&lt;27 weeks' gestation). (16)</td>
</tr>
<tr>
<td>European Association of Perinatal Medicine (2010)</td>
<td>If possible, delay clamping of the umbilical cord for at least 30–45 seconds with the infant held below the mother. (17)</td>
</tr>
<tr>
<td>International Confederation of Midwives (2003)</td>
<td>Endorses waiting until pulsations cease before cord clamping in low-risk pregnancies. (18)</td>
</tr>
</tbody>
</table>

Adapted from Raju. (6)
such as administration of fluid boluses and rapid volume expansion may become necessary. (2)(5)(6) These practices are best avoided in fragile populations, such as the extremely premature, and may be harmful.

Transfusion in DCC and Factors That Affect Transfusion

The total amount of whole blood in the fetal-placental circulation throughout gestation is estimated to be 110 to 115 mL/kg of fetal body weight, with approximately 30 mL/kg of this volume in the placenta at any one time. (10) In the debate on the use of DCC vs ECC, the first question that needs to be answered is whether, through the use of DCC, the neonate actually receives a placental transfusion. Research on this subject goes back to the 1960s, in the work of Alice Yao and John Lind. In their 1969 study, blood volume of infants and placental residual blood volume was measured in 111 full-term infants at varying cord clamping times. They found infant and placental blood volume distributions of 67% and 33% at birth, 80% and 20% at 1 minute, and 87% and 13% at 3 minutes, respectively, providing evidence that placental transfusion occurs (Figure 1). (22) A study of term infants in which placental transfusion was estimated by measuring infant weight gain in the first 5 minutes after birth while the cord was left intact found that the mean transfusion of blood was 81 mL, or 25 mL/kg, predicting that placental transfusion could account for 24% to 40% of the total potential blood volume at birth. (21) Present in this additional blood volume are iron-rich red blood cells and stem cells. (10)

Key factors that affect the volume of placental transfusion are time, gravity, uterine contractions, and onset of respirations. Studies have found that in an infant held at the level of the introitus, the beginning rate of placental transfusion is rapid and slows in a stepwise fashion; approximately 25% of the transfusion occurs in the first 15 to 30 seconds, 50% to 78% by 60 seconds, and the remaining amount by 3 minutes (Figure 2). (19) As this finding suggests, the location of the infant during DCC affects the volume of blood transferred as well. It is known that gravity affects speed of placental transfusion but not the overall amount of placental transfusion; placement on the maternal abdomen will slow transfusion compared with being held well below the level of the placenta. (10)(19) If the infant is held higher than cord venous pressure, no blood will flow to the infant at all, and, in fact, there may be reverse flow if the infant is held high enough. (2) Generally, it is thought that holding the infant within 10 cm above (on the maternal abdomen) to 10 cm below the placenta (near the level of the introitus) will lead to completion of placental transfusion within approximately 3 minutes (Figure 3). (19) The presence of uterine contractions will speed up placental transfusion as well, and the administration of uterotonic medications is safe during DCC. (10) As reported by Mercer and Erickson-Owens, in 1974 Yao found that the use of an intravenous uterotonic at birth increased the rate of placental transfusion without causing overtransfusion. (10) In regard to onset of respiration, the size of the transfusion is reduced if cord clamping occurs before the onset of respiration, and transfusion is accelerated by the onset of breathing. (2)

![Figure 1. Association between infant blood volume and placental residual blood volume at various times of cord clamping. (22)](image1)

![Figure 2. Stepwise nature of placental transfusion. Reprinted from Chaparro and Lutter (19) in which this figure was reproduced from van Rheenen et al. BMJ. 2006;333(7575):954-958.)](image2)
Concerns About Using DCC

Despite an increasing body of research that seems to argue for the safety and efficacy of the use of DCC (detailed in part 2 of this review), the adoption of DCC as a standard practice has been sluggish. Concerns about excessive transfusion, polycythemia, and hyperbilirubinemia, particularly in the term infant, exist. These specific concerns are addressed in part 2 of this review. Others are concerned that a delay in cord clamping will lead to a subsequent delay in resuscitation and poorer outcomes in asphyxiated infants or those infants in cardiorespiratory failure. (6) The question of the effect of a delay in cord clamping on the results of cord blood gases has been investigated as cord gases have become a tool for quality control in addition to having medicolegal implications. (2)(23) Finally, with more mothers opting for cord blood banking, concerns exist that a delay in cord clamping will interfere with and leave insufficient amounts of blood for cord blood banking. (2)(6)

Neonatal Resuscitation and DCC

The delay in initiation of resuscitation as a concern has prompted many to come up with the following solution: if resuscitation is required, start resuscitation while the cord is still intact. As suggested by Mercer and Erickson-Owens, “it does not seem logical to cut the cord immediately and remove a non-breathing infant from his only source of respiratory support.” (10) One such situation in which an asphyxiated infant may certainly benefit from DCC is the infant with a tight nuchal cord. In this situation, selective intermittent occlusion of the thin-walled umbilical vein occurs, preventing oxygenated blood from the placenta from flowing to the infant. At the same time, the thicker-walled arteries stay somewhat patent, allowing blood to flow out of the infant and into the placenta. Therefore, clamping the umbilical cord directly after delivery without allowing for some unimpeded placental transfusion may actually reduce the amount of circulating blood volume and promote the development of shock in these infants. (6) A similar example is an infant with shoulder dystocia. (10) It seems that, in particular, asphyxiated infants or those infants in cardiorespiratory failure would benefit from the additional oxygenated blood volume from a placenta that continues to perform gas exchange. (5) In 1969, Philip et al (24) found that in asphyxiated infants who had their cords cut early, residual placental blood volume was lower than that of nonasphyxiated infants who had their cords cut early. This finding suggested that a physiologic in utero transfusion had occurred in these asphyxiated infants. These results were confirmed a few years later by Yao and Lind, who found that not only was residual placental blood volume lower in term asphyxiated infants but also the mean blood volumes in these infants were significantly greater than the mean blood volumes of normal term infants whose umbilical cords were clamped at comparable times after birth. In fact, the mean blood volumes in these asphyxiated infants were close to the values of mean blood volumes in term, nonasphyxiated, late clamped infants. (25)

If advanced resuscitation is indicated, several groups have found that interventions can be initiated with the cord still intact with some imagination and flexibility. In fact, providing ventilation to inflate the lungs may increase venous return to the heart, thereby promoting placental transfusion. Aladangady et al were able to initiate resuscitation, including intubation of 2 infants, while allowing for DCC. (26) Options for initiation of resuscitation with an intact cord include beginning resuscitation between a mother’s legs or use of a portable trolley that can be placed at the mother’s bedside. (2)(20) In this way, the neonatal clinicians may assess the newborn and begin positive pressure ventilation if indicated. (2)

Cord Blood Gases and DCC

De Paco et al compared umbilical cord arterial and venous blood gases in term infants randomized to 2 minutes of DCC or ECC and found that the only significant difference
between groups was a slightly higher PaO₂ in the DCC group. (27) In a slightly different study design, Valero et al compared cord blood gases collected immediately after delivery to cord blood gases collected at the time that umbilical cord pulsation ceased in the same infants. (18) They found that cord gases collected after cessation of pulsation revealed significant decreases in pH, oxygen saturation, bicarbonate, and base excess, with an increase in lactate and PaCO₂. (18) Therefore, to obtain cord gas values that will truly reflect fetal status at the time of delivery, there is a need for collection of cord blood gases immediately after delivery. Andersson et al attempted to address the concern that accurate and reliable early cord blood gases could be obtained while still practicing DCC. (23) They introduced a technique in which cord gas sampling from the unclamped pulsating cord is obtained, allowing for cord gas sampling and a delay in cord clamping. In an evaluation of 189 women who underwent ECC (cord gas sampling performed per standard double clamp technique) and 193 women who underwent DCC (cord gas sampling performed using the new technique within 30 seconds of delivery), they found that although the proportion of obtained paired arterial and venous blood gas samples was lower in the DCC group, there was no significant difference between the 2 groups in the proportion of valid paired samples that were obtained, pH, PaCO₂, lactate, base deficit, or bicarbonate levels. The PaO₂ was significantly higher in the DCC group. (23)

Cord Blood Banking and DCC

Private and public cord blood banking has become more popular among delivering mothers in the past several years because the use of stem cells from the cord blood can be used for treatment of certain medical diseases. Current methods of cord blood banking rely on the collection of a sufficient volume of residual placental blood to be successful. (2) The DCC results in much smaller residual placental blood volumes and, therefore, less available blood for banking. Farrar et al found that after a physiologic transition, only approximately 20 ± 10 mL of blood remains in the placenta, which is rarely a sufficient amount for cord blood banking. (21) For the future, current stem cell transfusion technology is beginning to combine cord blood donations to obtain an adequate volume for transfusion, which would support the collection of these smaller volumes. (10) Currently, the American Academy of Pediatrics does not support the practice of cord blood banking unless there is a clear medical need within the family, (28) and the American College of Obstetricians and Gynecologists states that collection of cord blood should not alter routine practice for the timing of umbilical cord clamping. (19) (29) Ultimately, this decision is left to the delivering mother and her provider, who need to determine the best use and ultimate recipient for the cord blood.

Conclusions

Immediate clamping of the umbilical cord after delivery evolved into standard care without sufficient evidence to support or refute its practice. As reviewed here, a significant transfusion from the placenta to the newborn occurs when clamping is delayed. This transfusion may have important implications for the newborn’s successful postnatal physiologic transition. In part 2 of this article, we review the evidence from trials of early vs delayed umbilical cord clamping in preterm and term infants and their mothers. We also present alternative methods of placental transfusion and a summary of unanswered questions.

American Board of Pediatrics Neonatal–Perinatal Content Specifications

- Know the role of the placenta in gas exchange and oxygenation of the fetus.
- Know the factors affecting and regulating the systemic circulation in the fetus (including umbilical vessels) and newborn infant during the transitional period.

References


Amanda J. H. Kim, MD,* Jamie B. Warren, MD, MPH*

Educational Gaps

1. Clinicians should have an understanding of the currently available evidence about timing of umbilical cord clamping in both preterm and term infants.
2. Umbilical cord milking may provide a safe and effective alternative to delayed cord clamping.

Abstract

In the middle of the 20th century, practices regarding the timing of umbilical cord clamping changed from delaying cord clamping to clamping the umbilical cord soon after delivery of the infant. In the last several years, interest in reviving delayed cord clamping has led to an abundance of literature on the subject. On the basis of recent research, many professional organizations in the fields of obstetrics, midwifery, and pediatrics have started to recommend the use of delayed cord clamping for at least a subset of infants. In part 1 of this 2-part review, we presented the history of the delayed cord clamping debate, discussed the rationale behind the use of delayed cord clamping from a physiologic standpoint, detailed the factors that affect transfusion volume during a delay in cord clamping, and examined the concerns that exist regarding the use of delayed cord clamping. In part 2, we present the evidence surrounding timing of cord clamping for the preterm and term infant and maternal outcomes. Finally, we discuss alternatives to delayed cord clamping and present a summary of unanswered questions on the subject.

Objectives

After completing this article, readers should be able to:

1. Describe the available evidence about timing of umbilical cord clamping for preterm and term infants.
2. Discuss umbilical cord milking as an alternative to delayed cord clamping.
3. Identify future areas of research on the subject of delayed cord clamping.

Introduction

At one time the standard in the medical community, delayed cord clamping (DCC) gave way to early cord clamping (ECC) in the middle of the 20th century. In the last several years, interest in reviving DCC has led to an abundance of literature on the subject. In part 1 of this 2-part review, we presented the history of the DCC debate, the rationale behind the use of DCC from a physiologic standpoint, factors that affect transfusion volume during DCC, and concerns that exist regarding the use of DCC. In part 2 of the review, we examine the evidence comparing DCC to ECC in the preterm infant, term infant, and mother. We also discuss umbilical cord milking (UCM) as an alternative to DCC and present a summary of unanswered questions on the subject of DCC.

Review of the Evidence: Timing of Cord Clamping and the Preterm Neonate

Hematologic Outcomes

Although there is an abundance of literature supporting the practice of DCC in premature deliveries, the area of most clear benefit in preterm infants is in the hematologic realm. Most of the
randomized clinical trials (RCTs) of DCC in preterm infants have evaluated blood volume, red blood cell volume, hemoglobin and hematocrit, or rates of postnatal blood transfusions rather than reporting on iron stores (as is done in the term population). A study of preterm infants born between 24 and 33 weeks’ gestation in the United Kingdom found a significant increase in blood volume for vaginally delivered infants randomized to DCC (80.5 vs 61 mL/kg). (1) A number of RCTs have found significant improvements in early neonatal hemoglobin or hematocrit. (2)(3)(4)(5)(6) (7) Other studies have found a nonsignificant trend toward higher hematocrits in premature infants receiving DCC compared with their ECC counterparts. (8) One group reported on outcomes before and after implementing a DCC protocol for preterm infants delivered at their institution. Among very low-birth-weight (VLBW) and low-birth-weight neonates, the cohorts receiving DCC had higher hematocrits after delivery. (9) None of the studies that reported on polycythemia found an increased risk in preterm neonates who received DCC. (4)(5)(10)

Perhaps the most appealing outcome related to increased red blood cell volume after DCC is the effect on rates of postnatal blood transfusions. A prospective study of 32 extremely preterm infants found a significant decrease in rates of blood transfusions (P < .001) during the first month after birth for the DCC group. (7) An earlier trial of preterm infants in the United Kingdom delivered vaginally and randomized to 30 seconds of DCC or conventional cord clamping resulted in increased neonatal red blood cell volumes and significantly lower median blood transfusion volumes during the hospital course. (10) Among 40 VLBW neonates randomized to DCC or ECC in another study, 50% in the DCC group and 85% in the ECC group had received a transfusion by age 6 weeks. (11) However, some individual reports found no difference in the number of transfusions between infants receiving DCC and those receiving ECC. (3)(8)(9) A multicenter RCT of extremely premature infants found a nonsignificant trend toward fewer transfusions. (2) To our knowledge, there is no evidence in the literature that DCC has a negative effect on rates of transfusions among preterm infants. The most recent Cochrane review of DCC in preterm infants validated the positive results of most of these smaller studies; the pooled results of 7 trials (n = 392) revealed that fewer infants received transfusions (relative risk [RR], 0.61; 95% confidence interval [CI], 0.46-0.81) and the overall number of transfusions was less in the DCC groups. (12)

**Hyperbilirubinemia**

If DCC increases red blood cell volume, then it follows that there may be increased bilirubin levels. An RCT evaluating effects of DCC in neonates born at 30 to 36 weeks found that an increased proportion of infants were treated with phototherapy (53 vs 73%, P = .03); initial bilirubin level, age at onset of phototherapy, duration of phototherapy, and number of phototherapy courses were not different between the DCC and ECC groups of infants who required treatment. (3) However, most individual studies have not found significant differences in bilirubin levels or phototherapy rates among preterm infants receiving DCC. (4) (5)(7)(10) In the 2012 Cochrane review, Rabe et al (12) found a statistically significant increase in peak bilirubin levels of 0.88 mg/dL (15 mmol/L) in infants receiving DCC compared with infants receiving ECC. Rates of phototherapy were not reported in most studies included in the meta-analysis, but among those with data, there was a nonsignificant trend toward increased use of phototherapy in the DCC group. (12)

**Intraventricular Hemorrhage**

The potential for a rapid change in circulating blood volume at the time of delivery in very preterm infants receiving DCC initially raised concerns for an increased risk of intraventricular hemorrhage (IVH). However, clinical trials have found either decreased risk or no change in the risk of IVH (all grades). An RCT of 72 premature infants born before 32 weeks and randomized to ECC vs DCC found a significant decrease in rates of IVH among infants in the DCC group with an odds ratio of 3.5 (95% CI, 1.1-11). The difference was most pronounced among male infants. (8) Hofmeyr et al reported on rates of IVH among 38 infants younger than 35 weeks’ gestation randomized to DCC vs ECC. (13) Ultrasonography was performed at approximately 24 hours after birth (range, 6-72 hours), and the results, which were read masked to treatment group, revealed a significantly reduced rate of IVH among infants in the DCC group. (13) A more recent single-center report found no difference in incidence of IVH after implementing a DCC protocol for preterm deliveries. (9) In addition, Ibrahim et al found no difference in the incidence of IVH between infants receiving DCC and ECC. (7) The recent Cochrane review meta-analysis concluded that there was a decreased risk of IVH with data from 10 trials and 539 infants (RR, 0.59; 95% CI, 0.41–0.85). (12) Important to note, however, is the lack of power to determine differences in rates of severe (grade III or IV) IVH among infants randomized to DCC vs ECC.

**Hemodynamics**

The mechanism by which DCC may reduce the risk of IVH in preterm infants probably lies in the effect on hemodynamics and cerebral blood flow. An animal study using preterm catheterized lambs compared a group that
had their cords clamped after the onset of respiration (approximately 3-4 minutes; the DCC group) with a group that had their cords clamped before the onset of ventilation (the ECC group). The DCC lambs exhibited no significant change in heart rate after delivery and had a less severe decrease in right ventricular output. In contrast, carotid and pulmonary blood flows and heart rate underwent rapid fluctuations in ECC lambs, whereas these parameters remained stable in DCC lambs. (14) As described previously, the improved hemodynamic measurements may be attributed to the improved pulmonary blood flow with establishment of ventilation and subsequent improvement in preload for the left ventricle. In support of this statement, Zaramella et al used echocardiography to reveal that infants who received DCC of 4 minutes exhibited larger left ventricular end-diastolic diameters on day 3 after birth compared with infants who underwent ECC. (15) A prospective trial of 51 premature infants (24%<31% weeks’ gestation) randomized to 45 seconds of DCC vs ECC evaluated multiple hemodynamic parameters at a series of time points during the first 5 days after birth. Using serial Doppler evaluations, the investigators found that infants in the DCC group had increased superior vena cava blood flow at each time point and greater right ventricular output and stroke volume at 48 hours compared with infants in the ECC group. No significant differences were found in middle cerebral artery or superior mesenteric artery Doppler flows, ventricular shortening fraction, or incidence of patent ductus arteriosus between the groups. (16)

Differences in blood pressure with cord clamping timing were measured in a study in the late 1990s, with higher mean blood pressure (P < .01) through the first 4 hours after delivery reported among infants in the DCC group compared with the ECC group. (7) An RCT with 65 preterm infants randomized to DCC of 30 to 45 seconds vs ECC found overall higher diastolic blood pressure in infants receiving DCC and higher mean blood pressure among VLBW infants. (4) Baenziger et al evaluated cerebral oxygenation in preterm infants randomized to 60 to 90 seconds of DCC vs ECC. (6) They found that although cerebral blood volume was no different, cerebral regional tissue oxygenation was better at both 4 and 24 hours after delivery in infants receiving DCC. The infants receiving DCC also had a higher mean blood pressure at 4 hours but not at 24 hours; there was no significant difference in heart rate at any study time point. (6) Other studies have found no change in measured blood pressures after delivery. (2)(11) In the 2012 Cochrane review of DCC in preterm infants, Rabe et al found a nonsignificant trend toward higher mean arterial blood pressure at birth and at 4 hours after birth and significantly less need for inotropic support after delivery. (12) In summary, DCC appears to provide hemodynamic stability that may reduce fluctuations in cerebral blood flow and blood pressure that contribute to IVH.

