Evidence for Role of Genital Mycoplasmas in Preterm Birth and Neonatal Lung Injury

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Education Gaps

1. Although *Ureaplasma* respiratory colonization is a risk factor for bronchopulmonary dysplasia, there is a gap in knowledge concerning the impact on longer-term respiratory outcomes.

2. It remains to be determined in sufficiently powered randomized clinical trials whether eradication of ureaplasmas from the intra-amniotic cavity antenatally or the respiratory tract postnatally prevents preterm birth or ameliorates its short- or long-term complications.

Abstract

Although the genital mycoplasmas are common commensals in the vaginal flora of up to 80% of women, these organisms have been associated with adverse perinatal outcomes. These outcomes include chorioamnionitis, preterm premature rupture of the membranes (pPROM), preterm birth, and altered lung development contributing to the development of bronchopulmonary dysplasia in preterm infants with respiratory infection. This review focuses on the current knowledge of the evidence supporting a causal role of these organisms in these adverse outcomes and controversies, including whether treatment of affected pregnant women or their infants is warranted.

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

1. Discuss the role of genital mycoplasmas in the pathogenesis of preterm birth and neonatal lung injury that contributes to bronchopulmonary dysplasia.

2. Review the clinical presentation of ureaplasmal respiratory infection.

3. Discuss the diagnostic evaluation for suspected ureaplasmal infection.

4. Discuss the potential treatment options and status of clinical trials of antibiotic treatment to prevent adverse respiratory outcomes in preterm infants with *Ureaplasma* species respiratory infection.
INTRODUCTION

The genital mycoplasmas are bacteria included in the phylum Tenericutes, class Mollicutes, that are characterized by their small genome size, lack of a cell wall, limited biosynthetic capabilities, resistance to β-lactam antibiotics, and specific growth requirements. (1) There are 16 Mollicute species that have been isolated from humans, of which 6 species are considered pathogens: Mycoplasma pneumoniae, Mycoplasma fermentans, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma parvum, and Ureaplasma urealyticum. This review will focus on the species that have been linked to complications during pregnancy and adverse neonatal outcomes (2)(3) (Table 1). It will summarize the current evidence for a pathogenic role of these organisms, the clinical presentation in the neonate, and diagnostic methods, and the controversy about treatment of these organisms is reviewed.

Pathogenic Genital Mycoplasmas

M. hominis. M. hominis may be detected in the vagina or cervix in 20% to 50% of sexually active women in the presence or absence of Ureaplasma species (1); organism numbers increase in the presence of bacterial vaginosis. (3) In addition to bacterial vaginosis, M. hominis is associated with i) maternal conditions such as pyelonephritis, pelvic inflammatory disease, chorioamnionitis, early miscarriages, mid-trimester abortions, and postpartum endometritis; and ii) newborn conditions including congenital pneumonia, meningitis, and bacteremia. (1)(4) Genome sequencing of M. hominis isolates from infected amniotic fluid and placentas identified a microbial gene of unknown function that was used to identify women at highest risk for preterm birth. Although vertical transmission of M. hominis occurs, neonatal respiratory colonization rates are 5% to 10% and the association with bronchopulmonary dysplasia (BPD) is less established than with the more common Ureaplasma species. (6)

These organisms metabolize arginine as an energy source, and colonies may be identified in culture on agar medium by their characteristic fried egg appearance. M. hominis expresses a variable adherence antigen that may assist organisms to evade the host immune response by varying the size and phase of the surface antigen. The M. hominis genome contains 665 kb with 527 protein-encoding genes, making it the second smallest known self-replicating, free-living organism. (3)

M. genitalium. M. genitalium is a significant cause of male urethritis, female cervicitis, and pelvic inflammatory disease. (3) It is much less common than M. hominis or the Ureaplasma species, with detection in the vagina or cervix of 0% to 5% of women. The organisms attach to and invade epithelial cells by a terminal structure, the MgPa adhesion. Antigenic variation of the adhesion protein is a host immune response evasion mechanism. The 580-kb M. genitalium genome is the smallest of free-living organisms with only 485 protein-encoding genes. Because it is difficult to perform cultures of M. genitalium, molecular assays are most commonly used for detection. (1)

Ureaplasma Species. The Mycoplasma species U. parvum (serovars 1, 3, 6, and 14) and U. urealyticum (serovars 2, 4, 5, and 7-13) are the most common organisms detected in infected amniotic fluid and infected placentas with or without other organisms in extremely preterm (7) and late preterm/term

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>MORPHOLOGY</th>
<th>GENOME SIZE</th>
<th>METABOLIC SUBSTRATES</th>
<th>VAGINAL COLONIZATION</th>
<th>DISEASE ASSOCIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ureaplasma parvum</td>
<td>7-15 μm colonies, Agar</td>
<td>U. parvum: 750 kb</td>
<td>Urea</td>
<td>40%–80%</td>
<td>Urethritis, CAM, postpartum endometritis, PB, BPD, meningitis</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td></td>
<td>U. urealyticum: 947 kb, 27%–30% GC content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>200 μm colonies, “fried-egg” appearance</td>
<td>665 kb, 27% GC content</td>
<td>Arginine</td>
<td>20%–50%</td>
<td>PID, BV, CAM, congenital pneumonia, meningitis</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>Difficult to cultivate; PCR for detection</td>
<td>580 kb, 32% GC content</td>
<td>Glucose</td>
<td>0–5%</td>
<td>Urethritis, cervicitis, PID</td>
</tr>
</tbody>
</table>

BPD=bronchopulmonary dysplasia; BV=bacterial vaginosis; CAM=chorioamnionitis; GC=guanine-cytosine; PB=preterm birth; PCR=polymerase chain reaction; PID=pelvic inflammatory disease.
pregnancies (8) with chorioamnionitis. These species are associated with spontaneous preterm birth. (2) *U parvum* is the predominant species in clinical specimens from amniotic fluid, cord blood, neonatal respiratory secretions, and cerebrospinal fluid (9) but evidence for differences in serovar-specific virulence is currently lacking. However, evidence from epidemiologic studies and experimental infection models has demonstrated that these organisms contribute to complications of preterm birth including BPD. (9)(10) Detection of *Ureaplasma* species with culture and/or PCR in nasopharyngeal or tracheal aspirates is inversely related to gestational age, with the overall incidence being 35% among infants with birthweights less than 1,501 g. (10) Although most studies focused on colonization in the first 7 to 10 days of age, serial sampling suggests that the infection persists for weeks in many infants. The host and organism factors that determine persistent infection versus transient colonization are unknown.

Culture-independent studies of the lung microbiome have confirmed that the lower airways are not sterile, as previously believed. Although studies of the newborn lung microbiome are technically challenging in infants receiving mechanical ventilation, recent studies have confirmed the presence of *Ureaplasma* species in the context of the microbial community and relationship to BPD. Lal et al (20) observed that the lung microbiome was similar at birth in extremely low-birthweight infants and term infants. The predominant phyla on the first day after birth in both extremely low-birthweight and term infants were Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Tenericutes (*Ureaplasma*), Fusobacteria, Cyanobacteria, and Verrucomicrobia. In established BPD, the phylum Proteobacteria was increased, and phyla Firmicutes and Fusobacteria were decreased. In an analysis of lung microbiome characteristics in association with BPD severity, the infants who developed severe BPD exhibited greater bacterial community turnover with age, less abundant *Staphylococcus* in the first week after birth, and higher initial relative abundance of *Ureaplasma*. (21) Both host and microbial factors determine the susceptibility of preterm infants to *Ureaplasma* species respiratory tract colonization and persistence of infection. Critical host factors include developmental deficiencies and genetic mutations in innate immune defenses. The preterm lung is deficient in surfactant protein A, which is required for ureaplasmal phagocytosis, killing in vitro (22) and pathogen clearance, and modulating the inflammatory response in vivo. (23) Single nucleotide polymorphisms in relevant Toll-like receptors may affect both the susceptibility to *Ureaplasma* respiratory infection and the risk of developing BPD in infected preterm infants. (24) Ureaplasmas possess multiple host immune response avoidance mechanisms that facilitate establishing a chronic infection in the