**Resuscitation**

There is concern that use of DCC will delay necessary resuscitation or stabilization for preterm infants. However, no increased need for intubation or mechanical ventilation and no reports of decreased 5-minute Apgar scores among preterm infants receiving DCC have been found in clinical trials. (3)(7)(9)(11)

**Mortality and Other Morbidities**

Studies evaluating outcomes after DCC have not found any difference in mortality rates among preterm or VLBW infants, although these were not sufficiently powered to detect a difference. (8)(12) DCC has been associated with decreased risk of other major neonatal morbidities. Mercer et al found in their RCT of 72 premature infants born before 32 weeks that when compared with infants in the ECC group, infants in the DCC group were significantly less likely to have blood culture-proven late-onset sepsis during their neonatal intensive care unit stay (P = .03). (8) Among pooled data from 5 trials (n=241), Rabe et al found a decreased risk of necrotizing enterocolitis (RR, 0.62; 95% CI, 0.43-0.9). (12) An earlier study found that preterm infants who received 60 to 90 seconds of DCC had lower risk of respiratory distress syndrome compared with those receiving ECC. (17)

**Long-term Neurodevelopment**

Little is known about the effect DCC may have on long-term outcomes in preterm infants. Mercer et al performed developmental testing at 7 months’ corrected gestation among former preterm infants as a follow-up from their trial that reported decreased rates of IVH and late-onset sepsis among infants randomized to DCC. They found no significant difference in the Bayley Scales of Infant Development scores between infants receiving DCC and ECC, although there was a trend toward better motor scores in boys who received DCC. (18) It is notable that the infants who were available for follow-up in that study did not exhibit a difference in their rates of IVH, which may have accounted for the findings.

**Review of the Evidence: Timing of Cord Clamping and the Full-Term Neonate**

**Hematologic Outcomes and Iron Deficiency Anemia**

Much of the recent clinical research evaluating the effects of DCC in term infants has been performed in the developing world. Grajeda et al performed an RCT of DCC vs
ECC in Guatemala, finding no difference in hemoglobin levels at birth but a significantly greater hematocrit and hemoglobin level and significantly less frequent anemia at 2 months in the DCC group. (19) A large RCT in Mexico of 476 infants randomized to 2 minutes of DCC or ECC had a 75% follow-up rate at 6 months. Among infants who were followed up, those in the DCC group had increased mean corpuscular volume, increased ferritin levels (50.7 vs 34.4 ng/mL [113.9 vs 77.3 pmol/L] P < .001), and increased total body iron with improved iron stores by 27 to 47 mg compared with infants who received ECC. The benefit was most pronounced in exclusively breastfed infants not receiving iron supplementation and in infants born to mothers with low ferritin levels at delivery. (20) An Argentinean study of 276 infants had 92% follow-up (n=255) at 6 months for iron and hematologic studies. The investigators found that the serum ferritin level was significantly higher in infants who received 3 minutes of DCC compared with ECC (33.2 vs 20.9 ng/mL [74.6 vs 47.0 pmol/L]), but there was no difference in ferritin levels between infants with 60 seconds of DCC vs ECC. The mean hemoglobin at follow-up was no different in infants with ECC, 1 minute of DCC, or 3 minutes of DCC. However, the incidence of iron deficiency anemia was 3 times higher among infants receiving ECC compared with infants with 3 minutes of DCC. (21) A group of infants in Zambia randomized to DCC exhibited higher hemoglobin levels at 4 months compared with infants receiving ECC, although this difference did not persist at 6 months. (22) A large RCT in Brazil (n=325) followed up infants at 3 months (69% follow-up; n=224) with hemoglobin and ferritin levels and found a mean improvement of 23.3 ng/mL (50.1 pmol/L) in serum ferritin in infants receiving DCC (P = .04). (23) Jahazi et al in Iran found no difference in hematocrit at 2 or 18 hours after delivery between term infants born after 30 seconds vs 3 minutes of DCC. (24) However, the measured placental residual blood volume was 39.5% less in the late clamping group, and the neonatal blood volume was increased a mean of 7.1% in the late clamping group (P < .001). (24) Two separate RCTs from India, both looking at serum ferritin and hemoglobin levels at age 3 months, found conflicting results. Geethanath et al found no difference in serum ferritin or hemoglobin levels at age 3 months. (25) However, Gupta and Ramji found serum ferritin and hemoglobin levels to be significantly higher at age 3 months in the DCC compared with the ECC group, and infants receiving ECC were more than 7 times more likely to be anemic at age 3 months. (26)

Fewer studies on hematologic outcomes in term infants randomized to DCC vs ECC have been published in developed countries. A study from Sweden reported on markers of iron stores in 382 term infants randomized to at least 180 seconds of DCC vs ECC. The primary outcome was serum ferritin level at 4 months. The investigators found no difference between groups in infant hemoglobin levels at 4 months but a 45% higher ferritin level in the DCC group (95% CI, 23%-71%; P < .001). In addition, the risk of iron deficiency was significantly decreased in infants who received DCC (0.6% vs 5.7%, P = .01). (27) Meta-analyses have found favorable effects of DCC on risk of anemia and improved iron stores in term infants. An analysis of studies including term infants with at least 2 minutes of DCC found significant increases in hematocrit, ferritin, and iron stores and a decreased risk of anemia at 2 to 6 months compared with infants receiving ECC. (28)

The 2009 Cochrane review found improved iron stores and less risk of anemia among 3- to 6-month-old infants who had received DCC at delivery. (29)(30) These findings were confirmed in the 2013 updated review.

**Polycythemia and Hyperbilirubinemia**

Concern exists for an increased risk of polycythemia with increased placental transfusion at delivery in infants who undergo DCC. However, as with the preterm infant data, this concern has not been supported by results in clinical trials, with a number of studies revealing no difference in incidence of polycythemia between the DCC and ECC groups. (24)(31)(32) One study in Libya, in which infants in the DCC group did not undergo cord clamping until cord pulsation ceased, found that 3 of 50 infants had asymptomatic polycythemia that did not require treatment and no infants had symptomatic polycythemia. (33) A meta-analysis evaluating DCC in term infants found a significantly increased risk of polycythemia (defined as a venous hematocrit of >65%) in the first 48 hours after birth; however, when a sensitivity analysis was performed and only high-quality studies were included, the risk estimate for polycythemia remained similar, but statistical significance was lost (RR, 3.91; 95% CI, 1.0-15.3). (28)

Akin to the concern for polycythemia is the understandable fear of an increase in hyperbilirubinemia among term infants who receive DCC. An evaluation of individual RCTs reveals mixed results. Some found no difference in rates of jaundice or use of phototherapy. (22)(31)(33) (34) However, a Cochrane review on the subject in 2009 found an increased risk of hyperbilirubinemia requiring phototherapy when evaluating data on 1,762 infants from 5 RCTs (RR, 0.59; 95% CI, 0.38-0.92). (29) An update on that review in 2015 continued to reveal an increased risk of jaundice requiring phototherapy. (30) However, a meta-analysis by Hutton and Hassan in 2007 did
not find a difference in mean serum bilirubin levels or an increased risk of clinical jaundice or use of phototherapy in infants receiving DCC compared with infants receiving ECC. (28) Another meta-analysis by Mathew in 2011 confirmed these findings and did not find significant differences in hyperbilirubinemia, jaundice, or an increased need for phototherapy due to DCC. (32)

Additional Outcomes

No studies of DCC have found a difference in admission rates to the neonatal intensive care unit. (30)(32) Likewise, no difference in respiratory distress among term infants receiving DCC vs ECC has been detected. (31)(35) No difference in timing of cord separation was found among 551 term infants randomized to DCC vs ECC. (34)

Aside from evaluating RBC indexes and iron stores, few investigators have researched additional outcomes for term infants beyond the early neonatal period. A large (n= 382) follow-up group of term infants randomized at birth to 180 seconds of DCC vs ECC underwent the Ages and Stages Questionnaires at age 4 months. Overall, the scores were no different between the groups. However, among the subsection scores, infants who received DCC had increased problem-solving scores (mean difference of 2 points; P = .03) and lower personal-social scores (mean difference of 2 points; P = .01) compared with the infants receiving ECC. (31) The same set of infants had serial IgG levels measured at birth, 2 to 3 days, and 4 months, and parents reported on history of infections in the first 4 months after birth. The IgG levels were statistically, but not likely clinically, significantly higher in the infants receiving DCC at 2 to 3 days but not at other time points. There was no difference in reported infections. (31)

Review of the Evidence: Timing of Cord Clamping and Maternal Outcomes

In thinking about the risks and benefits of DCC vs ECC, we must also look at maternal outcomes. Because a main contributor to the evolution in the routine use of ECC was the addition of ECC to the active management of the third stage of labor procedures, the effect of DCC on maternal postpartum hemorrhage (PPH) must be known. The 2013 Cochrane review on maternal outcomes of DCC concluded that with data on 2066 women, no significant differences were apparent between the ECC and DCC groups for severe (≥1,000-mL blood loss) PPH. In terms of PPH with a blood loss of 500 mL or more, data from 2,260 women were analyzed, again revealing no significant differences between deliveries managed with ECC or DCC. No statistically significant differences were found in the need for maternal blood transfusion after ECC or DCC in 1,345 women. No trials in the Cochrane review reported on maternal death or severe maternal morbidity. (30)

Alternatives to DCC: UCM

Because of concerns about the amount of time DCC takes after the birth of an infant, particularly in the extremely premature population or in those infants who require immediate resuscitation, UCM has emerged as a possible alternative to DCC because roughly 15 to 20 mL of cord blood resides in the umbilical vein alone. (36) Research involving UCM at this time is limited to 5 RCTs dating back to 2008. Hosono et al were the first to perform an RCT to compare UCM and ECC in 40 infants born between 24 and 28 weeks’ gestation. (37) UCM was defined as milking 20 cm of umbilical cord 2 to 3 times before clamping at a rate of 20 cm per 2 seconds while the infant was held at or below the level of the placenta. (37) They found that infants in the UCM group were more likely not to have needed red blood cell transfusions and had a decreased number of red blood cell transfusions. The initial hemoglobin level and mean admission blood pressures were significantly higher in the UCM group, and no significant differences were found in major morbidities or mortality between the 2 groups. (37) In a secondary analysis of the same enrolled patients, they found that those infants in the UCM group had significantly higher systolic and diastolic blood pressures in the first 12 hours after birth, as well as significantly higher urine output in the first 72 hours after birth. (38) In a study with a similar patient population, cord milking methods, and size (n=50), Takami et al found that the hemoglobin level was significantly higher, mean arterial blood pressures were higher for the first 12 hours, and urine output was higher for the first 24 hours in the milked group compared with the ECC group. (39) They also found that left ventricular end-diastolic dimension, left ventricular cardiac output, and superior vena cava flow were higher in the milked group compared with the ECC group. (39) Rabe et al (36) compared the use of UCM to a 30-second delay in cord clamping in 58 preterm infants born at less than 33 weeks’ gestation. In this study, UCM was performed while the infant was held 20 cm below the level of the placenta and the cord was milked 4 times at a rate of 20 cm per 2 seconds. The authors concluded that this method of cord milking achieved a similar amount of placenta-fetal blood transfusion compared with a 30-second DCC because no significant differences were found in mean hemoglobin values for the first 42 days and
no significant differences were found in the number of infants undergoing red blood cell transfusion or the median number of transfusions in the first 42 days. (36) In general, in the preterm infants described in these studies, UCM appears to be safe, leads to higher hemoglobin levels, reduces the need for red blood cell transfusions, and facilitates the stabilization of blood pressure and urine output. (36)(37) (38)(39)

UCM has also been studied in the term population. Upadhyay et al compared UCM and ECC (<30 seconds) in 200 infants born at greater than 35 weeks’ gestation. (40) In this group, UCM was performed by first cutting the cord at a length of 25 cm from the umbilicus, holding the cord above the level of the infant, then milking the cord 3 times at a rate of 20 cm per 2 seconds, after which time the cord was clamped at 2 to 3 cm from the umbilicus. They found that infants undergoing UCM had significantly higher mean hemoglobin and ferritin levels at age 6 weeks, without increased polycythemia, serum bilirubin, or need for phototherapy. (40) Erickson-Owens et al compared ECC (<10 seconds) to UCM (5 times) and found in 24 term infants delivered by elective cesarean section that those undergoing UCM had smaller placental residual blood volumes and higher hematocrits at 36 to 48 hours after birth. (41) Although questions remain on how best to use UCM, these initial studies on the subject suggest that it may be a safe alternative to DCC, particularly when resuscitation needs to occur. (42)

Conclusions and Future Avenues of Exploration
Despite all the evidence that has been gathered, a great deal of which supports the safety and efficacy of DCC, many questions remain. These questions are summarized beautifully by Raju and Singhal (Table). (43)

Although questions remain on how best to use UCM, these initial studies on the subject suggest that it may be a safe alternative to DCC, particularly when resuscitation needs to occur. (42)

**Table. Remaining Questions**

<table>
<thead>
<tr>
<th>Maternal care</th>
<th>What is the best time to clamp the cord in relation to administration of uterotonic drugs?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resuscitation</td>
<td>How can the infant’s position in relation to the placenta be maintained, especially in cesarean deliveries?</td>
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<tr>
<td></td>
<td>Can resuscitation with the umbilical cord still attached to the undelivered placenta be performed?</td>
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<tr>
<td>Cord clamping</td>
<td>How long of a delay is ideal—30 seconds, 60 seconds, other duration?</td>
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<td></td>
<td>What should be the location of the infant in relation to the placenta during DCC during vaginal or cesarean deliveries?</td>
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<td></td>
<td>Should the exact time of cord clamping be documented in all deliveries?</td>
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<td></td>
<td>Up to what lower gestational age can benefits from DCC be demonstrated?</td>
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<tr>
<td>Clamping vs milking</td>
<td>Are there differential benefits between milking and DCC?</td>
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<tr>
<td></td>
<td>What is the appropriate length of cord to be milked?</td>
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<tr>
<td></td>
<td>How fast and how many times is milking appropriate?</td>
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<tr>
<td>At-risk infants</td>
<td>What should be done in infants at risk for fetal polycythemia (eg, born at high altitude, severe intrauterine growth restriction, infants of diabetic mothers, infants large or small for gestational age)?</td>
</tr>
<tr>
<td></td>
<td>Is DCC safe in multiple gestation pregnancies?</td>
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</table>

DCC=delayed cord clamping; HIV=human immunodeficiency virus. Adapted from Raju and Singhal. (43)
Delayed cord clamping reduces the incidence of intraventricular hemorrhage and late-onset sepsis in very preterm infants. 

References
44. Beard JL. Why iron deficiency is important in infant development. *J Nutr.* 2008;138(12):2534–2536
Hemoglobinopathies in the Neonate

Trinh Nguyen, DO

Educational Gaps

1. There is a need for recognition of the many different types of sickle cell disease and their varying severities.
2. Clinicians should understand how varying mutations in $\alpha$- or $\beta$-globin genes leading to altered globin production will cause varying presentations of thalassemia carrier state and disease.

Abstract

Hemoglobinopathies are a heterogeneous group of inherited disorders resulting from mutations in the globin genes. Transmission is autosomal. There are 2 main types of hemoglobinopathies, one of which comprises disorders of decreased or absent production of a globin gene. These disorders are known as thalassemias. Structural abnormalities resulting from single amino acid substitutions comprise the second group of hemoglobinopathies. Although delineated by quantitative and qualitative characteristics, they are not mutually exclusive. Thalassemias can have qualitative defects, whereas other structural abnormalities may have quantitative defects. Collectively, they are one of the most common causes of nonimmune hemolytic anemias with various frequencies distributed throughout the world.

Objectives

After completing this article, readers should be able to:

1. Understand that $\alpha$-thalassemias are more likely to present early—in utero or during the neonatal period—whereas $\beta$-thalassemia manifestations may present later.
2. Describe significance of cis- vs trans-mutation of the $\alpha$-globin gene.
3. Understand that there are many different mutations in the $\alpha$- and $\beta$-globin genes that will cause unstable hemoglobin variants, which may present in the fetus or neonatal period. However, these same variants may become less severe or resolve when globin gene switching occurs.
4. Understand that sickle cell disease manifestations are not likely to present in the first few months after birth when hemoglobin F is the most common hemoglobin present.
5. Describe methods used to diagnose hemoglobinopathies.

Hemoglobin Nomenclature and Expression

Hemoglobin is a tetramer of 2 $\alpha$-like and 2 $\beta$-like globins. Genes encoding the $\alpha$-like globins are located on chromosome 16 and are arranged 5'-$\zeta$-$\alpha_2$-$\alpha_1$-$3'$. (1) There are 2 $\alpha$-like chains per chromosome 16; hence, there are 4 $\alpha$-globin genes. The $\beta$-like globin genes are located on chromosome 11, arranged as 5'-$\epsilon$-$\gamma^A$-$\gamma^S$-$\delta$-$\beta$-$3'$. (2) There are only 2 $\beta$-globin genes. During development, switching among the various forms of hemoglobin occurs at precise times. (3) In the first 8 weeks of gestation, 3 embryonic forms of hemoglobin predominate: $\xi_2\zeta_2$ (hemoglobin Gower 1), $\xi_2\gamma_2$ (hemoglobin Portland), and $\alpha_2\epsilon_2$ (hemoglobin Gower 2). The $\gamma$-globin expression starts to increase at 8 weeks, forming hemoglobin F, a tetramer of $\alpha_2\gamma_2$, whereas the embryonic hemoglobin levels decrease. (3)(4)(5)(6) Hemoglobin F ($\alpha_2\gamma_2$) predominates throughout the rest of pregnancy and is the most common hemoglobin at birth, accounting for 70% to 90% of hemoglobin in the neonatal period. After birth, $\gamma$-globin production decreases, whereas $\beta$-globin production increases.

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At approximately 1 year, hemoglobin F is at adult levels of 1% to 2%, whereas the most common hemoglobin is adult hemoglobin, αβ2. In the postnatal period, there is also a slight increase in the production of δ-globin such that levels of the minor adult hemoglobin, hemoglobin A2 (αδ2), reach 2% to 3% by age 1 year.

Because switching of the globin chains are timed during development, a premature infant will have mostly hemoglobin F and lower hemoglobin A levels than a term neonate.

Thalassemias
There is often confusion regarding the nomenclature of thalassemias. The name of the specific thalassemia derives from the defective globin chain. α-Thalassemia results from defects in the α-globin gene, whereas β-thalassemia occurs from mutations in the β-globin gene (Table 1).

α-Thalassemias
α-Thalassemias result from mutations of 1 or more of the 4 α-globin genes such that there is decreased (α+) or no (α0) production of α-globin from the mutated gene (Figure). Most α-globin gene mutations are large segment deletions. (7)(8) Because α-globin gene expression begins in utero, mutations in the α-globin gene may have clinical consequences in the fetus and neonate, depending on the severity of the α-globin chain deficiency.

α-Thalassemia carrier occurs when one α-globin gene is mutated (α-α). There are 3 other normal-functioning α-globin genes. Carriers have no clinical manifestations. Mutations of 2 α-globin genes lead to α-thalassemia trait. Individuals with alpha Thalassemia trait have low mean corpuscular volume, low mean corpuscular hemoglobin, and normal or a very mild anemia. They are otherwise well and have normal growth and development.