**Respiratory Infection in Preterm Newborns**

Ureaplasmas can be transmitted to the fetus/newborn respiratory tract by vertical transmission at the time of birth, via an ascending infection through direct contact with infected amniotic fluid, or possibly a hematogenous route. (9) **Detection of Ureaplasma** species with culture and/or PCR in nasopharyngeal or tracheal aspirates is inversely related to gestational age, with the overall incidence being 35% among infants with birthweights less than 1,501 g. (10) Although most studies focused on colonization in the first 7 to 10 days of age, serial sampling suggests that the infection persists for weeks in many infants. The host and organism factors that determine persistent infection versus transient colonization are unknown.

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amniotic cavity and the neonatal respiratory tract. These include 1) phase and size variation of the multiple banded antigen, (1) 2) the ability to form biofilms, (25) 3) presence of multiple nucleases that may degrade neutrophil extracellular traps formed when activated neutrophils release granule proteins and chromatin that kill bacteria, (26) and 4) downregulation of various endogenous antimicrobial peptides. (27)

Respiratory tract colonization is associated with a pro-inflammatory response in the lung that is augmented by volutrauma and oxygen exposure. (9) The effects of intra-amniotic exposure to *U. urealyticum* 2 days before delivery and subsequent mechanical ventilation for 14 days were assessed in a 125-day preterm baboon model of BPD. Preterm baboons exposed in utero to *Ureaplasma* with persistent postnatal ureaplasmal infection had higher tracheal aspirate inflammatory cytokines at birth and at 14 days of age, and more severe BPD changes, including extensive fibrosis compared with intra-amniotic exposed animals in which the infection cleared, and noninfected controls. (18) Transforming growth factor-β1 was elevated in tracheal aspirates of infants who progressed to BPD and was increased in autopsy lung specimens from *Ureaplasma*-infected preterm infants (28)(29) and in the infected preterm baboon model. Upregulation of transforming growth factor-β1 signaling during lung development as a result of inflammation contributes to alveolarization arrest, airway remodeling, and fibrosis, all of which are characteristic of BPD. (9)

*Ureaplasma* Respiratory Colonization as an Independent Risk Factor for BPD

As a result of improvements in neonatal care, BPD is now a disease limited to the most immature infants, and is seen in 30% of infants born at 28 weeks’ gestation or earlier. (30) The “new” BPD (characterized by more uniform inflation, fewer but larger alveoli, and less severe, but persistent inflammation) results from interruption of normal developmental signaling during the saccular stage of lung development. This injury is often initiated in utero by an intrauterine infection and a subsequent dysregulated inflammatory response. (30)

Three meta-analyses of more than 40 studies over the past 30 years have confirmed that *Ureaplasma* respiratory colonization is an independent risk factor for BPD. (10)(31) (32) In the most recent meta-analysis in 2014, *Ureaplasma* respiratory tract colonization led to a 3-fold higher risk for BPD at 28 days and 2-fold higher risk at 36 weeks’ post-menstrual age. (9)(10) Remarkably, this association has remained unchanged over the past 3 decades, despite many changes in neonatal care.

**CLINICAL MANIFESTATIONS IN INFANTS**

Infants with *Ureaplasma* respiratory colonization are more likely born extremely preterm to women with pregnancies complicated by chorioamnionitis and preterm labor or pPROM (33)(34) (Table 2). The vertical transmission rate increases with longer duration of membrane rupture, (35) while infants delivered for maternal indications have the lowest rate of respiratory tract colonization. (36) Interestingly, infants with *Ureaplasma* respiratory colonization may present with a peripheral blood leukocytosis, (37) but exhibit mild respiratory distress syndrome (33) that evolves rapidly in the first 2 weeks of age into early BPD with radiographically evident emphysematous changes and histologic early fibrosis and disordered elastin. (29)(38)

**TABLE 2. Characteristic Clinical and Laboratory Findings in Ureaplasma-Positive Preterm Infants**

<table>
<thead>
<tr>
<th>CLINICAL PRESENTATION</th>
<th>LABORATORY/RADIOGRAPHIC FINDINGS</th>
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<tbody>
<tr>
<td>POL or pPROM</td>
<td>Bistratified inflammatory pattern chorioamnionitis</td>
</tr>
<tr>
<td>GA&lt;28 weeks</td>
<td>Leukocytosis at birth</td>
</tr>
<tr>
<td>Mild RDS, but worsening gas exchange requiring increased respiratory support in 2nd week after birth</td>
<td>Early radiographic emphysematous changes</td>
</tr>
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GA=gestational age; POL=preterm onset of labor; pPROM=preterm premature rupture of membranes; RDS respiratory distress syndrome.

Diagnosis

Laboratory detection of Ureaplasma species in neonates can be achieved on culture or using molecular methods such as the PCR assay in tracheal and nasopharyngeal aspirates, pleural fluid, lung tissue obtained at autopsy, urine, blood, or cerebrospinal fluid. (40) Because ureaplasmas are extremely sensitive to adverse environmental conditions, particularly dessication and heat, specimens should be inoculated directly into appropriate transport media such as 10B broth or universal transport media for mycoplasmas, ureaplasmas, and chlamydiae available from various commercial suppliers. The specimens should then be placed on ice or refrigerated if immediate transportation to the laboratory is not possible. If a specimen must be shipped and/or if the storage time is likely to exceed 24 hours before it is processed, the specimen in transport medium should be frozen at ~80°C to prevent loss of viability and to minimize bacterial overgrowth.

Growth of ureaplasmas in vitro requires a complex medium containing serum, growth factors such as yeast extract, and urea as a metabolic substrate. Serially diluted specimens should be inoculated into 10B broth and subcultured directly onto A8 agar. Broths should be incubated at 37°C under atmospheric conditions while agar plates should be incubated in room air supplemented with 5% to 10% carbon dioxide for at least 7 days. An alkaline color change from yellow to pink in 10B broth as a result of urea hydrolysis is most likely attributable to Ureaplasma species, but M hominis may coexist with Ureaplasma in clinical specimens. Brown granular colonies 10 to 60 μm in diameter visualized on A8 agar under a stereomicroscope after 24 to 48 hours of incubation are diagnostic of Ureaplasma species. MYCOSCREEN PLUS and MYCOFAST US (Wescor Inc, Logan, UT) are commercial kits available for detection and quantitation of Ureaplasma species and M hominis based on the color change of specific wells containing substrates and inhibitors. PCR assays may be performed to distinguish U parvum from U urealyticum, but identification to the species level is not necessary for clinical diagnostic purposes. Details of various nucleic acid amplification tests and their applications for detection of mycoplasmas and ureaplasmas have been discussed in depth in a recent review. (1) Antimicrobial susceptibility testing for ureaplasmas of clinical isolates to macrolides, tetracyclines, and fluoroquinolones can be performed by broth microdilution, (41) but is not routinely performed because resistance to macrolides, the agents of choice for use in most neonatal infections, is uncommon and such testing is available in only a few specialized reference laboratories.

Treatment

Current Status of Recommendations for Treatment of Ureaplasma Infection in Preterm Infants. Trials of antibiotic therapy with erythromycin in the first few weeks of age often failed to eradicate respiratory colonization and had no impact on the development of BPD in Ureaplasma-infected infants. (9) Recent retrospective studies demonstrated that azithromycin treatment failed to improve outcomes when initiated after 2 weeks of age in symptomatic, culture-proven Ureaplasma-positive infants. (42)(43)