When the 2 α-globin gene mutations occur on the same chromosome, this is called a cis-mutation. This is clinically significant because there is a higher chance for this carrier to have an offspring with a more severe phenotype if the fetus inherits another α-globin mutation from the other parent. When 2 α-globin gene mutations occur on opposite sides of chromosome 16 (trans-mutation), there is no risk of passing along 2 mutated α-globin genes to the offspring. cis-Mutations are more often seen in Asians, whereas trans-mutations are typically seen in the African American population. (5)(10)

Mutations in 3 α-globin genes cause hemoglobin H disease (α/α). Here, the decrease in α-globin chains in utero and in the postnatal period allows excess γ- and β-chains to form tetramers γ4 (hemoglobin Bart) and β4 (hemoglobin H). Hemoglobin H disease is most prevalent in Asia, specifically Southeast Asia, where inheritance of a cis-mutation from a parent with α-thalassemia with another α-globin gene deletion results in 3 α-globin gene deletions. When the third α-gene mutation allows for glutamine in place of a stop codon at position 142, the result is transcription of another 31 amino acids causing hemoglobin Constant Spring (αCS, α142, Term→Gln). This abnormally longer α-globin chain is unstable and, when inherited with a cis-mutation (α/αααα), results in a more severe form of hemoglobin H disease. (11)

The most severe form of α-thalassemia occurs when all 4 α-globin genes are mutated and no α-globin chains are produced (α/αααα). Most develop hemoglobin Bart hydrodrops fetalis and die in utero. (12)

Geographic Distribution
α-Thalassemias occur with greater frequency in Asia, the Mediterranean, and Africa. Up to 40% of the population of China and Southeast Asia are reportedly carriers for α-

<table>
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<tr>
<th>Hemoglobinopathy</th>
<th>Genetics</th>
<th>Severity</th>
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<tr>
<td>A-Thalassemias</td>
<td></td>
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<tr>
<td>HbH</td>
<td>---/α---</td>
<td>Variable degrees of hemolytic anemia; some require transfusions</td>
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<tr>
<td>HbH-CS</td>
<td>---/αCSα</td>
<td>More severe than HbH and require transfusions more often</td>
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<tr>
<td>Hemoglobin Bart hydrops fetalis</td>
<td>---/---</td>
<td>Severe, most die in utero</td>
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<tr>
<td>β-Thalassemias</td>
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<tr>
<td>Cooley anemia</td>
<td>ββ0 ββ0</td>
<td>Transfusion dependent</td>
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<tr>
<td>β-Thalassemia (intermedia)</td>
<td>β+ β+ or β+ β0</td>
<td>Variable degrees of hemolytic anemia and may require transfusions</td>
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<tr>
<td>Other hemoglobinopathies</td>
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<tr>
<td>HbCC</td>
<td>β+ β+</td>
<td>Mild hemolytic anemia</td>
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<tr>
<td>HbEE</td>
<td>β+ β+</td>
<td>Mild hemolytic anemia</td>
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HbCC=hemoglobin CC; HbEE=hemoglobin EE; HbH=hemoglobin H; HbH CS=hemoglobin H Constant Spring.
thalassemia. There are many variants of cis-mutations, mostly in China and Southeast Asia. In southern China, -/-SEA is the most common cause of hydrops fetalis, whereas in the Philippines or Thailand, -/-FIL and -/-THAI are common. The Mediterranean region has a cis-mutation (-/-MED) that is also associated with hydrops fetalis in this region. Hemoglobin Constant Spring is also very common in Southeast Asia. Any combination of these cis-mutations will have a 25% chance of having an offspring with hemoglobin Bart hydrops fetalis.

With the migration of people from areas of high frequencies of a-thalassemia, there is increasing incidence of a-thalassemia syndromes in areas of the world with previously low or no diagnoses. In the last 30 years, there has been a 2,000% increase in Asian immigration to the United States. In California, a-thalassemia has become the most common nonsickle hemoglobinopathy. (11)

Clinical Manifestations of a-Thalassemia

a-Thalassemia carriers do not have phenotypic manifestations of the one a-globin gene mutation. Growth and development are not affected. Individuals with the a-thalassemia trait have low mean corpuscular volume and mean corpuscular hemoglobin but may have normal hemoglobin to slight anemia. Because a-globin chain synthesis starts in utero, 3- or 4-a-globin mutations will present during development and in the neonatal period. With less a-globin chains to stoichiometrically pair with y-globin chains in utero and b-globin chains in the postnatal period, there is an increase in hemoglobin Bart (y4) and hemoglobin H (b4). These tetramers have increased oxygen affinity and do not readily deliver oxygen to the tissues. Hypoxia ensues. (6) Ineffective erythropoiesis and extramedullary hematopoiesis occur to ameliorate the anemia with resultant hepatosplenomegaly and dysmorphisms secondary to ineffective erythropoiesis occurring in the flat bones, including the skull, sternum, and ribs. Both hemoglobin Bart and hemoglobin H are unstable and precipitate in the red blood cells, causing direct damage to the red blood cell membrane and further exacerbating the red blood cell turnover. Indirect and total bilirubin levels are elevated. Cholelithiasis and splenomegaly occur. (14) Srisupundit et al studied red blood cell indices in midpregnancy fetuses and found a much lower hemoglobin level, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration in hemoglobin Bart compared with normal or a-thalassemia trait. (15) Hemoglobin H Constant Spring, as noted previously, has a more severe phenotype with moderate to severe hemolysis due to the relatively more unstable hemoglobin Constant Spring. Homozygous hemoglobin Constant Spring, however, causes a mild anemia with jaundice and splenomegaly. (11)

Both a- and b-thalassemias result in increased iron absorption through the gastrointestinal tract with resultant iron overload. It is important not to confuse thalassemia with iron deficiency anemia and avoid therapeutic iron, further exacerbating the iron overload. Most a-thalassemias are not transfusion dependent; however, occasional transfusional support may be required, especially during acute infections, particularly parvovirus, which can precipitate an aplastic crisis. Avoidance of drugs or foods that cause oxidant stress is important to prevent worsening hemolysis. Folic acid supplementation is recommended because of the rapid cell turnover.

Hemoglobin Bart hydrops fetalis was almost universally fatal. Most die in utero. Manifestations from high cardiac output failure from chronic hypoxia and anemia include massive organomegaly, ascites, edema, heart failure, and pleural and pericardial effusions. (16)(17) In regions where the carrier rate of the cis-mutations are high, particularly the Guangdong Province of China (4.1%), Southeast Asia (4.5%), and Hong Kong (4.5%) (-/-SEA), this
4-α-globin gene mutation accounts for approximately 90% of nonimmune hydrops fetalis. (7)

There have been many reports of congenital anomalies associated with hemoglobin Bart hydrops fetalis, mostly limb, nervous, and urogenital systems. Findings include hypospadias, limb malformation, microcephaly, hydrocephalus, hypoplasia of the lungs, and cardiac defects. (5)(6)(18)(19)

Noninvasive methods, including ultrasonographic monitoring of the fetal cardiothoracic ratio, placental thickness, and middle cerebral artery peak systolic velocities, which are all increased in hydrops fetalis, help to identify such fetuses in high-risk pregnancies. (20)(21) Molecular genetic testing of fetal cells obtained by amniocentesis and chorionic villous sampling is available to confirm diagnosis. In utero transfusions have been successful in some pregnancies with cessation of development of hydrops and prevention of progressive disease. Generally, neurologic sequelae have resulted in hydrops fetalis cases that survive at birth. With intrauterine transfusions, long-term follow-up is ongoing, but morbidity and mortality appear to be minimized, and neurologic function is not as severely compromised. Long-term transfusions remain the mainstay of therapy for these individuals, however. There have been reports of successful stem cell transplantation. (5)(22)(23)

Pregnancies with hydrops fetalis have higher risk of worsening anemia for the mother, premature delivery, preeclampsia, and antepartum and postpartum hemorrhage. (7)(18)(19)(24) Both mother and fetus should be followed up closely.

β-Thalassemias

β-Thalassemias are commonly seen in newborns of Asian Indian, Middle Eastern, and Southeast Asian descent. More than 200 mutations in the β-globin gene have been identified. (3) Most are point mutations. Mutations in one β-globin gene is β-thalassemia trait. These individuals have mild microcytic hypochromic anemia, target cells on peripheral blood smear, and increased hemoglobin A₂ (α₂β₂). There is no transfusion dependence for β-thalassemia trait.

Mutations of both β-globin genes are disease states—β⁺ if there is some production of the β-globin chain, and β⁰ if there is no production of the β-globin chain. Homozygous β⁰-thalassemia, also known as Cooley anemia, is a severe hemolytic anemia characterized by ineffective erythropoiesis with resultant hepatosplenomegaly, skeletal hypertrophy, chronic hemolytic anemia, total and indirect hyperbilirubinemia, cholelithiasis, and growth and developmental delay. β⁺-Mutations have varying manifestations, depending on available residual β-globin chains. Those with the β⁺-phenotype range from being asymptomatic to exhibiting some evidence of the disease. β⁺-individuals are typically transfusion independent, although they may have periods requiring transfusional support, such as during infections and puberty. Iron overload occurs in both β⁺- and β⁰-thalassemias, and iron supplementation should be avoided unless iron deficiency is documented.

β-Globin production accounts for approximately 10% of β-like globin in utero. After birth, β-globin production increases with a corresponding increase in hemoglobin A, whereas γ-globin, and therefore hemoglobin F, decreases to adult levels by 1 year. (6) As such, neonates do not manifest signs or symptoms of β-thalassemia. Transfusions are initiated early once diagnosis is made of Cooley anemia to avoid ineffective erythropoiesis; however, these infants are usually discharged from the newborn nursery or neonatal intensive care unit by then. (4)(25)

Structural Mutations of α– and β-Globin Genes

Hemoglobin S

Hemoglobin S is the most common hemoglobinopathy in the United States. It is common among descendants of Africans, Americas (Central and South America), Middle East, India, and the Mediterranean (Turkey, Greece, and Italy). Although the exact prevalence of sickle cell disease is not known, the estimated frequency of sickle cell trait is 1:12 African Americans, and an estimated 90,000 to 100,000 of the US population is affected by sickle cell disease. One in 500 African American births are affected by sickle cell disease, whereas 1 in 36,000 Hispanic American newborns have the disorder. (26)(27)

Hemoglobin S results from a point mutation of the β-globin gene at codon 6 of the amino acid sequence, causing a valine to replace a glutamic acid. Because β-globin expression is not the predominate globin chain until a few months after birth, sickle cell disease signs and symptoms do not manifest for a few months beyond the postnatal period. In addition, with the dominant hemoglobin at birth being hemoglobin F, persistence of this hemoglobin F during infancy and into childhood protects against sickling. As hemoglobin F wanes, increased hemoglobin S will induce sickling in times of stress, including infections and dehydration. Dactylitis, a condition characterized by swelling of the hands and feet due to vaso-occlusion of the distal vessels supplying these areas, is typically the first manifestation of sickle cell disease. (25)

Sickle cell anemia is a homozygous sickle cell disease (hemoglobin SS) that accounts for 60% to 70% of all sickle cell disease. The remaining sickle cell diseases
include coinheritance of the hemoglobin S with another \( \beta \)-globin variant.

Sickle cell hemoglobin SC disease results from inheritance of hemoglobin S from one parent and a hemoglobin C from the other parent. Sickle cell \( \beta^+ \)-thalassemias result from hemoglobin S coinheritance with \( \beta^+ \) or \( \beta^0 \)-thalassemia. Compound heterozygotes for hemoglobin S and hemoglobin E, D (D-Punjab), O (O-Arab), and any other \( \beta \)-globin variant will result in sickle cell variant disease with varying degrees of severity.

The more severe phenotypes of sickle cell disease include sickle cell anemia (SS disease) and sickle cell \( \beta^0 \)-thalassemia. There are more cases of hemolysis, anemia, pain, infection, and cerebrovascular accident. Once a diagnosis is made, prophylactic antibiotics should be administered as soon as possible. Sickle cell hemoglobin SC and sickle cell S–\( \beta^+ \)-thalassemia have a milder phenotype. (28)

Hemoglobin C

Hemoglobin C mutation occurs mostly in West Africa, where the estimated gene frequency is approximately 25%. (3) Hemoglobin C trait is clinically asymptomatic. Homozygous hemoglobin C results in a mild hemolytic anemia, cholelithiasis, and splenomegaly. Aplastic crisis may occur after parvovirus infection. Hemoglobin SC is a sickle cell disease characterized by milder phenotype but higher frequency of aseptic necrosis of the femoral head and proliferative retinopathy compared with the more severe sickle cell anemia genotype. (3)

Hemoglobin E

Hemoglobin E is a \( \beta \)-globin mutation that is very prevalent in Asia. (8) Hemoglobin E trait is clinically asymptomatic. Homozygous hemoglobin E results in a mild hemolytic anemia, cholelithiasis, and splenomegaly. Aplastic crisis from parvoviral infection is a concern. Compound heterozygotes for hemoglobin E and hemoglobin S result in sickle cell SE disease, which is typically mild. Compound heterozygote hemoglobin E–\( \beta \)-thalassemia has a variable phenotype that ranges from mild to severe with transfusion dependence. (8)

Other

Several rare mutations in the \( \alpha \)- or \( \beta \)-globin genes can cause hemolysis in the neonatal period. Hemoglobin Hasharon results when the \( \alpha \)-globin mutation leads to a histidine substituting for aspartate at position 47. When this \( \alpha \)-globin mutation is paired with \( \gamma \)-globin, neonatal hemolysis ensues. When paired with \( \beta \)-globin, however, hemoglobin Hasharon is stable, thus accounting for the cessation of hemolysis as adult hemoglobin (\( \alpha_2\beta_2 \)) increases in the postnatal period. (9)

Hemoglobin Poole results from a glycyne replacing the tryptophan at position 130 of the \( \gamma \)-globin chain. This results in an unstable \( \gamma \)-globin and hemolysis of the fetal hemoglobin. Neonates have a hemolytic anemia that subsides once \( \gamma \)-expression is replaced by \( \beta \)-globin. (29)

A large deletion of 3 \( \beta \)-like globins, \( \gamma \delta \beta \)-thalassemia, also causes neonatal hemolysis, which stops once \( \delta \gamma \)-expression decreases after birth. Older children and adults with \( \gamma \delta \beta \)-thalassemia will have a \( \beta \)-thalassemia trait phenotype. (29)(30)(31)

Newborn Screening

Several techniques are used to screen for hemoglobinopathies in the newborn screen, namely, gel electrophoresis

<table>
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<tr>
<th>Table 2. Newborn Screening Results</th>
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<td><strong>Result</strong></td>
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<td>FA</td>
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<td>FVA</td>
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<td>FV,X2</td>
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V=any hemoglobin variant.
via isoelectric focusing, high-performance liquid chromatography, and citrate agar electrophoresis. (13)(32)(33) Hemoglobin Constant Spring is measured via capillary electrophoresis because it will be missed on high-performance liquid chromatography. (11)

Results of the newborn screen are reported with the most predominant hemoglobin type first, followed by subsequently decreasing amounts of X hemoglobin (Table 2). Because hemoglobin F is the predominant hemoglobin at birth, it is usually listed first, even if the child has a hemoglobinopathy. For example, an otherwise healthy newborn without sickle cell disease or trait will have newborn screening results noted as FA for fetal hemoglobin followed by hemoglobin A. In a child with sickle cell anemia, the newborn screen would show hemoglobin FS, again with hemoglobin F as the predominant hemoglobin at birth followed by hemoglobin S. This FS pattern is typically seen in newborns with either hemoglobin SS disease or hemoglobin S-β0 disease.

At birth, state screening will miss β-thalassemia trait because β-globin chain production is not maximal yet, and the most common hemoglobin is still hemoglobin F. By age 1 year, individuals with Cooley anemia will have essentially 100% hemoglobin F and no hemoglobin A due to absent β-globin production.

Newborn screening allows for identification of some, but not all, hemoglobinopathies. Early recognition of newborns with sickle cell anemia provides the opportunity to start antibiotic prophylaxis. (34)(35) Newborn screening also helps ascertain parents who are carriers, especially for hemoglobin S trait. Genetic counseling of these carrier parents regarding the risk of having a child with sickle cell disease is important. (34)(35)(36)(37)

Although all 50 states include sickle cell in their newborn screening, only a few mandate thalassemia testing. With the increased immigration of the Asian population to the United States, more cases of thalassemias have been found. (37) In utero transfusions have afforded lower morbidity and mortality in hemoglobin Bart hydrops fetalis but pose an ethical dilemma and financial stress on the family, medical personnel, and society. (38) Identifying, educating, counseling, and screening at-risk populations for hemoglobin H disease and hydrops fetalis allow for prevention and early intervention. (39)

Conclusions

The hemoglobinopathies comprise a group of autosomal disorders resulting from mutations of α-like and β-like globin genes on chromosomes 16 and 11, respectively. Resulted decreased quantity or structural mutations of the globin gene compromise hemoglobin stability, and poor oxygen delivery, hypoxia, and hemolysis ensue. Complications from chronic hemolysis include anemia, poor growth and development, heart failure, cholelithiasis, organomegaly, and extramedullary expansion. Variable expression of the mutations provides for a diverse phenotype ranging from the asymptomatic state to severe transfusion dependence, presenting as early as during in utero development.

American Board of Pediatrics Neonatal–Perinatal Content Specifications

- Know the clinical and laboratory features of neonatal hemoglobinopathies, including the thalassemias.
- Know the indications for and approaches to screening for hemoglobinopathies in the newborn population.
- Know the methodologies and interpretations of screening for hemoglobinopathies in the newborn population.

References

associated with pregnancy.

35. National Heart, Lung, and Blood Institute, Division of Blood Disease and Resources. Evidence-Based Management of Sickle Cell Disease. Bethesda, MD: National Institutes of Health; 2014
**NeoReviews Quiz Requirements**

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1. An Asian pregnant woman who is at 28 weeks of gestation visits your office for consultation. She notes that α-thalassemia disease and carrier status run in her family. She is unaware of her own status and does not remember having any problems with anemia. Which of the following statements regarding α-thalassemia and carrier status is correct?
   - A. An individual needs at least 3 α-globin genes that are mutated to be considered a carrier.
   - B. Hemoglobin H disease occurs when one parent passes on one α-globin gene mutation and the other passes on one H-globin gene mutation.
   - C. For carriers with α-globin gene mutations, Asian individuals tend to have cis-mutations, whereas trans-mutations (on opposite sides of chromosome 16) are more likely to be seen in African Americans.
   - D. When trans-mutations of the α-globin gene are passed on from both parents to a fetus, the most likely outcome is hydrops fetalis and in utero death.
   - E. Individuals who have the hemoglobin Constant Spring version of α-thalassemia are essentially carriers without significant clinical symptoms.

2. From further interview with the mother, it is determined that her family moved recently to the United States from Thailand. Her partner, the father, has a Mediterranean background. Which of the following is correct regarding the potential for α-thalassemia in their infant?
   - A. Even if both parents are carriers of α-thalassemia, it will not be possible for their infant to have α-thalassemia disease due to their different ethnic origins, although their child will have a 25% chance of having carrier status.
   - B. Individuals from both Asian and Mediterranean regions do not have cis-mutations; therefore, the most severe forms of α-thalassemia are not seen in their offspring.
   - C. Because migration of people from high areas of α-globin gene mutations to the United States has occurred with dilution of the gene mutation pool, the incidence of α-thalassemia in the United States has decreased markedly in the past 2 decades.
   - D. Up to 40% of the population of China and Southeast Asia are reportedly carriers for α-thalassemia.
   - E. Hemoglobin Constant Spring is most common in individuals of Mediterranean origin and is not seen in individuals of Asian origin.

3. A male infant is born to a mother who is a carrier for α-thalassemia. He is tested and found to have hemoglobin Constant Spring. Which of the following characteristics are likely to be found in hemoglobin Constant Spring?
   - A. Although there may be slight anemia found in the neonatal period, symptoms of hemoglobin Constant Spring are likely to resolve by 2 months and may be helped by iron supplements.
   - B. Hemoglobin Constant Spring is not associated with anemia but presents clinically as seizures and encephalopathy.
   - C. The most severe form of disease is found in homozygous hemoglobin Constant Spring, which is associated with hydrops and fetal death.
   - D. Hemoglobin Constant Spring is clinically similar to β-thalassemia major and may require transfusions during infancy.
   - E. Hemoglobin Constant Spring is caused by a mutation corresponding to the same location as a mutation in the β-globin gene.

4. A female infant is born with a point mutation in one β-globin gene only. Which of the following is likely to be true?
   - A. She is likely to have mild microcytic hypochromic anemia and target cells on peripheral blood smear.
   - B. She will likely require a blood transfusion in the first month after birth.
   - C. She will have hepatosplenomegaly, direct hyperbilirubinemia, and skeletal hypertrophy.
D. She will likely be iron deficient for life and require constant supplementation to avoid transfusions.
E. She will have a normal hemoglobin, hematocrit, and red blood cell indexes for the first year after which there may be a mild macrocytic anemia.

5. A family is in a prenatal counseling visit and notes that sickle cell disease runs in their family. Which of the following regarding disease states with hemoglobin S is correct?

A. Approximately 1 in 10,000 African American infants and 1 in 100,000 Hispanic American infants have sickle cell disease.
B. Sickle cell anemia is caused by homozygous hemoglobin SS or by coinheritance of hemoglobin S with another β-globin variant
C. Sickle cell disease typically presents with symptoms during early infancy, with significant anemia and pain crises in the first 3 months after birth.
D. The most severe phenotype of sickle cell disease occurs in hemoglobin SC disease.
E. Dactylitis often presents at birth in both hemoglobin SC disease and sickle cell S–β+–thalassemia.
Transfusion in Extremely Low-Birth-Weight Premature Neonates: Current Practice Trends, Risks, and Early Interventions to Decrease the Need for Transfusion

Lisa A. Hensch, MD,* Alexander J. Indrikovs, MD,* Karen E. Shattuck, MD†

Educational Gap

Because of the potential adverse outcomes of giving or withholding transfusions in extremely low-birth-weight neonates, clinicians should be aware of the results of clinical trials that assess transfusion strategies and methods to prevent and treat anemia.

Abstract

The goal of this review is to familiarize readers with current issues related to red blood cell transfusion and alternative strategies in the extremely low-birth-weight neonatal population. We discuss benefits associated with packed red blood cell transfusion and unique adverse outcomes in this fragile group. Alternative strategies for the prevention of anemia requiring transfusion are also reviewed.

Objectives

After completing this article, readers should be able to:

1. Understand the outcomes of clinical trials investigating liberal vs restrictive transfusion strategies.
2. Know the unique adverse outcomes associated with transfusion in this population.

Introduction

Extremely low-birth-weight (ELBW) infants, those weighing less than 1,000 g, are one of the most fragile and unstable populations in the hospital. It is estimated that approximately 90% of these infants will require at least one transfusion. (1) Transfusion requirements in this population have a number of causes, most significantly anemia of prematurity. Anemia of prematurity is a multifactorial event that affects preterm neonates with low birth weights in the first few weeks after birth. Phlebotomy and diminished erythropoietin levels due to decreased production in the premature liver and increased hemoglobin clearance play critical roles in the development of anemia with a nadir of 7 to 8 g/dL (70-80 g/L) occurring at the postnatal age of 4 to 6 weeks. This is in sharp contrast to the physiologic anemia of infancy, which occurs in term neonates with a nadir of 10 to 12 g/dL (100-129 g/L) of hemoglobin at 8 to 10 weeks of extrauterine life. (2)(3) Among neonatologists, individual and institutional transfusion practices may vary widely, with the greatest influences being need for respiratory support and postnatal age. (4)

Red blood cell transfusion is the mainstay of treatment for anemia of prematurity. (2) To obtain blood products for transfusion, whole blood is collected from a donor and processed into 3 components: packed red blood cells, plasma, and platelets. Red blood cells are separated from platelet-rich plasma by primary centrifugation followed by removal of the platelet-rich plasma. Preservative (CPD, CP2D, CPDA-1) and additive (AS-1, AS-3, AS-5) solutions are added to red blood cells to reduce hemolysis and allow packed red blood cells to be stored for 35 to 42 days. These units should have a hematocrit of 55% to 65%. (5) During storage of the red cell units, several biochemical and morphologic changes occur. These changes include decreased adenosine triphosphate, decreased 2,3-diphosphoglycerate (2,3-DPG), oxidative changes, and the accumulation of potassium. Collectively, these changes are referred to as “the storage lesion.” (6) The decrease of 2,3-DPG results in
an increased affinity for oxygen and less oxygen offloading in hypoxic tissues. Regeneration of 2,3-DPG occurs in adults during a period of hours, and it is reasonable to conclude that this happens in neonates as well, although this has not been well studied. (7) A scanning electron microscope image of red blood cell deformities after storage from a study by D’Alessandro et al is provided in the Figure. (8) Irradiation of units is performed to prevent transfusion-associated graft-vs-host disease and amplifies the changes associated with the storage lesion. Washing can remove excess extracellular potassium temporarily, and should be considered when a red blood cell unit aged less than 7 to 14 days or irradiated for less than 12 to 24 hours is not available. (9) For small volume transfusions (15 mL/kg), the potassium dose for a neonate who receives a stored unit is small compared with the 2- to 3-mEq/kg daily requirements of the neonate. (7) By using a single, dedicated unit until its expiration for all small transfusions for a neonate, donor exposure, and therefore the risk of transfusion-transmitted infections, can be reduced. Fresh or washed red blood cell units are indicated for large, rapid transfusions. (10) In addition, units can be leukoreduced to prevent the transmission of cytomegalovirus. (7)

Current Practice Trends in the Neonatal Population

Guidelines for transfusion triggers in this delicate and heavily transfused population are currently the topic of increasing interest and study. Although more restrictive transfusion strategies in adults and older pediatric patients reveal favorable outcomes as found in the Transfusion Requirements in Critical Care (11) and Transfusion Requirements in the Pediatric Intensive Care Unit (12) studies, the same may not hold true for the neonatal population. The Iowa trial by Bell et al (13) sought to answer critical questions regarding restrictive vs liberal transfusion in neonates. One hundred low-birth-weight infants were enrolled in this randomized clinical trial to determine whether the number of red blood cell transfusions to preterm neonates could be reduced without an increase in adverse outcomes. This study found that there was no difference between the restrict and liberal groups in avoiding transfusions but found significant differences in the development of neurologic consequences in the restrictive group. Infants assigned to the restrictive groups were more likely to develop intraparenchymal brain hemorrhage and periventricular leukomalacia, and to have more frequent apneic episodes. (13)

Figure. Red blood cells after storage. Scanning electron microscope images reprinted with permission from D’Alessandro et al. (8) A. Red blood cells after 42 days of storage (original magnification ×7,500; scale bar = 1 μm). B. Red blood cells after 28 days of storage, panoramic view (original magnification ×2,000; scale bar = 10 μm). C. Red blood cells after 42 days of storage (original magnification ×2,000; scale bar = 10 μm).
The Premature Infants in Need of Transfusion (PINT) study by Kirpalani et al (14) also tried to answer the questions of restrictive vs liberal transfusion in 451 ELBW infants. Transfusion triggers were based on the postnatal age and the need for respiratory support. In contrast to the Iowa trial, (13) the study found that infants in the restrictive group were more likely to avoid transfusion (89% vs 95% transfused) and that there was no statistically significant difference between the groups regarding morbidity, including survival rates, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), and brain injury. (14) However, a long-term study on subsequent outcomes in this group raised questions about cognitive delay in the infants assigned to the restrictive transfusion group. (15)

Guidance regarding restrictive vs liberal transfusion practices remains controversial. (4)(16) As such, the decision to transfuse must be primarily based on clinical information and careful consideration of the expected benefits vs associated risks. The need for transfusion depends on a number of clinical factors, particularly oxygen requirements, use of respiratory support, and postnatal age. (4) With the clinical status of the neonate in mind, Roseff et al (17) provide commonly used guidelines based on symptoms (tachypnea and tachycardia) and respiratory requirements in addition to hematocrit. A comparison of these guidelines (17) vs the criteria used in the Iowa (13) and PINT (14) trials is given in the Table. Current guidelines from the American Association of Blood Banks recommend that neonates receive transfusions at a dose of 10 to 15 mL/kg for 1 to 2 hours and that the transfusion be completed within 4 hours. (18) Transfusion at this dose is estimated to increase the hemoglobin concentration by 2 to 3 g/dL (20-30 g/L). (19)

Red blood cell transfusions in preterm neonates are given for a wide variety of manifestations of symptomatic anemia, including tachypnea, dyspnea, poor weight gain, and bradycardia. (20) In an international survey of neonatologists, 51% reported having transfusion guidelines within the units where they practiced. These clinicians reported that the need for oxygen and respiratory support was very influential in the decision to transfuse. Variation for transfusion thresholds was most significant in the first week after birth. (4) A study found that 4 neonatal intensive care units within one health care system had vastly different rates of transfusion despite the use of the same transfusion guidelines. The neonatal intensive care unit with the lowest transfusion rate had guidelines to decrease anemia, including cord clamping and milking, darbepoetin use, and decreasing phlebotomy blood losses. (21)

**Adverse Outcomes Associated With Transfusion in the Neonatal Population**

In recent years, several studies have highlighted the association between red blood cell transfusion in neonates and adverse outcomes unique to this population. The most serious of these are BPD, necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), and ROP. Multiple factors play into the cause of each disorder; however, the contribution of transfusion to iron-mediated infection and oxidative stress has been proposed as a possible unifying cause. (22)

**Bronchopulmonary Dysplasia**

In 1967, Northway et al (23) described a set of neonates who developed chronic lung disease after mechanical ventilation for respiratory distress syndrome. These infants have unique clinical characteristics and radiographic findings. (23) Primary risk factors for the development of BPD are immaturity and oxygen toxicity. (24) Studies have emerged revealing an association between BPD and red blood cell transfusion. (24)(25)(26)(27) In a study of very low-birth-weight infants, a correlation was found between red blood cell transfusion and development of BPD, as well as persistence of oxygen requirements after 36 weeks’ gestational age. This finding was attributed to increased rate of transfusion in infants with oxygen requirements; however, both iron and free radical damage were postulated as possible mechanisms. (28) In a study by Zhang et al of 231 preterm infants, packed red blood cell transfusion was found to be associated with BPD, and an increased number of transfusions was found to be associated with disease severity. (26)

**Transfusion-Related NEC**

In 2006, Mally et al (29) described a subset of premature neonates who developed NEC, which was temporally associated with transfusion (<48 hours). These neonates had an older postnatal age and were more stable immediately before onset of NEC than their counterparts who developed NEC not associated with transfusion. (29) In a follow-up study, the same institution found that these cases of NEC occurred around a mean postconceptual age of 31 weeks and that onset of symptoms occurred at a mean of 5 hours after transfusion, similar to transfusion-related acute lung injury. (30) Josephson et al (31) found a subset of neonates with late-onset NEC who developed clinical symptoms at a median of 37 days. In contrast to the prior study, these infants were less stable than those who developed early NEC (<4 weeks). They additionally found these infants were more premature, had lower birth weights, had lower hematocrits, and more frequently required surgery. (31) In a near-infrared spectroscopic evaluation, Marin et al
Table. **Comparison of Transfusion Criteria in Guidelines vs Trials**

<table>
<thead>
<tr>
<th>Hematocrit ≤45%</th>
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<td>ECMO Cyanotic Heart Disease</td>
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<thead>
<tr>
<th>Roseff et al. (^a) (17)</th>
<th>Iowa Trial (^b) (13)</th>
<th>PINT Trial (^c) (14)</th>
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<td>&gt;35% Hood (O_2)</td>
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<td>CPAP/intermittent Ventilation with MAP ≥6-8 cm H(_2)O</td>
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<tr>
<td>(O_2) Nasal Cannula</td>
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<tr>
<td>CPAP/intermittent Ventilation with MAP &lt;6 cm H(_2)O</td>
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<td>Significant Apnea, Bradycardia, Tachycardia, or Tachypnea</td>
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<td>Low Weight Gain</td>
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<th>Roseff et al. (^a) (17)</th>
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<tr>
<td>ECMO Cyanotic Heart Disease</td>
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| Capillary Hematocrit ≤40.5% OR Central Hematocrit <36.6% Respiratory Support |
| Capillary Hematocrit <36% OR Central Hematocrit <32.7% Respiratory Support |
| Capillary Hematocrit <30% OR Central Hematocrit <27% Respiratory Support |
| Capillary Hematocrit <34.5% OR Central Hematocrit <31.2% Respiratory Support |
| Capillary Hematocrit <30% OR Central Hematocrit <27% Respiratory Support |
| Capillary Hematocrit <25.5% OR Central Hematocrit <23.1% Respiratory Support |
| Capillary Hematocrit <22.5% OR Central Hematocrit <20.4% Respiratory Support |

CPAP=continuous positive airway pressure; ECMO=extracorporeal membrane oxygenation; MAP=mean airway pressure; PINT=Premature Infants in Need of Transfusion.

\(^a\)Adapted from Roseff et al. (17)

\(^b\)Adapted from stated guidelines in Bell et al. (13)

\(^c\)Adapted from Kirpalani et al. (14) Guidelines converted to estimated hematocrit from hemoglobin in grams per liter.
(32) were able to demonstrate that there was a greater variation in mesenteric blood flow in the infants developing NEC related to transfusion as opposed to transfused infants who did not develop NEC. (32) Withholding feeding during transfusion has been reported to be protective; (33) however, larger clinical trials are needed to evaluate the magnitude of this effect.

**Intraventricular Hemorrhage**

The development of IVH in the preterm neonate is a multifactorial event that may be causally associated with early red blood cell transfusions. (34) A study found that there was an association between red blood cell transfusion and progression of grade 1 IVH to grade 3 or 4 hemorrhage. (35) Christensen et al (36) found that the rate of severe IVH was noted to decrease with implementation of more restrictive transfusion policies, but a direct link between red blood cell transfusion and the development of IVH has yet to be made.

**Retinopathy of Prematurity**

ROP is a leading cause of visual impairment in preterm neonates that occurs secondary to a disruption of neurovascular growth. Risk factors include high oxygen supplementation, low birth weight, and respiratory distress syndrome. (37) Studies have found an independent association between red blood cell transfusion and development of ROP, whether dose dependent (38) or related to frequency of transfusion. (40) In a study by Dani et al, (40) results indicated a 47% incidence of ROP in infants weighing less than 1,000 g and that iron from packed red blood cell transfusions independently contributed to its development, echoing the results of prior studies. (39) Despite this apparent association, a study examining a more restrictive transfusion policy failed to find a decreased incidence of ROP. (42)

When evaluating these potential adverse outcomes before transfusion of ELBW infants, it is important to remember that these neonates are often those with the most additional risk factors for these outcomes and for transfusion. Of particular note, both aforementioned trials examining the use of liberal vs restrictive transfusion policies did not find a decreased incidence of BPD, NEC, IVH, or ROP. (13) Pediatric red blood cell transfusions have been associated with increases in redox active iron, heme, and free radicals, which increase with increasing storage age and are postulated to play a role in the pathophysiology of these diseases. (22) The Age of Red Blood Cells in Premature Infants (ARIPI) randomized trial, which evaluated neonatal morbidities associated with increasing age of red blood cell units, did not identify a decrease in these outcomes when fresh units (mean, 5.1 days) vs older units (mean, 14.6 days) were used. However, because of the practice of limiting donor exposures, red blood cell units may be used until their outdated and additional studies may be needed to evaluate the effects of greater storage times. (43) Ongoing research is crucial to examine the potential links between adverse outcomes and red blood cell transfusions and to clarify the underlying mechanisms.

**Early Prevention Methods to Decrease the Need for Transfusion**

**Erythropoietin**

The preterm neonate, unlike the full-term infant, relies on the liver for erythropoietin production. The liver is less sensitive than the kidneys to anemia; therefore, the amount of erythropoietin produced is insufficient to compensate for the severe degrees of anemia seen in very low-birth-weight neonates. To complicate matters, clearance of erythropoietin in this population is increased. (20) Erythropoiesis-stimulating agents have been developed as a means of reducing red blood cell transfusions, and recombinant human erythropoietin (rhEPO), the first of these, has been extensively studied in preterm neonates as a means to avoid red blood cell transfusions. An early study by Ohls et al (44) reported that a combination of rhEPO (200 U/kg/d) and parenteral iron (1 mg/kg/d) during a 2-week period resulted in decreased transfusions in neonates weighing less than 750 g. (44) In a subsequent meta-analysis of 21 studies on the efficacy of reducing transfusions by the administration of rhEPO, Vamvakas and Strauss (45) found that the use of rhEPO was associated with only a modest decrease in transfusions. In addition, they found that application of conservative criteria for transfusion, minimizing iatrogenic blood loss, and therapy with supplemental iron were effective strategies for reducing transfusion and cautioned that more clinical trials were needed to make a definitive conclusion about the use of rhEPO. (45) Ohls et al (46) reported their results using darbepoetin alfa, a newer hypoglycosylated analog with a half-life that is 13- to 14-fold longer than that of rhEPO. This study found that neonates receiving either agent received fewer transfusions and had fewer donor exposures than those receiving placebo. (46) Questions remain regarding the clinical risk-benefit ratio of rhEPO therapy. Some studies have found an increase of ROP associated with higher cumulative doses of rhEPO, (47)(48) whereas others have found that rhEPO may have neuroprotective effects. (49)(50) Although research into the safety and efficacy of rhEPO is ongoing, several promising nonpharmacologic approaches to reducing the need for transfusion are gaining increasing attention. (51)
Delayed Cord Clamping and Cord Milking

Current obstetric practice includes immediate clamping of the umbilical cord after birth. Leaving the cord unclamped for a period after birth results in placental transfusion as long as the neonate is held below the level of the uterus. Delayed cord clamping results in decreased need for subsequent transfusion and appears to decrease the risk of IVH and NEC. (52) In a randomized clinical trial, Oh et al (53) were able to demonstrate effective placental transfusion by demonstrating increased hematocrits in the delayed cord clamping group 4 hours after delivery. Hosono et al (54) found that milking the umbilical cord 2 to 3 times for a period of 4 to 6 seconds was associated with increased blood pressure after birth, decreased respiratory support, and decreased rate of transfusions. (54) A meta-analysis of 7 randomized clinical trials confirmed that umbilical cord milking was associated with higher initial hemoglobin level and lower risk of oxygen requirement at 36 weeks but no associated decrease in need for red blood cell transfusion. (55) Rabe et al (56) found that milking the umbilical cord 4 times was comparable to delayed cord clamping for 30 seconds. Further studies are needed to continue to evaluate the efficacy and safety of delayed cord blood clamping and umbilical cord milking, but initial studies are promising. Delayed cord clamping is reviewed in more detail elsewhere in this issue.

Autologous Placental Transfusion

Given the successes seen with umbilical cord blood milking and delayed clamping, one may assume that autologous blood from the umbilical cord may also be an alternative to allogeneic blood components. Eichler et al (57) studied the possibility of obtaining cord blood for transfusion purposes. Their study found umbilical cord blood collection to be technically difficult and 6 times as expensive as allogeneic red blood cells. In addition, umbilical cord blood could often not be processed because of low volume. (57) In a later study, Jansen and colleagues (1) found umbilical cord blood collection to be most efficient and effective for neonates between 29 and 31 weeks of gestation. Collection in infants at less than 29 weeks’ gestation had insufficient yields. For neonates between 29 and 31 weeks’ gestation, the authors determined they could cover 41% of their transfusion needs in the first 30 days with autologous blood. (1) Until methods can be improved and safety can be verified, transfusion of autologous cord blood in neonates is unlikely to gain widespread acceptance. (58)

Decreasing Iatrogenic Blood Loss

Iatrogenic blood loss is a primary contributor to the need for red blood cell transfusion in the ELBW population. (51) Laboratory phlebotomy losses are typically 15% to 30% of an infant’s total blood volume in the first 6 weeks after birth. Researchers have observed a direct correlation between phlebotomized and transfused volumes. (3) In an investigation of phlebotomy overdraw, one study found that significant overdraw could be partially attributed to lack of fill-lines on collection devices, use of syringes rather than standard tubes, and severity of illness. This study also found a significant variation between phlebotomists, and, surprisingly given their fragile clinical status, that overdraw was increased in infants who were of lighter weight and more critically ill. (59) Improving technologies also provide a means for reduction in overdraw. When an umbilical artery catheter is being used, the use of in-line chemistry and blood gas monitors can reduce laboratory blood loss by 25%. (60) Similarly, the introduction of a bedside blood gas analyzer, not in-line, led to an estimated 30% reduction in phlebotomy losses, which was associated with a 43% reduction in red blood cell transfusion. (61) Arterial catheters are associated with increased blood loss, (62) and because of their association with infection, it is recommended that they be removed as soon as they are no longer clinically necessary. (51) Placental blood also provides an opportunity to decrease red blood cell transfusion in this setting. Fetal blood is separate from maternal blood in the placenta and can be used for initial laboratory testing. (51) Initial blood testing, including complete blood cell count, cultures, metabolic screens, blood glucose, and blood gases, can be reliably drawn from the placenta, decreasing subsequent transfusions and initial need for vasopressor support. This technique can be used even when delayed clamping or cord milking strategies are used. (63)

Conclusion

Transfusion strategies in the neonatal population are a topic of increasing concern and study. Although studies in older populations have led to more restrictive guidelines, studies in preterm infants have not been as clear. Many of the benefits of transfusion in this population have been established and serve as the basis of practice; however, increasing recognition of adverse outcomes, such as transfusion-related NEC, BPD, IVH, and ROP, has raised important questions about whether some transfusions are appropriate. Alternative strategies, both pharmacologic and nonpharmacologic, have been proposed to help combat anemia of prematurity. Both delayed umbilical cord clamping and cord milking are effective in increasing hematocrits and subsequently decreasing transfusion. Likewise, improvements in practice and technology can help reduce the degree of iatrogenic blood loss and subsequent transfusion. Ongoing research into
guidelines and alternative therapies will help to improve care, decrease unnecessary transfusion, and reduce the incidence of adverse outcomes associated with transfusion.