Azithromycin in Preterm Infants. Previous studies may have failed to show microbiologic efficacy in part because of lack of preliminary pharmacokinetic/pharmacodynamic studies of erythromycin and azithromycin in the preterm population to determine effective dosage. We have focused on the azalide azithromycin because its immunomodulatory properties make it an ideal candidate for therapy to prevent Ureaplasma-mediated lung injury in preterm infants. We conducted pharmacokinetic/pharmacodynamic studies characterizing the population pharmacokinetics, safety, tolerability, and microbiologic effects of 10 and 20 mg/kg intravenous single-dose azithromycin and 20 mg/kg every 24 hours for 3 days multiple dose in preterm neonates born at 24 to 28 weeks’ gestation who are at high risk for Ureaplasma respiratory tract colonization and BPD. (44)(45)(46) The disposition of azithromycin in plasma was biphasic, suggesting that the antibiotic’s pharmacokinetics follow a 2-compartment model. Compared with the single-dose groups, the 20 mg/kg multidose effectively eradicated Ureaplasma in all subjects who were colonized before the dose. The short course azithromycin regimen appeared safe, with no deaths or serious adverse events attributed to the drug. Azithromycin has been associated with increased risk for cardiovascular death in older adults, due to its proarrhythmic potential, (47) and infantile hypertrophic pyloric stenosis in infants. (48) Therefore it cannot be recommended for routine use in preterm infants with suspected Ureaplasma infection until further randomized trials confirm its safety and efficacy.

Other macrolides may be beneficial in the treatment of Ureaplasma respiratory colonization. In a placebo-controlled trial of a 10-day course of 20 mg/kg per day clarithromycin, there was a 68.5% eradication rate 2 days after the last dose in treated colonized infants. In addition, the BPD rate was greatly reduced in treated colonized infants compared with the placebo group (3% vs 36%). (49) However, this study had several limitations: it excluded infants smaller than 750 g who have the highest Ureaplasma colonization rate and risk for BPD; the duration of mechanical ventilation was brief in...
all subjects; culture status was based solely on nasopharyngeal cultures so it did not differentiate between upper and lower respiratory tract infection; and organism clearance was not assessed in the placebo group. Although there have been case reports of torsades de pointes in adults treated with clarithromycin, (50) the risk of idiopathic hypertrophic pyloric stenosis with clarithromycin is unknown. Therefore, data on the safety and efficacy are insufficient to recommend the use of clarithromycin for improving pulmonary outcomes in Ureaplasma-infected preterm infants.

**SUMMARY**

Epidemiologic and experimental evidence has been accumulating to support a causal role of the genital mycoplasmas, particularly *Ureaplasma* species, in subclinical intrauterine infections. Such infections contribute to chorioamnionitis, pPROM, and preterm birth, and neonatal morbidities including neonatal lung injury. Future research should use comprehensive “omic” approaches to identify host and microbial factors that identify pregnancies at risk for preterm birth. Clinical trials are needed to address whether maternal and/or neonatal therapy targeting *Ureaplasma* infection is safe and effective in eradicating the organisms and preventing adverse consequences.

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

- Know the epidemiology, pathogenesis, and prevention of perinatal infection with *Mycoplasma* and *Ureaplasma*.
- Know the clinical manifestations, diagnostic features, management, and complications of perinatal infection with *Mycoplasma* and *Ureaplasma*.

**References**

42. Anbu Chakkarapani A, Paes B, Shivvana S. Macrolides do not affect the incidence of moderate and severe bronchopulmonary dysplasia in symptomatic ureaplasma-positive infants. Acta Paediatr. 2015;104(10):e427–e432
1. A 24-year-old gravida 1, para 0 woman presents at 24 weeks 3 days of gestation with preterm premature rupture of membranes (pPROM). If amniocentesis were to be performed, which of the following mycoplasma species would be MOST likely to be detected in the amniotic fluid?
   A. Mycoplasma hominis.
   B. Ureaplasma urealyticum.
   C. Mycoplasma genitalium.
   D. Ureaplasma parvum.
   E. Mycoplasma pneumonia.

2. The woman undergoes further evaluation and treatment. It is known that genital mycoplasmas are common commensals of the vaginal flora and have been associated with adverse pregnancy outcomes. Which of the following statements about the pathogenic role of these organisms during pregnancy is TRUE?
   A. Mycoplasma hominis is strongly associated with pPROM in primiparous women.
   B. Ureaplasma parvum and Ureaplasma urealyticum (Ureaplasma species) are an important cause of chorioamnionitis but not pPROM or preterm labor.
   C. Mycoplasma genitalium is associated with bacterial vaginosis and preterm labor.
   D. Ureaplasma species is usually detected as early as 10 to 12 weeks of gestation in the amniotic fluid.
   E. Mycoplasma and/or Ureaplasma are detected in up to 81% of women with pPROM, and their presence is associated with lower gestational age at delivery.

3. The woman is admitted to the hospital for latency antibiotics and observation. She develops a fever 5 days into her hospital stay and is diagnosed with chorioamnionitis. Which of the following placental pathology results BEST describes the characteristic findings seen in Ureaplasma species infection?
   A. Placental destruction with callous trophoblast cell apoptosis.
   B. Necrotizing funisitis with chronic villitis.
   C. Bistratified inflammatory pattern.
   D. Chronic villitis with Hoffbauer cells.
   E. Villous inflammation and sclerosis with trophoblastic necrosis.

4. Ureaplasma colonization of the respiratory tree has been shown to be an independent risk factor for bronchopulmonary dysplasia (BPD). Which of the following statements is CORRECT regarding Ureaplasma respiratory colonization and risk for BPD?
   A. Transforming growth factor β1, which contributes to alveolarization arrest, airway remodeling, and fibrosis, is increased in autopsy lung specimens from Ureaplasma-infected preterm infants.
   B. In the most recent meta-analysis in 2014, Ureaplasma respiratory tract colonization increased the risk for BPD at 36 weeks’ postmenstrual age by 4-fold.
   C. Respiratory tract microbiome studies indicate that infants who developed severe BPD have a higher abundance of Staphylococcus in the first week after birth and lower initial relative abundance of Ureaplasma.
   D. Treatment with azithromycin has been shown to improve the outcome of BPD at 28 days.
   E. When it occurs in the presence of other early-onset bacterial infections, Ureaplasma respiratory colonization is associated with a decreased likelihood of development of BPD.
5. Because of the history and presentation, you decide to evaluate the infant for *Ureaplasma* infection. Which of the following statements is CORRECT regarding the laboratory detection of *Ureaplasma* species?

A. Tracheal and nasopharyngeal aspirates are the only biological samples in which *Ureaplasma* species can be detected in the preterm neonate.

B. Brown granular colonies 10 to 60 μm in diameter visualized on A8 agar under a stereomicroscope after 24 to 48 hours of incubation are diagnostic of *Ureaplasma* species.

C. Because of their robust resistance to adverse environmental conditions, specimens can be kept on the specimen retrieval device and kept in room temperature for up to 48 hours before laboratory evaluation.

D. Growth of *Ureaplasma* in vitro is generally quick and results in positive culture detection within 48 hours in most cases.

E. An alkaline color change from yellow to pink in 10B broth is indicative of *Mycoplasma hominis*, but not *Ureaplasma* species.
A Focus on Microbiome Completeness and Optimized Colonization Resistance in Neonatology

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Education Gaps

1. The potential for microbiome-based complementary therapies when antibiotics are required is not fully realized.

2. Colonization resistance can be a tool for minimizing health risks during the neonatal period.

Abstract

The human microbiome contributes a majority of genes and significant metabolic capacity to the newborn. The infant’s bacteria, archaea, viruses, and fungi are also critical for immateuration and neurologic development. Because a microbiota is highly malleable, it is an ideal target for improving infant health. Yet, management of this major biological resource to reduce health risk for the infant has been comparatively neglected to date. This review discusses the opportunities for a more holistic, ecological approach to infant health with an emphasis on the microbiome, which includes 1) the benefits of microbiome completeness (microbial seeding and feeding), as well as 2) optimized colonization resistance. The latter can better protect against infectious as well as noncommunicable diseases by shifting pathogen load requirements for producing disease, protecting mucosal barriers, and optimizing immune homeostasis.

Objectives

After completing this article, readers should be able to:

1. Discuss the neonatal microbiome and its impact on infant and later-life health.

2. Consider mutualistic and commensal microbiota and their regulation of barrier (eg, gut, skin, lung, urogenital) function and immune maturation.

3. Use colonization resistance as a primary infectious disease prevention strategy.

4. Discuss commonalities between noncommunicable and communicable diseases with a focus on barrier function and immune maturation.

AUTHOR DISCLOSURE

Dr Dietert has disclosed no financial relationships relevant to this article. Dr Dietert is a consultant with Seed Health. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.