American Board of Pediatrics Neonatal–Perinatal Content Specifications

- Know the factors regulating erythropoiesis in the fetus and neonate, including erythropoietin.
- Know the causes of and approaches to management of an infant with anemia of prematurity.
- Recognize the causes of iron deficiency anemia and various prevention measures.

References

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34. Christensen RD. Associations between “early” red blood cell transfusion and severe intraventricular hemorrhage, and between “late” red blood cell transfusion and necrotizing enterocolitis. Semin Perinatol. 2012;36(4):283–289
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1. A 5-day-old male infant who was born at 24 weeks' gestational age, with a birth weight of 600 g, is noted to have a hematocrit of 26%. He is receiving mechanical ventilation and has hypotension. The team decides to perform a red blood cell transfusion. Which of the following statements regarding the processing of red blood cells for transfusion in neonates is correct?
   A. Preservative and additive solutions are added to the red blood cell component to reduce hemolysis and extend storage to approximately 35 to 42 days.
   B. The goal hematocrit of stored red blood cells is 75% to 85%, which will be diluted during the actual transfusion.
   C. During storage of red blood cell units, there is an increase in 2,3-diphosphoglycerate levels.
   D. During storage of red blood cell units, the level of calcium increases and potassium levels decrease, such that these levels should be monitored closely after transfusion.
   E. Irradiation of red blood cell units is only performed if the donor had a suspicion of infection to reduce bacterial load.

2. You are reviewing the evidence for transfusion practices for extremely low-birth-weight (ELBW) preterm infants in your neonatal intensive care unit (NICU). Which of the following is true regarding studies that have been performed regarding this issue?
   A. There is conclusive evidence that a more liberal transfusion policy in ELBW infants leads to a higher incidence of necrotizing enterocolitis and bronchopulmonary dysplasia.
   B. The Premature Infants in Need of Transfusion (PINT) study by Kirpalani et al concluded that although retinopathy of prematurity rates were lower in the liberal transfusion group, NICU mortality was also higher in that group.
   C. The PINT study by Kirpalani et al found that infants in the restrictive group were more likely to avoid transfusion than the liberal group, although most infants in both groups received transfusion.
   D. The Iowa trial by Bell et al found that the risk of transfusion decreased by 50% in their restrictive group compared with the liberal group.
   E. In both the Iowa trial and PINT study, neurodevelopmental outcomes at 18 months revealed a favorable trend toward improved outcomes in the restrictive group compared with the liberal group.

3. A 25-week-gestational-age female infant is receiving packed red blood cell transfusion at age 10 days. Which of the following statements concerning the association of neonatal morbidities and transfusion is correct?
   A. Red blood cell transfusion has been associated with bronchopulmonary dysplasia, with one study indicating an association of increased number of transfusions related to increased severity of pulmonary disease.
   B. Transfusion-related necrotizing enterocolitis has been described only for the subset of preterm infants that are between 34 and 37 weeks' gestational age.
   C. Transfusion in the first day after birth is a strong predictor of grade 2 intraventricular hemorrhage but does not appear to have any association with progression to grade 3 or 4 hemorrhage.
   D. The PINT trials found that risk of retinopathy of prematurity appears to be decreased when transfusion occurs in the first few days after birth but increased when transfusion occurs after the first month.
   E. Restrictive transfusion policies have found decreased retinopathy of prematurity in several studies.

4. A male infant is born at 26 weeks' gestational age after spontaneous preterm labor. The patient is prescribed a protocol of recombinant human erythropoietin (rhEPO) injections. Which of the following is correct regarding erythropoietin and rhEPO use in preterm neonates?
   A. The main source of endogenous erythropoietin production in preterm neonates is the adrenal glands.
   B. A newer form of erythropoietin, darbepoetin alfa, has a half-life that is 13- to 14-fold longer than that of rhEPO.
C. Similar doses of darbepoetin alfa given across 2 weeks have 10 to 20 times the effect of increasing red blood cell mass than rhEPO.
D. For infants younger than 26 weeks' gestational age, a paradoxical effect of increased blood transfusion has been seen when rhEPO is administered during the first 2 weeks after birth.
E. One potential benefit of supplemental erythropoietin has been the observation that retinopathy of prematurity is significantly less common for higher cumulative doses of rhEPO.

5. A woman presents to the labor and delivery room at 28 weeks of gestation and is in preterm labor. During the process of antenatal counseling, she notes that she would like to avoid blood transfusions for her infant if at all possible. Which of the following statements regarding strategies to reduce transfusion is correct?
   A. Delayed cord clamping reduces the need for transfusion in preterm neonates but is associated with a higher risk of intraventricular hemorrhage and necrotizing enterocolitis.
   B. In a recent meta-analysis of umbilical cord milking, the percentage of infants receiving transfusion and the number of transfusions administered were lower in the cord milking group.
   C. Autologous placental transfusion appears to be an effective strategy for reducing other transfusions but only in infants born before 28 weeks' gestational age.
   D. Laboratory phlebotomy losses are typically 15% to 30% of an infant's total blood volume in the first 6 weeks after birth.
   E. No association has been found between phlebotomy losses and the amount of red blood cell transfusion for preterm infants.
Biomarkers of Neonatal Sepsis

Clarissa Deleon, MD,* Karen Shattuck, MD,* Sunil K. Jain, MD*

Objective Disclosure
Drs Deleon, Shattuck, and Jain have disclosed no financial relationships relevant to this article. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.

Abstract
Neonatal sepsis is an important cause of morbidity and mortality in infants, and diagnosis of neonatal sepsis remains challenging. The diagnostic standard for neonatal sepsis is blood culture. Sensitivity of blood culture may be affected by antepartum antibiotic exposure or volume of blood collected for culture. The present review highlights the importance of various biomarkers that can be used in combination with hematologic scoring to diagnose neonatal sepsis.

Objectives After completing this article, readers should be able to:
1. Identify various biomarkers of sepsis.
2. Understand the role of a receiver operating characteristic (ROC) curve to identify appropriate cut-off values of individual biomarkers of sepsis.
3. Determine the possible role of combinations of biomarkers in the diagnosis of neonatal sepsis.
4. Recognize the limitations of individual biomarkers in diagnosing neonatal sepsis.

Introduction
Despite significant advances in the care of newborn infants, sepsis remains a leading cause of neonatal morbidity and mortality, particularly among very low-birth-weight (VLBW) preterm infants. The overall incidence of neonatal sepsis ranges from 1 to 5 cases per 1,000 live births with a mortality rate of approximately 5% to 10%. (1) The Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Network and the Vermont Oxford Network define neonatal early-onset sepsis (EOS) as the onset of signs and symptoms of sepsis with an associated positive culture result at or before age 72 hours. Late-onset sepsis (LOS) is defined as the onset of signs or symptoms of sepsis after age 72 hours. (2) The incidence of EOS in the United States is estimated to be 0.98 cases per 1,000 live births overall and 10.96 cases per 1,000 live births among VLBW infants. (3) In addition, more than one-fifth (21%) of VLBW infants have at least one episode of late-onset culture-proven sepsis. (4)

The diagnosis of neonatal sepsis is challenging because early signs and symptoms are often subtle and nonspecific, yet prognosis depends on early detection and treatment. Furthermore, the symptoms of many noninfectious common neonatal conditions can mimic those of sepsis, complicating the diagnosis of sepsis. (5)

The gold standard for diagnosing sepsis is a positive result on culture from blood or another sterile body fluid, such as cerebrospinal fluid (CSF) or urine. It has been estimated that cases with a positive blood culture result represent less than 40% of all neonatal sepsis (6) because of inadequate blood volume sampled, transient or low-grade bacteremia, or antibiotic transferred from a mother who received antibiotics during the intrapartum period. A single aerobic blood culture of sufficient volume (1 mL) has a 98% probability to isolate an organism even in infants with low-level bacteremia (4 CFU/mL). (7)

The complete white blood cell (WBC) count with differential is routinely used to assist in the diagnosis of sepsis; however, multiple studies have determined that the WBC, immature-to-total neutrophil (I/T) ratio, and platelet count have low sensitivities and specificities. (8)(9) Two very large retrospective, multicenter database studies (8)(9) found that low WBC counts and high I/T ratios were associated with increasing odds of infection...
in EOS. High and low WBC counts, high absolute neutrophil count, high I/T ratios, and low platelet counts were associated with LOS. However, a single blood cell count–derived index did not have proven sensitivity to reliably include or exclude EOS or LOS in neonates. The absolute immature neutrophil count and absolute neutrophil count have suboptimal sensitivity and decreased predictive accuracy for EOS because elevation does not consistently distinguish an inflammatory response from a noninfectious origin. The I/T ratio is a more sensitive indicator of sepsis; however, single assessments have a better negative predictive value (NPV) (99%) than positive predictive value (PPV) (25%). The I/T ratio is elevated in a quarter to half of presumptively uninfected neonates. Overall, neutrophil indexes seem to be more helpful for excluding infants without infection than for including infants with infection. (8)(9)

Given the low incidence of culture-positive sepsis and poor predictive value of individual complete blood cell indexes, rapid diagnostic biomarkers of sepsis to differentiate septic from nonseptic neonates may allow timely discontinuation of antibiotic therapy, thus avoiding their prolonged use and preventing emergence of antibiotic-resistant bacteria. In addition, prolonged empiric antibiotic use has been associated with increased risk of necrotizing enterocolitis, LOS, and death. (10)(11) Recent studies have found improved sensitivity and NPV of various biomarkers of sepsis compared with complete blood cell indexes. (12)(13)(14)(15)(16)(17)(18)

Acute-phase proteins, components of the complement system, chemokines, cytokines, adhesion molecules, and cell surface markers have all been investigated as biomarkers of neonatal sepsis. (14) The most widely studied and most researched markers include C-reactive protein (CRP), interleukin (IL) 6, IL-8, procalcitonin (PCT), and tumor necrosis factor α (TNF-α). In this article, we summarize the role of various biomarkers in the diagnosis of neonatal sepsis.

Ideal Biomarker for Neonatal Sepsis

Because of the high mortality of neonatal sepsis, the clinician’s goal is to identify and treat sepsis as early as possible. An ideal biomarker of neonatal infection would therefore have a very high sensitivity (approaching 100%) to identify all infants with infection and a high NPV (approaching 100%) to confidently rule out infection in those with a negative test result. (12)(14)(15)(18)(19)(20) Conversely, an ideal test would also have a relatively good specificity to minimize the unnecessary use of antibiotics in those with false-positive test results. Studies investigating biomarkers of infection determine appropriate cutoff values for each individual test through the use of a receiver operator characteristic (ROC) curve, which plots sensitivity against the false-positive rate (1–specificity) for a range of potential diagnostic cutoff levels. An example of a ROC curve is shown in the Figure. A test with no diagnostic value would be represented as a straight line from the bottom left-hand corner to the upper right-hand corner and is represented by the dotted line in the Figure. (21) An ideal test would produce a line starting at the bottom left-hand corner and sharply ascend to 100% sensitivity, following the y-axis closely. (21) The inflexion point (the point closest to the left uppermost corner) represents the cutoff point with the highest combined sensitivity and specificity or the highest diagnostic accuracy and is represented as point A in the Figure. Accuracy can be defined as the highest number who are diagnosed correctly as having or not having sepsis divided by the total number studied. (12) Most studies cited in this article and included in the Table use the inflexion point as the appropriate cutoff level from their ROC curves. (22)(23)(24)(25)(26)(27)(28)(29)(30)(31)(32)(33)(34)(35)(36) However, many clinicians would argue that in the case of neonatal sepsis, sensitivity is more important than specificity to positively identify all those with infection and confidently rule out infection in those with a negative test result. (12)(20) This point of highest sensitivity with a lower specificity is represented as point B in the Figure. In general, a specificity greater than 85% is preferred, (14)(15)(18) but some authors would accept a specificity of 50% or higher. (12) It is important to identify a specific cutoff value using a ROC curve for each diagnostic biomarker to allow comparison of results among different neonatal centers. (14)

In addition, an ideal biomarker would be available quickly after the onset of symptoms of infection to discontinue unnecessary antibiotic treatment in nonseptic patients while awaiting culture results.

C-Reactive Protein

CRP is an acute-phase reactant that has been extensively studied as a marker for sepsis. Acting through the humoral immune system, CRP is important in the recognition and clearance of bacterial pathogens. CRP increases within 6 to 18 hours after a stimulus and peaks 8 to 60 hours later. (1)(2)(37) CRP is synthesized by the fetus and newborn, and concentrations were once thought to be unaffected by gestational age. (1) More recent studies, however, have found levels to be decreased in preterm infants. (38)(39) There is minimal transplacental passage of maternal CRP, (1) making it an ideal candidate as a marker of EOS in neonates.

CRP levels increase rather slowly after an infectious stimulus, making its sensitivity low during the early stages of infection. (40) In one study attempting to distinguish
early bacterial sepsis from respiratory diseases of the newborn, CRP at the time of presentation was of no diagnostic value with a sensitivity of 33%. (24) Ng et al (23) serially measured a combination of CRP and other cytokines in VLBW infants suspected of having LOS and found that of 101 suspected episodes of sepsis (45 ultimately with proven infection) CRP (using a cutoff value of 12 mg/L [114.3 nmol/L]) had a poor sensitivity (60%) despite excellent specificity (100%) at the time of initial sepsis workup.

CRP, however, was the best single marker at 24 and 48 hours, with an overall sensitivity of 84% and a specificity of 96%. Benitz et al (40) measured serial CRP levels in newborns evaluated for infection and found that initial CRP had poor sensitivity (35%-65%); however, sensitivity increased with serial measurements (98%). They also found that 3 serial normal CRP levels had high NPV for EOS (99.7%) and LOS (98.7%). Therefore, although the sensitivity of a single CRP level is not sufficient to justify withholding antibiotics, 3 consecutive normal values indicate that sepsis is highly unlikely. (40)

CRP can be used as a specific and late biomarker of neonatal infection; (14) in fact, CRP is more sensitive and specific in diagnosing neonatal sepsis than the more commonly used modalities of the total neutrophil count and I/T ratio. (41) However, caution should be used because the CRP level is also elevated in noninfectious conditions, such as meconium aspiration, intraventricular hemorrhage, and perinatal asphyxia. (37)(39)(40)(42)

Figure. Receiver operating characteristic curve. See text for details.

**Interleukin 6**

IL-6 is a proinflammatory cytokine produced by mononuclear phagocytes, endothelial cells, and fibroblasts in response to inflammation. Its level peaks 2 to 3 hours after stimulation and returns to baseline after 6 to 8 hours. (42) IL-6 is the major inducer of hepatic acute-phase protein synthesis, including CRP and fibrinogen. (43) The concentration of IL-6 is not influenced by gestational age. (32)(38)(42) It has a high sensitivity in diagnosing both EOS and LOS. (23)(24)(28)(29)(30) (31)(32)(33)(34)(35)(36)(38)(44)

Umbilical cord blood levels of IL-6 are elevated in newborn infants with EOS. (32)(36) Messer et al (32) prospectively measured IL-6 plasma concentrations in 157 newborn infants on admission to the neonatal intensive care unit and in the cord blood of 131 newborns. The sensitivity and NPV of IL-6 in diagnosing blood culture–positive and clinical sepsis was 100% in those infants who had an early measurement of IL-6 at birth (cord blood) or within 1 hour after birth. In addition, they found that high sensitivity and NPV persisted until the 12th hour of age. Krueger et al (36) reported umbilical cord IL-6 to have a sensitivity of 87% and a specificity of 90% in 71 term and 100 preterm infants. When only preterm infants were included in the analysis, IL-6 sensitivity increased to 96% and specificity increased to 94%. Kuster et al (33) serially measured IL-6 levels in 101 VLBW infants who were older than 48 hours and found a sensitivity of 86% and a specificity of 83% on the day of diagnosis.

Ng et al (23) serially measured biomarkers in VLBW infants with suspected LOS (>72 hours). At the onset of infection (day 0), IL-6 was found to be the single most accurate marker of LOS, with a sensitivity of 89% and a specificity of 96%. The sensitivity and specificity of IL-6 decreased to 67% and 89%, respectively, 24 hours later (day 1) and to 58% and 84%, respectively, 48 hours later (day 2) (Table). Attempting to distinguish early bacterial sepsis from respiratory diseases of the newborn, Kallman et al (24) measured IL-6 at the time of presentation in newborns being evaluated for sepsis or respiratory symptoms. They found that IL-6 could be useful in distinguishing proven and clinical sepsis from transient tachypnea of the newborn but not from respiratory distress syndrome.
## Characteristics of Published Studies of Biomarkers of Neonatal Sepsis

<table>
<thead>
<tr>
<th>Source</th>
<th>Definition of Sepsis</th>
<th>No. of Patients With Sepsis</th>
<th>No. of Controls (n)</th>
<th>Sample Studied</th>
<th>Diagnostic Test</th>
<th>Cutoff Concentration</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buck et al [44]</td>
<td>a. Blood culture–positive sepsis: blood culture positive with abnormal WBC count and CRP level</td>
<td>11</td>
<td>54</td>
<td>Term and preterm newborns</td>
<td>IL-6</td>
<td>&gt;10 pg/mL</td>
<td>73</td>
<td>85</td>
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<td></td>
<td>b. Clinical sepsis: abnormal WBC and CRP level with ≥3 clinical signs</td>
<td>15</td>
<td></td>
<td>IL-6</td>
<td>CRP</td>
<td>≥10 mg/L</td>
<td>73</td>
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<td></td>
<td>c. Infection: abnormal WBC count and CRP level with &lt;3 clinical signs</td>
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<td></td>
<td>IL-6</td>
<td>CRP</td>
<td>≥10 mg/L</td>
<td>68</td>
<td>85</td>
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<tr>
<td></td>
<td>d. Blood culture–positive sepsis, clinical sepsis and infection combined</td>
<td>67</td>
<td></td>
<td>IL-6</td>
<td>CRP</td>
<td>≥10 mg/L</td>
<td>73</td>
<td>85</td>
<td>86</td>
<td>72</td>
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<tr>
<td>Messer et al [32]</td>
<td>Infected clinical symptoms, positive blood and/or CSF culture result, abnormal WBC count and CRP level, and probably infected (clinical symptoms, abnormal WBC count and CRP level)</td>
<td>&lt;1 Hour</td>
<td>18</td>
<td>181 Term and preterm newborns</td>
<td>IL-6</td>
<td>&gt;100 pg/mL</td>
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<td>All</td>
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<td>IL-6</td>
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<td>CRP + IL-6</td>
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<td>IL-6</td>
<td>CRP</td>
<td>≥15 mg/L + &gt;100 pg/mL</td>
<td>100</td>
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<tr>
<td>Ng et al [23]</td>
<td>2 Positive blood cultures; pneumonia with clinical symptoms, positive chest radiographic result, and 2 positive sputum cultures; positive culture from sterile site (peritoneal fluid, CSF, systemic fungal infection); NEC (with or without positive blood culture)</td>
<td>45</td>
<td>56</td>
<td>VLBW &gt;72 hours day 0, initial sepsis evaluation</td>
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<td>TNF-α</td>
<td>CRP + TNF-α</td>
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<th>Diagnostic Test</th>
<th>Cutoff Concentration</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
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<td>Doelner et al (38)</td>
<td>Sepsis (clinical signs and positive blood or CSF culture result), clinical sepsis (clinical signs with elevated I/T ratio and CRP level), pneumonia (chest radiographic abnormalities with elevated I/T ratio and CRP level)</td>
<td>24</td>
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Table. (Continued)
### Table. (Continued)

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<td>a. Proven sepsis: clinical sign(s) and positive blood or CSF culture result</td>
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<td>Franz et al b,c (27)</td>
<td>a. Proven sepsis: clinical sign(s) or maternal amniotic infection and positive blood or CSF culture result</td>
<td>7f/17f</td>
<td>30f/219f</td>
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<td>Martin et al (35)</td>
<td>Positive blood or CSF culture result or clinical symptoms (oliguria, metabolic acidosis, or hypoxemia) and abnormal WBC count and CRP level on admission and/or at 24 hours</td>
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<td>Laborada et al (28)</td>
<td>a. Defined: clinical suspicion with positive blood, CSF, or urine culture result</td>
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Table. (Continued)

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<th>Source</th>
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<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
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<td>Santana Reyes et al (29)</td>
<td>Positive blood culture result with compatible clinical symptoms using a clinical score for sepsis</td>
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<td>Franz et al (22)</td>
<td>Culture-proven infection (≥1 clinical sign, positive blood culture result and CRP level &gt;10 mg/L, ≥95.2 nmol/L 12–60 hours after initial evaluation), and clinical infection (≥1 clinical sign, and CRP level &gt;10 mg/L, ≥95.2 nmol/L 12–60 hours after initial evaluation)</td>
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Continued
Buck et al (44) studied IL-6 levels in 222 term and preterm infants and found IL-6 to have a sensitivity of 73% and 87% in diagnosing blood culture–positive sepsis and clinical sepsis, respectively. In this study, 3 of the 11 infants with blood culture–proven sepsis and 2 of the 15 infants with clinical sepsis had undetectable IL-6 levels on admission; however, all the infants in these 2 groups with negative IL-6 levels had elevated CRP levels on admission. These results suggest that the IL-6 test result was already negative because of its short half-life but the level was elevated before the elevated CRP level because IL-6 stimulates CRP production. IL-6 had a poor sensitivity at initial evaluation in the study by Prashant et al; (31) however, when survivors were compared with nonsurvivors, IL-6 was found to predict mortality with a sensitivity of 100% at 48 hours after antibiotics using a cutoff value of 109 pg/mL. This study defined sepsis as a positive blood culture and controls as an initial suspicion of sepsis but a negative culture result. Because of its rapid decrease to baseline, IL-6 can be considered an early and sensitive biomarker of neonatal sepsis. (14)

Interleukin 8
IL-8 is a proinflammatory cytokine produced by monocytes, macrophages, and endothelial cells and has kinetics similar to IL-6. (14) IL-8 levels are not affected by gestational or postnatal age of infants. (14)(15)(26) Similar to IL-6, IL-8 has been found to be more sensitive but less specific than CRP at the onset of suspected infection. (21)(25)(26)(27)(28)(31)(45) In addition, those studies that measured serial IL-8 levels found sensitivity to decrease and specificity to increase at 24 to 48 hours compared with the time of initial evaluation. (28)(31)

Santana et al (46) found IL-8 cord blood levels to be a good predictor of EOS with a sensitivity of 78%, specificity of 91%, PPV of 100%, and NPV of 84%. Berner et al (45) likewise found cord blood levels of IL-8 to be significantly elevated in septic newborn infants, with a sensitivity of 91% and a specificity of 93% using a cutoff value of 300 pg/mL. Orlikowsky et al (47) compared detergent-lysed whole-blood IL-8 to standard plasma IL-8 concentrations in diagnosing neonatal sepsis and found that sensitivity improved to 97% from 71% and specificity increased to 95% from 93% at 6 hours after the onset of sepsis. Furthermore, they reported a laboratory result turnaround time of only 50 minutes and the need for only a small volume of blood (50 μL).

Tumor Necrosis Factor α
TNF-α is a very early proinflammatory cytokine that stimulates IL-6 production (20) and is elevated in neonatal sepsis. (48) A meta-analysis (49) revealed that TNF-α is
moderately accurate in the diagnosis of EOS (sensitivity, 66%; specificity, 76%) and LOS (sensitivity, 68%; specificity, 89%). Although TNF-α is an important mediator in the inflammatory response, it is much less useful as a diagnostic biomarker of neonatal sepsis than other cytokines, such as IL-6 and IL-8. (21)(23)(32)(45)(46)(48)(50)

Procalcitonin

PCT is an acute-phase reactant produced by monocytes and hepatocytes 4 to 6 hours after exposure to bacterial products. (16)(17) Because PCT levels increase physiologically during the first few days after birth, (17)(39) its usefulness as a biomarker of EOS is limited. Age- and gestation-specific reference intervals have been determined by Chiesa et al, (39) who found preterm infants to have an earlier and higher peak PCT level compared with term infants.

In a recent meta-analysis that involved 1,959 neonates, PCT had a pooled sensitivity of 81% and a specificity of 79% in diagnosing neonatal sepsis. For EOS, PCT had a pooled sensitivity of 76% and a specificity of 76%. For LOS, PCT had a pooled sensitivity of 90% and a specificity of 88%. Although this analysis revealed PCT to have high diagnostic accuracy, the authors noted marked statistical heterogeneity in the included studies and urged caution in the interpretation of the results. (51)

Combination of Biomarkers

Because there is no single biomarker that can be used to reliably diagnose neonatal sepsis, many investigators have used combinations of biomarkers to improve the sensitivity and specificity in diagnosing EOS and LOS. IL-6 and IL-8 appear to be the most promising early markers of infection; (17) however, because levels of these cytokines decrease quickly after initial response, ideal efficacy may be achieved when used in combination with CRP levels, which increase more slowly but remain elevated for a longer period.

In newborn infants, Buck et al (44) found the sensitivity of IL-6 in CRP-negative newborns and the sensitivity of CRP in IL-6-negative newborns on admission to be 100% in newborns with blood culture–positive or clinical sepsis. They concluded that the combination of IL-6 and CRP is the ideal tool for the early diagnosis of neonatal infection. One weakness of this study is that it is unclear how the cutoff value of 10 pg/mL for IL-6 was chosen because no ROC curve was generated. Doellner et al (38) similarly concluded that the combination of IL-6 and CRP is superior in diagnosing neonatal sepsis than either parameter alone. However, this study chose the cutoff value of 50 pg/mL for IL-6 without a ROC curve.

(38) Laborada et al also concluded that the combination of IL-6 and CRP is the best diagnostic test for the detection of sepsis in the first 24 hours in their study of 105 term and preterm infants. (28)

Franz et al (25) found the combination of IL-8 and CRP to be more reliable than PCT to diagnose EOS in neonates with a sensitivity of 91% and a specificity of 73%. This same group has since published several different studies in which they were able to reduce unnecessary antibiotic therapy in newborns with the use of IL-8 and CRP. (22)(26)(27)

In the study evaluating EOS in 709 term and preterm newborns, the study population was divided into 2 periods. (27) In the first period, IL-8 levels were analyzed retrospectively in infants with suspected EOS who were prescribed antibiotics when they had an elevated CRP or I/T ratio per the unit routine standard. The sensitivity and specificity of IL-8 and CRP combined were 92% and 74%, respectively, in diagnosing culture-proven and clinical EOS. In the second period, infants with suspected EOS were prescribed antibiotics if IL-8 and/or CRP levels were elevated. The sensitivity and specificity of IL-8 and CRP combined were 92% and 77%, respectively, in diagnosing culture-proven and clinical EOS. Of note, in both study periods, infants with presumed septic shock were prescribed antibiotics immediately before laboratory results were available. The authors reported an apparent reduction of unnecessary antibiotics of 40% in the second period.

In the study evaluating LOS by the same group (26) in 1,386 term and preterm newborns, the study design and methods were identical to the EOS group. (27) In the first period, the sensitivity and specificity of IL-8 and CRP combined were 93% and 80%, respectively, in diagnosing culture-proven and clinical LOS. In the second period, the sensitivity and specificity of IL-8 and CRP combined were 100% and 83%, respectively, in diagnosing culture-proven and clinical LOS. Again, of note, in both study periods, infants with presumed septic shock were prescribed antibiotics immediately before laboratory results were available. The authors reported an apparent reduction of unnecessary antibiotics of 73% in the second period.

In a multicenter randomized clinical trial by the same group, (22) IL-8 and CRP were used to reduce unnecessary antibiotic therapy in a study of 1,291 term and preterm infants with suspected EOS. There was a 13.5% reduction in unnecessary antibiotic treatment, and IL-8 needed to be measured in 7 patients with suspected infection to save 1 patient from unnecessary antibiotic treatment.

In newborn infants, Buck et al (44) found the sensitivity of IL-6 in CRP-negative newborns and the sensitivity of CRP in IL-6-negative newborns on admission to be 100% in newborns with blood culture–positive or clinical sepsis. They concluded that the combination of IL-6 and CRP is the ideal tool for the early diagnosis of neonatal infection. One weakness of this study is that it is unclear how the cutoff value of 10 pg/mL for IL-6 was chosen because no ROC curve was generated. Doellner et al (38) similarly concluded that the combination of IL-6 and CRP is superior in diagnosing neonatal sepsis than either parameter alone. However, this study chose the cutoff value of 50 pg/mL for IL-6 without a ROC curve.
Important to note, however, is that the numbers of infants with infection who were not detected at initial evaluation were similar between the groups, with 14.5% missed in the IL-8 group and 17.3% missed in the standard group.

Other investigators have proposed more complex combinations. One drawback of this approach currently is its limited clinical applicability. Silveira and Procanoy (34) measured IL-6, TNF-α, and IL-1β in 117 newborns who were younger than 5 days and found that the combination of IL-6 and TNF-α provided a sensitivity of 98.5% in diagnosing neonatal sepsis. Ng et al (23) serially measured a combination of cytokines, CRP, and E-selectin and found that the combination of IL-6 and CRP on day 0 with either TNF-α on day 1 or CRP on day 2 had the best overall sensitivity (98%), specificity (91%), PPV (90%), and NPV (98%) for the diagnosis of LOS in VLBW infants. (23) Kuster et al (38) serially measured IL-1 receptor antagonist, IL-6, CRP, and circulating intercellular adhesion molecule 1 in 101 VLBW infants who were older than 48 hours and concluded that IL-1 receptor antagonist and IL-6 can together be used to predict neonatal sepsis 1 or more days before clinical diagnosis.

In summary, biomarkers of sepsis are screening tests that may augment clinical evaluation and possibly allow early discontinuation of antibiotic treatment in well-appearing neonates. None has been proven sufficiently accurate to restrict initiation of empiric antibiotic treatment. In a sick-appearing neonate, despite a negative screening test result, antimicrobial therapy should not be delayed. Clinical signs of sepsis remain the most important criteria for the use of antimicrobial agents.

Conclusion

Although multiple studies have revealed a positive correlation between biomarkers and neonatal infection, clinicians are hesitant to withhold antibiotic treatment because no currently known biomarker or combination of biomarkers can consistently diagnose 100% of infected cases. Furthermore, essentially all biomarkers researched are nonspecific for infection and can be elevated in other inflammatory processes. However, some biomarkers, when used in combination, have had high NPVs and may be helpful in the decision to discontinue antibiotic therapy in an asymptomatic neonate while awaiting culture results.

Reports in the literature on the use of biomarkers of sepsis, such as CRP, IL-6, IL-8, PCT, and TNF-α, to aid in the diagnosis of neonatal sepsis are inconsistent. Most studies involve small sample sizes at single medical centers, which makes it difficult to apply the results to a broader neonatal population. There is much variation in study design, often including EOS and LOS together and infants of various gestational ages. In addition, the differing methods of laboratory measurement and different cutoff values of the same marker further limit the clinical applicability. More important, the definition of sepsis varies widely across studies and the population chosen as the controls (healthy neonates vs infants initially evaluated for sepsis but ultimately determined to be not infected), which can significantly influence statistical data on inflammatory biomarkers.

In summary, biomarkers of sepsis are screening tests that may augment clinical evaluation and possibly allow early discontinuation of antibiotic treatment in well-appearing neonates. None has been proven sufficiently accurate to restrict initiation of empiric antibiotic treatment. In a sick-appearing neonate, despite a negative screening test result, antimicrobial therapy should not be delayed. Clinical signs of sepsis remain the most important criteria for the use of antimicrobial agents.

References


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Case 1: Respiratory Distress and Poor Perfusion Within 24 Hours of Delivery

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Case Presentation

A term male infant born to a second gravida mother is referred to our hospital for new-onset respiratory distress. He was born by normal vaginal delivery and had Apgar scores of 8, 9, and 9 at 1, 5, and 10 minutes, respectively. The newborn begins breastfeeding immediately after birth and is shifted to the mother’s side. After 18 hours of delivery, he is noted to have fast breathing. A pediatric consultation is sought; a provisional diagnosis of congenital heart disease is made, and the patient is referred to our hospital. The antenatal period is uncomplicated. The results of targeted imaging for fetal anomalies performed at 20 weeks of gestation are reportedly normal. At admission, the newborn weighs 2.74 kg and has a temperature of 96.8°F (36°C), a heart rate of 175 beats per minute, a respiratory rate of 70 breaths per minute, and a blood pressure in the right upper limb of 58/26 mm Hg. The capillary filling time is prolonged to 4 seconds with weak and feeble pulses, and there is moderate to severe intercostal and substernal recessions with an audible grunt. The baseline saturation is 85% in room air, which improves to greater than 95% on hood oxygen. On systemic examination, he has an increase in precordial activity, visible jugular vein pulsations, systolic murmur (grade III), liver 4 cm below the costal margin, no crepitations or wheeze, depressed sensorium, and normal level anterior fontanel.

The infant is immediately given ventilatory support, intravenous fluids, antibiotics, diuretics, and inotropes. On investigation, the total leukocyte count is 11,800/µL (11.8 × 10⁹/L) with 48% neutrophils, the C-reactive protein level is 0.2 mg/dL (1.9 nmol/L), and arterial blood gas reveals pH 6.9, PaO₂ of 34 mm Hg, PaCO₂ of 24 mm Hg, and base excess of −26 mEq/L (−26 mmol/L). Chest radiography reveals massive cardiomegaly with a cardio-thoracic ratio (CT) ratio of 0.8 (Figure 1). Functional echocardiography reveals dilation of all 4 chambers with right more than left, deviation of interventricular septum to left, tricuspid and mitral regurgitation, and moderate-sized duc--tus arteriosus with bidirectional shunt. The inferior vena cava is dilated, and the rest of the intracardiac anatomy is normal. A clinical feature identified via another investigation performed at this stage confirms the diagnosis.

Case Discussion

Early-onset respiratory distress with tachycardia, feeble pulses, delayed capillary filling time, hepatomegaly, and cardiomegaly confirmed cardiogenic shock. Echocardiographic features of dilated cardiac chambers with septal deviation, tricuspid regurgitation, and bidirectional shunt in ductus arteriosus were suggestive of persistent pulmonary hypertension of the newborn. Clinical features, the findings on chest radiography, and 2-dimensional echocardiography ruled out the possibilities of obstructive total anomalous pulmonary venous connection, Ebstein anomaly, coarctation of aorta, and congenital or

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aspiration pneumonia. Presentation with the first 24 hours and no seizures or vomiting at onset were odd for the diagnosis of inborn errors of metabolism.

Bruit heard over the skull and fontanels with clinical features of hyperdynamic circulation (precardial activity and visible jugular pulsations), all-chamber dilatation on the echocardiogram, and increased flow in the superior vena cava with head ultrasonography revealing an echolucent structure in the posterior region of the midline superior to the tentorium cerebellum and thalami confirmed the diagnosis of vein of Galen malformation (VOGM) (Fig 2). Doppler ultrasonographic evaluation revealed high-velocity blood flow situated posterior to the pineal gland with multiple feeders to the vein of Galen (Video). Pulmonary hypertension was secondary to increased blood flow and high-output cardiac failure.

The Condition

VOGM was first described in 1895 by Steinhel, who referred to it as varix aneurysm. It accounts for less than 1% of arteriovenous malformations and is estimated to be seen in only 2.5 of 100,000 live births. VOGM manifests in the neonatal period in approximately 60% of all pediatric cases. (1) VOGMs arise as a result of direct arteriovenous communications between the primitive choroidal arterial network and the median prosencephalic vein on Markowski. The abnormal flow causes persistence of embryonic vein and prevents the development of the vein of Galen. The principal feeder vessels to VOGMs are posterior choroidal artery, anterior choroidal artery, middle cerebral artery, anterior cerebral artery, and posterior cerebral artery. (2)

The pathologic features observed with VOGMs consist of ischemic, hemorrhagic mass effects of the malformation and of restriction of cerebrospinal fluid absorption. The cerebral ischemia is due to intracranial “steal” phenomena, and manifestations include hydrocephalus, cerebral edema, and hypoxia, leading to neonatal seizures. Hemorrhages are due to thrombosis of the dilated vein of Galen, causing subarachnoid hemorrhage, germinal
matrix–intraventricular hemorrhage, and intracerebral hemorrhage. Mass effects included hydrocephalus secondary to obstruction, usually at the level of aqueduct and compression of other structures. Neonates usually present as asymptomatic cardiomegaly to high-output severe congestive cardiac failure that is refractory to medical management. Cardiomegaly, cyanosis, low-volume peripheral pulses, bounding carotids, continuous cranial bruit (which is more prominent on posterior cranium), and prerenal azotemia are the common clinical clues. Malformations such as supernumerary digits, hypospadias, transposition of great vessels, coarctation of aorta, Turner syndrome, and blue rubber bleb nevus syndrome are associated with it. (3)

Diagnosis
VOGMs should be suspected in neonates with unexplained high-output congestive cardiac failure. Ultrasonography is an important modality that enables the detection of VOGMs. Doppler ultrasonography can be used to reveal the hemodynamic changes associated with the malformation as in our index case. Contrast-enhanced axial computed tomography of the brain usually reveals a well-defined, multilobulated, intensely enhancing lesion located within the cistern of velum interpositum. In contrast-enhanced computed tomography, the presence of a central thrombus and peripheral circulating blood produces a pathognomonic target sign. Magnetic resonance imaging is gaining popularity as the modality of choice for initial assessment of VOGMs. Magnetic resonance angiography remains the gold standard for diagnosis of VOGMs. (4)

Management
VOGM is termed the Gordian knot of cerebrovascular surgery. Lesions are embolized from the arterial side to avoid venous hypertension using embolic agents, such as coils, cyanoacrylates, and detachable balloons. Transvenous and transtorcular coil embolization of the venous sac have been used to achieve flow reduction in high-flow fistulas and multiple fistulas. (5)(6)

Clinical Course of the Index Newborn
The newborn was treated with diuretics, restricted fluids, and inotropes. He had seizures that were controlled with phenobarbital. Because the management involved invasive procedure with poor long-term prognosis, parents opted for no surgical intervention, and the infant died after 50 hours after birth.

Lessons for the Clinician
• In all neonates presenting with high-output cardiac failure, auscultation of the skull for bruit and head ultrasonography are necessary.
• In a newborn with high-output cardiac failure, when echocardiography is not suggestive of left to right shunts, we should look for extra cardiac shunts, such as vein of Galen malformation, portosystemic shunt, and arteriovenous malformations.
• Neonates who present with symptomatic vein of Galen should undergo immediate embolization of the vessels, although the outcome is guarded.
References

The reader is encouraged to write possible diagnoses for each case before turning to the discussion. We invite readers to contribute case presentations and discussions by contacting NeoReviewsEditorial@aap.org. Please be sure to include "IOSITN" in the subject line.

Author Disclosure
Drs Todd, McLaughlin, Gallizzi, and Halley have disclosed no financial relationships relevant to this article. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.

Case 2: A 7-Day-Old African American Boy With Fever, Poor Feeding, and Lethargy
Stephanie Todd, MD,* Lauren McLaughlin, MD,† Gina Gallizzi, MD,* Tina Halley, MD*

Case Presentation
A 39-week-old African American male neonate presents to the emergency department 7 days after birth for evaluation of fever, poor feeding, and lethargy. Prenatal and birth histories are unremarkable; however, an aunt with upper respiratory tract viral symptoms has been caring for the infant. Physical examination findings at presentation are normal, but the infant is febrile with a rectal temperature of 38.1°C (100.6°F). Evaluation in the emergency department includes blood, urine, and cerebrospinal fluid (CSF) analysis. Initial complete blood cell count is notable for a white blood cell count of 11,700/μL (11.7 × 10^9/L), hemoglobin level of 14.0 g/dL (140 g/L), hematocrit of 39.0% (0.39), and platelet count of 36 × 10^3/μL (36 × 10^9/L). CSF is notable for a white blood cell count of 14/μL (0% neutrophils, 45% lymphocytes, and 48% monocytes), a red blood cell count of 33/μL, and normal protein and glucose concentrations. The infant is admitted to the hospitalist service while receiving empiric ampicillin and cefotaxime. On hospital day (HD) 2, reverse transcriptase polymerase chain reaction is positive for enterovirus in the CSF; antibiotic treatment is discontinued. The infant remains febrile but is clinically well-appearing without signs of tachycardia, poor perfusion, hepatosplenomegaly, rash, or neurologic deficit. On HD 3, he deteriorates with hepatomegaly and abdominal distention. Further laboratory analysis is significant for elevated liver function test results (aspartate aminotransferase, 519 U/L; alanine aminotransferase, 140 U/L). On HD 4, he develops coagulopathy with a low fibrinogen level (60 mg/dL [1.8 μmol/L]), high prothrombin time and partial thromboplastin time (31.1 and 95.8 seconds, respectively), and an elevated D-dimer level (4.87 μg/mL) consistent with disseminated intravascular coagulation. The patient is transferred to the neonatal intensive care unit. On HD 5, he deteriorates further with ongoing coagulopathy and cytopenias refractory to blood products, including fresh frozen plasma and cryoprecipitate, and significant metabolic acidosis refractory to intravenous bicarbonate and fluid resuscitation. He develops respiratory insufficiency that requires intubation on HD 6. On this same day, a ferritin level is checked and is markedly elevated at 81,820 ng/mL. Treatment for his condition is ordered; however, he dies before initiation of the therapy. Genetic testing is performed to confirm the diagnosis.

Case Discussion
The Diagnosis
The patient is clinically diagnosed as having hemophagocytic lymphohistiocytosis (HLH) because he met the necessary 5 of 8 criteria to establish the diagnosis: fever, splenomegaly, bicytopenias, hypofibrinogenaemia, and elevated ferritin level. An autopsy confirms the diagnosis of enterovirus-associated HLH with

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hemophagocytosis noted in the liver, spleen, and bone marrow (Figs 1 and 2). He has no evidence of myocarditis. Molecular testing is consistent with familial HLH (FHLH) due to a defect in PRFI, a gene that affects perforin expression. This is the most common genetic defect associated with FHLH in African American patients. (1)

The Condition
Enterovirus is one of the most commonly diagnosed infections in the neonatal period, especially in the summer and fall. Its clinical severity ranges from mild, asymptomatic illness to severe, potentially fatal, multisystemic disease, particularly if present in the first 2 weeks after birth. (2)(3) Case fatality rates for severe neonatal disease in these first 2 weeks after birth are generally estimated at up to 40%, but estimates as high as 83% have been reported. (2) Myocarditis and hepatitis with coagulopathy carry the greatest risk of mortality. Fortunately, the prognosis for survivors is good, with few sequelae. Early identification of enterovirus, particularly in septic neonates, is therefore useful to help target therapy and prognosis. Diagnosis is typically made with polymerase chain reaction because of rapid results and often improved sensitivities when compared with viral culture. In neonates, serum, urine, and CSF samples yield sensitivities greater than 90%. (3) Treatment of enteroviral infections is mostly supportive, although immunoglobulin therapy has been used with varying results. Antivirals are still being investigated for use in neonates with life-threatening, multisystemic enteroviral disease.

As stated, the mortality risk of neonates who develop severe enterovirus infection in the first 2 weeks after birth is high. HLH presenting in the neonate also carries a high mortality rate (up to 60%). (4) When HLH is associated with enterovirus in the neonate, the prognosis is similarly poor, with reports of approximately 40% survivability. (4)(5)

HLH is a syndrome of hyperinflammation caused by ineffective and
uncontrolled immune activation of histiocytes, natural killer cells, and cytotoxic lymphocytes, leading to cytokine storm. There are 2 forms of HLH: primary HLH or FHLH, which occurs in a patient with a known gene mutation, and secondary or sporadic HLH, which occurs in those patients who lack an associated genetic mutation. Both types can be triggered by inflammatory conditions, such as infection, malignant tumors, and rheumatologic conditions; infections are a common trigger in the neonatal period. To make a diagnosis of HLH, a genetic mutation or 5 of the following 8 criteria must be present: fever, splenomegaly, bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, low or absent natural killer cell activity, ferritin level greater than 500 ng/mL (1,123 pmol/L), and elevated soluble CD25 level. Similarly, enterovirus can also cause fever, cytopenias, organomegaly, and elevated ferritin levels. Clinically, liver dysfunction and coagulopathy are commonly seen in both HLH and viral-induced neonatal sepsis, making it difficult to recognize one disease entity from the other. (6) The management of these 2 conditions, however, is distinctly different. Controlling or eliminating the infectious trigger is important, but patients with HLH also require control of the hyperinflammatory state with immunotherapy. FHLH is eventually universally fatal without definitive treatment, which is hematopoietic stem cell transplantation.

Lessons for the Clinician
• Neonates presenting with enterovirus within 14 days after birth have a higher risk of mortality when hepatitis with coagulopathy or myocarditis is present.
• In neonates, the overlap in symptoms and clinical presentation between enterovirus and hemophagocytic lymphohistiocytosis (HLH) often makes it difficult to distinguish one disease entity from the other. Neonates with hepatosplenomegaly, cytopenias, and evidence of liver dysfunction should be examined further to exclude HLH. Ferritin is a useful screening test for HLH. A ferritin level greater than 10,000 ng/mL (22,470 pmol/L) is 90% sensitive and 96% specific for HLH. (7)
• Because of the high mortality risk associated with both of these conditions in the early neonatal period, a high index of suspicion and early intervention are paramount to successful treatment and survivability.
• Infections can trigger both familial and secondary HLH. Genetic testing should always be performed to guide future family planning, even if an infectious trigger is identified.

References
Electronic Fetal Monitoring Case Review Series

Electronic fetal monitoring (EFM) is a popular technology used to establish fetal well-being. Despite its widespread use, terminology used to describe patterns seen on the monitor has not been consistent until recently. In 1997, the National Institute of Child Health and Human Development (NICHD) Research Planning Workshop published guidelines for interpretation of fetal tracings. This publication was the culmination of 2 years of work by a panel of experts in the field of fetal monitoring and was endorsed in 2005 by both the American College of Obstetricians and Gynecologists (ACOG) and the Association of Women’s Health, Obstetric and Neonatal Nurses (AWHONN). In 2008, ACOG, NICHD, and the Society for Maternal-Fetal Medicine reviewed and updated the definitions for fetal heart rate patterns, interpretation, and research recommendations. Following is a summary of the terminology definitions and assumptions found in the 2008 NICHD workshop report. Normal values for arterial umbilical cord gas values and indications of acidosis are defined in the Table.

Assumptions From the NICHD Workshop

- Definitions are developed for visual interpretation, assuming that both the fetal heart rate (FHR) and uterine activity recordings are of adequate quality
- Definitions apply to tracings generated by internal or external monitoring devices
- Periodic patterns are differentiated based on waveform, abrupt or gradual (e.g., late decelerations have a gradual onset and variable decelerations have an abrupt onset)
- Long- and short-term variability are evaluated visually as a unit
- Gestational age of the fetus is considered when evaluating patterns
- Components of fetal heart rate FHR do not occur alone and generally evolve over time

Definitions

Baseline Fetal Heart Rate

- Approximate mean FHR rounded to increments of 5 beats/min in a 10-minute segment of tracing, excluding accelerations and decelerations, periods of marked variability, and segments of baseline that differ by >25 beats/min
- In the 10-minute segment, the minimum baseline duration must be at least 2 minutes (not necessarily contiguous) or the baseline for that segment is indeterminate
- Bradycardia is a baseline of <110 beats/min; tachycardia is a baseline of >160 beats/min
- Sinusoidal baseline has a smooth sine wave-like undulating pattern, with waves having regular frequency and amplitude

Baseline Variability

- Fluctuations in the baseline FHR of two cycles per minute or greater, fluctuations are irregular in amplitude and frequency, fluctuations are visually quantitated as the amplitude of the peak to trough in beats per minute
- Classification of variability:
  - Absent: Amplitude range is undetectable
  - Minimal: Amplitude range is greater than undetectable to 5 beats/min
  - Moderate: Amplitude range is 6 to 25 beats/min
  - Marked: Amplitude range is >25 beats/min
Accelerations
- Abrupt increase in FHR above the most recently determined baseline
- Onset to peak of acceleration is <30 seconds, acme is ≥15 beats/min above the most recently determined baseline and lasts ≥15 seconds but <2 minutes
- Before 32 weeks’ gestation, accelerations are defined by an acme ≥10 beats/min above the most recently determined baseline for ≥10 seconds
- Prolonged acceleration lasts ≥2 minutes but <10 minutes

Late Decelerations
- Gradual decrease in FHR (onset to nadir ≥30 seconds) below the most recently determined baseline, with nadir occurring after the peak of uterine contractions
- Considered a periodic pattern because it occurs with uterine contractions

Early Decelerations
- Gradual decrease in FHR (onset to nadir ≥30 seconds) below the most recently determined baseline, with nadir occurring coincident with uterine contraction
- Also considered a periodic pattern

Variable Decelerations
- Abrupt decrease in FHR (onset to nadir <30 seconds)
- Decrease is ≥15 beats/min below the most recently determined baseline lasting ≥15 seconds but <2 minutes
- May be episodic (occurs without a contraction) or periodic

Prolonged Decelerations
- Decrease in the FHR ≥15 beats/min below the most recently determined baseline lasting ≥2 minutes but <10 minutes from onset to return to baseline
- Decelerations are tentatively called recurrent if they occur with ≥50% of uterine contractions in a 20-minute period.
- Decelerations occurring with <50% of uterine contractions in a 20-minute segment are intermittent.

Sinusoidal Fetal Heart Rate Pattern
- Visually apparent, smooth sine wavelike undulating pattern in the baseline with a cycle frequency of 3 to 5/minute that persists for ≥20 minutes.

Uterine Contractions
- Quantified as the number of contractions in a 10-minute window, averaged over 30 minutes.
- Normal: ≤5 contractions in 10 minutes
- Tachysystole: >5 contractions in 10 minutes

Interpretation
A three-tier Fetal Heart Rate Interpretation system has been recommended as follows:
- Category I FHR tracings: Normal, strongly predictive of normal fetal acid-base status and require routine care. These tracings include all of the following:
  - Baseline rate: 110 to 160 beats/min
  - Baseline FHR variability: Moderate
  - Late or variable decelerations: Absent
  - Early decelerations: Present or absent
  - Accelerations: Present or absent
- Category II FHR tracings: Indeterminate, require evaluation and continued surveillance and reevaluation. Examples of these tracings include any of the following:
  - Bradycardia not accompanied by absent variability
  - Tachycardia
  - Minimal or marked baseline variability
  - Absent variability without recurrent decelerations
  - Absence of induced accelerations after fetal stimulation
  - Recurrent variable decelerations with minimal or moderate variability
  - Prolonged decelerations
  - Recurrent late decelerations with moderate variability

Table. Arterial Umbilical Cord Gas Values

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PCO2, mm Hg (35 to 70)</th>
<th>PO2, mm Hg (≥20)</th>
<th>Base Excess, mEq/L ≤-10 to ≥-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference rangea</td>
<td>≥7.20</td>
<td>&lt;60</td>
<td>≥20</td>
<td>≤-10 to ≥-10</td>
</tr>
<tr>
<td>Respiratory acidosis</td>
<td>&lt;7.20</td>
<td>&gt;60</td>
<td>Variable</td>
<td>≤-10</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>&lt;7.20</td>
<td>&lt;60</td>
<td>Variable</td>
<td>≥-10</td>
</tr>
<tr>
<td>Mixed acidosis</td>
<td>&lt;7.20</td>
<td>&gt;60</td>
<td>Variable</td>
<td>≥-10</td>
</tr>
<tr>
<td>Patient</td>
<td>7.33</td>
<td>51.0</td>
<td>15.0</td>
<td>-0.1</td>
</tr>
</tbody>
</table>

– Variable decelerations with other characteristics, such as slow return to baseline
– Category III FHR tracings: Abnormal, predictive of abnormal fetal acid-base status and require prompt intervention. These tracings include:
  – Absent variability with any of the following:
    ■ Recurrent late decelerations
    ■ Recurrent variable decelerations
    ■ Bradycardia
  – Sinusoidal pattern


We encourage readers to examine each strip in the case presentation and make a personal interpretation of the findings before advancing to the expert interpretation provided.

**Case Presentation**
**History**
A 34-year-old, gravida 1, para 0 woman at 29 weeks’ 6 days’ gestation presents for comprehensive fetal anatomy ultrasonography with previous findings of an echogenic intracardiac focus. She had an abnormal first trimester screen result with a 1:45 risk for Down syndrome that was reduced to less than 1:10,000 after reassuring cell-free fetal DNA screening results. She also had a normal maternal serum α-fetoprotein level at 15 weeks’ gestation. She had no significant medical, surgical, family, or social history.

During her comprehensive anatomy ultrasonography, the fetus was noted to be appropriately grown (1,272 g; 33%) with normal amniotic fluid and a left lateral placenta. A spontaneous fetal deceleration was visualized, lasting greater than 90 seconds with a fetal heart rate below 100 beats per minute. No evidence of nuchal cord or abnormal placental cord insertion was noted. She was then placed on continuous monitoring for 1 hour, and no additional decelerations were noted. The tracing is shown in Figure 1.

![Figure 1. EFM strip 1.](image-url)
Findings from EFM Strip #1 are as follows:

- Variability: Moderate
- Baseline rate: 140 beats per minute
- Episodic pattern: None
- Periodic pattern: None
- Uterine contractions: Irregular, mild per palpation
- Interpretation: Category I, normal tracing, predictive of normal acid-base status
- Differential diagnosis: Transient compression of the umbilical cord during ultrasonography
- Action: It was decided that she would return for twice weekly nonstress tests (NSTs) for the next 2 weeks, and further follow-up would be determined at that time.

Case Progression
The patient returned for twice weekly NSTs, which revealed no decelerations and remained category I tracings. She presented for her scheduled NST appointment at 32 weeks’ gestation with symptoms of leakage of fluid and was found to have a blood pressure of 160/98 mm Hg. She was sent to the labor and delivery room for evaluation.

On presentation, her blood pressure was 178/106 mm Hg, heart rate was 75 beats per minute, respiratory rate was 18 breaths per minute, and temperature was 98.4°F (36.9°C). She denied symptoms of headache or right upper quadrant pain. She noted occasional episodes of spots in her vision. She reported leakage of fluids for the last 5 days. A sterile speculum examination was performed, which confirmed intact membranes, and a vaginal examination revealed a closed, 0% effaced cervix at zero station and posterior position. Her fetal tracing at admission was category I with a baseline heart rate of 140 beats per minute. Her blood pressures were observed on bedrest and returned to 160/110 mm Hg or less without antihypertensives. Laboratory studies were performed to evaluate for preeclampsia, and the results were within normal limits except for a platelet count of 144 × 10^9/L (144 × 10^9/L) (reference range, 150-400 × 10^9/L), a mildly elevated aspartate aminotransferase level (43 U/L; reference range, 0-41 U/L), and an elevated spot urine protein to creatinine ratio of 0.48 mg/dL (reference range, <0.2 mg/dL). Given concern for preeclampsia, she was given her first dose of betamethasone (12.5 mg intramuscularly).

Figure 1. EFM strip 1.

Figure 2. EFM strip 2.
The patient ambulated to the restroom, and on returning to bed and being placed on the fetal monitor, the patient had an audible fetal deceleration with a nadir of 75 beats per minute lasting for approximately 4 minutes. The patient was repositioned with resolution of the deceleration. The fetal heart tracing afterward had minimal to moderate variability and a baseline heart rate of 150 beats per minute and returned to a category I tracing 10 minutes after the deceleration. Approximately 3 hours later, another prolonged deceleration was noted to 60 beats per minute that lasted for 4 minutes followed by another similar deceleration an hour later. The patient was repositioned, given supplemental oxygen by face mask, and given fluid boluses during this time. Frequent uterine activity was noted but not perceived by the patient. The tracing of the third deceleration is shown in Figure 2.

Findings from EFM Strip #2 are as follows:

- Variability: Moderate in the beginning of the tracing to minimal in the last few minutes
- Baseline rate: 140 beats per minute
- Episodic pattern: None
- Periodic pattern: Prolonged deceleration with a nadir to 50 beats per minute
- Uterine contractions: Unable to determine, palpation is required to assess contraction frequency and intensity, although not perceived by patient
- Interpretation: Category II
- Differential diagnosis: Tachysystole, umbilical cord compression, abnormal placental umbilical cord insertion
- Action: The patient was repositioned, given oxygen by face mask, and received a bolus of fluid with fetal tracing returning to category I

Because of the occurrence of these fetal decelerations in the setting of preeclampsia, the possible need for urgent delivery was discussed with the patient and her significant other; however, continued observation was recommended to allow for antenatal corticosteroid administration. The fetal tracing after the deceleration is shown in Figure 3.
Findings from EFM Strip #3 are as follows:

- Variability: Moderate
- Baseline rate: 140 beats per minute
- Episodic pattern: None
- Periodic pattern: None
- Uterine contractions: Every 2 to 8 minutes, mild by palpation
- Interpretation: Category I
- Differential diagnosis: Normally oxygenated infant
- Action: Continued fetal monitoring

Repeat vaginal examination findings were found to be unchanged. For the next couple hours, blood pressures remained 160/110 mm Hg or less with rare systolic blood pressure in the low 160s mm Hg. The patient continued to be asymptomatic. The following tracing was then noted in Figure 4.

Findings from EFM Strip #4 are as follows:

- Variability: Moderate in the beginning of the tracing to minimal in the last few minutes
- Baseline rate: 150 beats per minute
- Episodic pattern: None
- Periodic pattern: Prolonged deceleration with nadir to 50 beats per minute
- Uterine contractions: Unable to determine, palpation is required to assess contraction frequency and intensity, although not perceived by the patient
- Interpretation: Category II
- Differential diagnosis: Tachysystole, umbilical cord compression, abnormal umbilical cord insertion
- Action: The patient was repositioned and fetal status returned to overall reassuring with moderate variability

Because of the recurrent nature of the apparently spontaneous and prolonged fetal heart rate decelerations, a cesarean delivery was performed out of concern for fetal well-being while the tracing was reassuring rather than to wait for another deceleration and a more emergent procedure. The overall suspicion was for a velamentous cord insertion (VCI). The patient’s blood pressures remained 160/110 mm Hg or less, and she continued to be asymptomatic from...
a preeclampsia perspective. The pediatric team was called and available at the time of delivery because of the category II tracing and prematurity.

**Outcome**

A viable female weighing 1,415 g was delivered by primary low transverse cesarean delivery, with Apgar scores of 8 at 1 minute and 9 at 5 minutes. A VCI with vessels traversing a 4-cm portion of membranes to an accessory lobe was found (Figure 5). No abruption was noted. The umbilical arterial cord gas analysis revealed no evidence of acid-base disturbance (Table). The infant was transferred to the neonatal intensive care unit because of prematurity, required supplemental oxygen via high-flow nasal cannula, and was weaned to room air by day 5. She was diagnosed as having mild stage I retinopathy of prematurity bilaterally and hyperbilirubinemia that resolved with standard phototherapy. The mother received 24 hours of magnesium sulfate for seizure prophylaxis post partum because of preeclampsia with severe features based on blood pressure criteria. Her blood pressures were managed with labetalol, 200 mg twice daily, and nifedipine XL, 30 mg once daily. The mother was stable for discharge home on postoperative day 4. The infant was discharged home on day 31.

**Discussion**

Most umbilical cords insert into the placenta in a central location. VCI occurs when the umbilical vessels insert into the amnion and chorion before they reach the placental margin and are not protected by the Wharton jelly. The umbilical vessels can also insert into the placental edge, termed a *marginal cord insertion*. The prevalence of VCI is approximately 1.5% and marginal insertion approximately 6.3% in singleton gestations. (1) Abnormalities of umbilical cord insertion are seen more commonly with multiple gestations and pregnancies achieved with assisted reproductive technology. (2)

Umbilical cords that lack Wharton jelly are more vulnerable to injury and compression. Cord occlusion causes increases in fetal afterload and decreases in fetal arterial
oxygen content, leading to a vagal reflex and bradycardia. (3) Accordingly, variable decelerations are seen in higher frequency with VCI compared with normal cord insertion and may be seen in the setting of uterine quiescence, likely as a response to fetal movement. (4)(5) Abnormal cord insertion has been associated with fetal growth restriction, preterm labor, abnormal intrapartum fetal heart rate pattern, placental abruption, and neonatal death. (6) It has been recommended that pregnancies complicated by VCI should be monitored with antenatal testing and growth surveillance and fetal monitoring intrapartum. (7) Because of the increased frequency of decelerations noted in these gestations, there is an increased risk of cesarean delivery. (4)

References

American Board of Pediatrics Neonatal–Perinatal Content Specification
• Know the significance, interpretation, and management of abnormalities or changes in fetal heart rate patterns during labor (eg variable decelerations, late decelerations, sinusoidal patterns, bradycardia, and tachycardia).
Extremely Preterm Infant, Pronounced Dead, Comes to Life, But Outcome Is Compromised

Maureen E. Sims, MD

A 23³/₇ weeks¹, 592-g female infant was born to a 19-year-old-mother with a benign prenatal course until the development of preterm labor. When contractions began, the mother was hospitalized and received tocolysis and a full course of antenatal corticosteroids. After 3 days of hospitalization, the membranes spontaneously ruptured, labor progressed, and her footling breech fetus was delivered by emergency cesarean section. The neonatologist discussed medical intervention, comfort care, and data on mortality and morbidity in infants of similar gestation with the parents before delivery. The parents wanted resuscitation and full medical intervention after birth if the estimated gestational age and birth weight before birth were validated after delivery and if no unforeseen findings that would alter the outcome appeared. The fetal heart rate (HR) pattern was unremarkable, with the fetal HR being 145 beats per minute immediately before birth. At delivery, the infant had a similar HR, palpable pulses, and respiratory effort. At 30 seconds after birth, a 2.5-mm endotracheal tube (ETT) was inserted through which positive pressure ventilation was started because the respiratory effort was weak. The HR decreased to 58 beats per minute at 1 minute at which point the neonatologist discontinued the resuscitation. At 40 minutes the nurse recorded a HR of 40 beats per minute. At an hour the parents were informed that their child died and the infant was sent to the morgue. The page in the medical records noting pronouncement of death was absent.

In the morgue a few minutes after arrival while the infant still had the ETT in place, a weak cry was noted by the pathology personnel. The newborn intensive care staff was summoned and noted a weak respiratory effort, some grunting, and a HR of 150 beats per minute. The plaintiff neonatologist pointed out that the ETT could not have been initially correctly placed since neither crying nor grunting can occur when an infant is intubated. The defense neonatologist maintained that the ETT probably was displaced during transport to the morgue. The plaintiff neonatologist contended that a fetus with respiratory effort and a HR of 140 beats per minute at birth that steadily decreased would probably have responded positively to medical intervention providing that the ETT was properly placed. Furthermore, an infant who self-resuscitates despite an hour of deprivation of ventilation, oxygen, fluids, glucose, or thermal intervention supports the excellent reserve and viability of the infant at birth. These findings underscore the lack of appropriate ventilation secondary to esophageal intubation at birth. The plaintiff neonatologist further expressed that although an emergency umbilical

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venous catheter with volume infusion should be part of the resuscitation process when the HR continues to be low, it would not have helped in this situation because ventilation was not achieved.

The infant was brought back from the morgue to the newborn intensive care unit, where she was intubated, placed on the ventilator, and had umbilical catheters placed. The parents were not informed of the long-time deprivation of oxygen, ventilation, and thermal support that the infant incurred for an hour and the poor outcome that would be reasonably expected in this scenario. They were not provided the option of comfort care. The plaintiff neonatologist pointed out that the physician had an ethical obligation to discuss the option of medical intervention at this point and to offer comfort care because the infant’s chances of a very poor outcome were fairly certain. The defense argued that the outcome was dismal regardless of the postdelivery events because the infant was extremely premature. After receiving a bolus of normal saline when umbilical catheters were placed, the first blood pressure was 68/33 mm Hg with a mean of 56 mm Hg, the HR was 140 beats per minute, the respiratory rate was 39 breaths per minute, the temperature was 82.4°F (28°C), and the saturation was 96% on 26% inspired oxygen on low ventilation settings. The first blood gas on the ventilator had the following results: pH, 7.09; $\text{PCO}_2$, 36 mm Hg; $\text{PO}_2$, 158 mm Hg; and base excess, −19 mEq/L (−19 mmol/L).

Her course in the newborn intensive care unit included a spontaneous bowel perforation, a patent ductus arteriosus, and a grade 3 intraventricular hemorrhage with posthemorrhagic progressive ventriculomegaly requiring a ventriculoperitoneal shunt, which became infected. The examination findings of the placenta were normal. On follow-up examinations, the child was profoundly impaired.

The following failures were stated during the mediation conference: failure to properly intubate and ventilate after birth; failure to ensure that the infant was without signs of life before declaring her dead; failure to provide treatment, including monitoring, oxygen, ventilation, fluids, glucose, and maintenance of a thermal neutral environment; and failure to adequately counsel the parents after the infant was determined to be alive in the morgue. The case settled out of court.

**Discussion**

Extremely preterm (<28 weeks’ gestation) infants comprise less than 1% of all live births. Survivors of extreme prematurity have higher rates of many adverse health outcomes compared with term controls, which are carried into early adulthood. However, most survivors lead productive and healthy lives. Adverse outcomes in extremely preterm infants are a function of immaturity, the conditions that cause preterm birth, and postdelivery complications. Disentangling the effects of immaturity from the other components and estimating the contribution of the various entities are difficult, but epidemiologic studies have helped to shed light on this. Most of the studies on outcome of extremely premature infants reflect data from infants born in facilities with comprehensive resuscitation resources and personnel who are sophisticated in the resuscitation process. In the above discussed case, no conditions were identified during prenatal or intrapartum care, such as maternal hypertension, intrauterine growth restriction, anomalies, infection, antepartum hemorrhage or prolonged rupture of the membranes, or fetal distress. This infant was a singleton female who had the benefit of antenatal corticosteroids. Her lack of prenatal and intrapartum identifiable factors underscores the negative effects of the unfortunate events during the first hour after birth that affected her adverse outcome.

**Suggested Reading**


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**American Board of Pediatrics Neonatal–Perinatal Content Specifications**

- Recognize the controversies associated with treating extremely premature infants.
To submit cases for the NeoReviews Visual Diagnosis column, please e-mail a short summary of the case to NeoReviewsEditorial@aap.org, where your summary will be forwarded to the appropriate editor. Be sure to include “VisDx” in the subject line of your email.

Extensive Capillary Malformation and Hemihypertrophy in a 37-Week-Gestation Infant

Grace H. Nam, MD,* T. Allen Merritt, MD, MHA,* Douglas Deming, MD,* Robin D. Clark, MD,* June-Anne Gold, MBBS*

The Case
A newborn at 37\(\frac{5}{7}\) weeks’ gestation with multiple port-wine stains on the face, trunk, and extremities (Figs 1 and 2) presents to the emergency department after transport from the birth hospital.

Prenatal and Birth Histories
- 19-year-old, gravida 1, para 1 woman with pregnancy-induced hypertension at 35 weeks
- Early and consistent prenatal care
- A 20-week ultrasonography revealed hydrocephalus, cysts within the cardiac ventricles, and pericardial effusion
- Prenatal laboratory test results were negative, including for group B Streptococcus

Birth History
- Female infant born at 37\(\frac{5}{7}\) weeks’ gestation by cesarean section (for nonreactive fetal heart tracing, category 2 strip)
- Apgar scores of 6 and 9 at 1 and 5 minutes, respectively
- Infant needed continuous positive airway pressure via T-piece device during resuscitation only

Figure 1. Hemihypertrophy of infant’s right side.

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Birth weight of 3.54 kg (75–90th percentile), head circumference of 37 cm (>97th percentile), and length of 50 cm (50–75th percentile)

Admission to the neonatal intensive care unit because of multiple port-wine stains on face, trunk, and extremities

No family history of port-wine stains

Physical Examination

- Vital signs within normal limits for gestational age
- Infant transitioned to room air with normal oxygen saturations
- Extensive capillary telangiectasia and cutis marmorata on the trunk, back, legs, and arms (1.5 cm long on right leg) (Fig 3)
- A port-wine stain on the right forehead extending across the midlines (Fig 4)
- A 1-cm skin tag in the sacral region with a dimple noted
- No vesicles, rash, or petechiae

Laboratory Studies

- Serum electrolytes, normal
- Blood urea nitrogen, 10 mg/dL (3.6 mmol/L)
- Creatinine, 0.3 mg/dL (26.5 μmol/L)
- Liver function test results, normal
- Cholesterol, 109 mg/dL (2.8 mmol/L)
- Triglycerides, 121 mg/dL (1.4 mmol/L)
- Total IgM, 11 mg/dL (110 mg/L)
- Hemoglobin, 18.4 g/dL (184 g/L)
- Hematocrit, 51.2% (0.51)
- Platelet count, $170 \times 10^9/\mu$L ($170 \times 10^9/L$)
- Total bilirubin, 4.4 mg/dL (75.3 μmol/L)
The infant began feeding expressed mother’s milk on day 2, and feedings were progressively advanced. She had enlarging head circumference.

**Imaging**

The infant underwent imaging studies of the brain and heart, and skeletal survey radiography revealed the right femur, tibia, and fibula to be greater in length than those on the left, enlarged right facial bones, and a larger right cerebrum and ventricle compared with the left. Echocardiography did not confirm ventricular cystic structures or pericardial effusion (Figs 8 and 9).

**Differential Diagnosis**

- Klippel-Weber-Trenaunay syndrome
- Macrocephaly-capillary malformation (MCAP) syndrome
- Arteriovenous malformations
- Smith-Lemli-Opitz syndrome
- PIK3CA mutation
- Congenital lipomatosus overgrowth, vascular malformations, and epidermal nevi (CLOVE) syndrome with hemimegalencephaly
- Proteus syndrome

**Actual Diagnosis**

This infant was diagnosed with MCAP syndrome because she met the Martinez-Glez diagnostic criteria (Table 1). (1) Subsequent findings of developmental delay support the diagnosis.

Chromosomal array found a 483-kb interstitial deletion at 19p13.11 (hg19:17,357,704-17,840,956). This deletion includes 19 genes. The deletion in this case does not include the PIK3R2 gene (hg19:18,263,987-18,281,342), which has been associated with another megalencephaly syndrome and is nearby. (2) This syndrome is called megalencephaly, polymicrogyria, polydactyly, hydrocephalus, or MPPH syndrome. Interrogation of the DECIPHER database (https://decipher.sanger.ac.uk/) revealed that there are no other patients described in the literature with the same deletion. The parents declined to be tested for this deletion, and fibroblast culture for PIK3CA gene analysis is currently pending.

**The Experts**

MCAP is one of a group of megalencephaly syndromes caused by germline or somatic mutations in genes in the (PI3K)-AKT-mTOR pathway. (3) Class IA PI3K dimers...
are composed of a p110 catalytic subunit and a p85 regulatory subunit, each of which has 3 isoforms encoded by 3 genes. Germline mutations in 5 of these genes, \textit{PIK3CA}, \textit{PIK3CB}, \textit{PIK3CD}, \textit{PIK3R1}, and \textit{PIK3R2}, have been found in human cancers. MCAP is due to mutations in \textit{PIK3CA}, whereas \textit{PIK3R2} causes a different megalencephaly phenotype with polydactyly and without capillary malformations. (3) This patient has a deletion near the \textit{PIK3R2} gene, which raises the possibility of disruption of a promoter, regulatory element, or transcription factor that affects genes in this pathway (Fig 10).

MCAP, a genodermatosis, was first described in 1997 (4)(5)(6). It is characterized by macrocephaly, partial or asymmetric overgrowth, and patchy, reticular capillary malformations. It can be associated with developmental delay, hydrocephalus, syndactyly or polydactyly, connective tissue disorders, and neonatal hypotonia. Neuroimaging abnormalities can include white matter alterations, cerebral asymmetry, ventriculomegaly, cerebellar tonsillar herniation, cortical dysplasia, and polymicrogyria. (7)(8)

The latest and most comprehensive diagnostic criteria for MCAP syndrome were published in 2010 by Martinez-Glez et al. (1) Diagnosis depends on whether at least 3 major and 2 minor criteria are present (Table 1).

### Table 1. Martinez–Glez Criteria for Megalencephaly Capillary Malformation

<table>
<thead>
<tr>
<th>Major Criteria (at Least 3)</th>
<th>Minor Criteria (at Least 2)</th>
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<tbody>
<tr>
<td>- Macrocephaly</td>
<td>- Developmental delay</td>
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<tr>
<td>- Capillary malformation(s)</td>
<td>- Midline capillary malformation</td>
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<tr>
<td>- Overgrowth or asymmetry</td>
<td>- Neonatal hypotonia</td>
</tr>
<tr>
<td>- Neuroimaging abnormalities</td>
<td>- Syndactyly or polydactyly</td>
</tr>
<tr>
<td>- Ventriculomegaly</td>
<td>- Connective tissue abnormalities</td>
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<tr>
<td>- Cavum septum pellucidum or cavum septum vergae</td>
<td>- Frontal bossing</td>
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<tr>
<td>- Frontal bossing</td>
<td>- Hydrocephalus</td>
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<tr>
<td>- Cerebellar tonsillar herniation</td>
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Figure 9. Head magnetic resonance image showing enlargement of the right lateral ventricle with right hemimegalencephaly.

Figure 10. Oligonucleotide single-nucleotide polymorphism results: 483-kb deletion at 19p13.11. A total of 19 genes were found in the deleted region and 13 were OMIM annotated genes. None were associated with a similar phenotype to case subject. Picture courtesy of https://genome.ucsc.edu/.
Table 2. Phenotypic and Neurologic Findings of this Case Compared With Other Genodermatosis With Hemi hypertrophy in Differential Diagnosis

<table>
<thead>
<tr>
<th>Case Subject</th>
<th>Cutaneous Findings</th>
<th>Neurologic Findings</th>
<th>Other</th>
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<tbody>
<tr>
<td></td>
<td>Multiple port-wine stains</td>
<td>Macrocephaly</td>
<td>Hemi hypertrophy</td>
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<td></td>
<td>One midline port-wine stain</td>
<td>Right lateral ventriculomegaly</td>
<td>Body overgrowth or hemihypertrophy</td>
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<td></td>
<td>Capillary malformations</td>
<td>Frontal bossing</td>
<td>Postaxial polydactyly</td>
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<td>Midline capillary malformation</td>
<td>Hydrocephalus</td>
<td>Overgrowth of bones and soft tissues</td>
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<td></td>
<td>Connective tissue abnormalities</td>
<td>Cerebellar tonsillar ectopia</td>
<td>Asymmetry of body (usually hyper- or hypoplasia of the extremities)</td>
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<td>Cutis marmorata</td>
<td>Cortical brain abnormalities</td>
<td>Overgrowth of bones, skin and other tissues</td>
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<td>Vein malformations (varicose veins)</td>
<td>Cerebellar tonsillar herniation</td>
<td>Non-progressive and proportionate overgrowth</td>
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<td>Skin atrophy</td>
<td>Open mouth expression</td>
<td>Seizures</td>
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<td>Ulceration at lesional sites</td>
<td>Vision loss</td>
<td>Intellectual disability</td>
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<tr>
<td></td>
<td>Can have vascular overgrowth</td>
<td>Cranial asymmetry</td>
<td>Intellectual disability</td>
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<tr>
<td></td>
<td>Cerebral or cerebellar asymmetry</td>
<td>Dysgenesis of the corpus callosum</td>
<td>Seizures</td>
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<td>* Cavum septum pellucidum</td>
<td>Neuronal migration defects</td>
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This infant met all 4 major criteria and at least 3 minor criteria (including midline capillary malformation, neonatal hypotonia, syndactyly or polydactyly, and/or developmental delay). As previously described, this infant had a 483-kb interstitial deletion 19p13.11 (hg19:17,357,704-17,840,956), which includes 19 genes. This deletion does not include the PIK3CA gene; however, although the deletion is situated near the PIK3R2 gene, suggesting possible disruption of a promoter, regulatory element, or transcription factor that affects transcription and translation of the associated genes.

**Treatment**

A multidisciplinary approach to MCAP syndrome and other genodermatoses depends on symptoms present in each patient. Supportive care for these patients represents current medical management. As mentioned, there is the possibility of modulation of the PI3K-AKT-MTOR pathway. Therapy with PI3K/mTOR pathway inhibitors, such as everolimus, is currently being evaluated under an investigational protocol for patients with CLOVE syndrome, another PIK3CA-related disorder. This offers the possibility and hope for a novel treatment for other overgrowth syndromes in this pathway. (9)

**Lessons for the Neonatologist**

Multiple syndromes with vascular malformations and hemihypertrophy can lead to difficulties with diagnosis (10)(11) (Table 2). Historically, any overgrowth condition with a vascular component was given the diagnosis of Klippel-Weber-Trenaunay syndrome, but these conditions are now being recognized as separate entities. Previously there was no systematic manner in which to distinguish these syndromes. In our case presentation, this patient was initially diagnosed as having Klippel-Weber-Trenaunay syndrome by the primary team. However, with more information, a more accurate diagnosis was reached. In conducting a literature review, we were able to develop a comprehensive approach to systematic diagnosis. An important aspect of establishing the precise diagnosis includes obtaining affected skin tissue to send for biopsy, rather than sending blood tests because the blood and unaffected tissue may not contain the causative mutation(s). Although these syndromes remain rare, establishing a diagnosis is crucial in management and counseling. This case highlights the importance of following up on genetic screening tests performed on patients who appear to have a clear-cut diagnosis. The microarray results in this case provide an intriguing view into the underlying genotype for this patient’s phenotype. Although it may give a sense
of relief to both the physician and the family to have a diagnosis, care must be taken when new, inconsistent information is discovered. This case highlights the complexity of overlapping phenotypes and genotypes in this spectrum of megalencephaly and overgrowth syndromes, from Klippel-Trenaunay syndrome to CLOVE syndrome to Proteus syndrome.

References

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Answer Key for May 2015 NeoReviews: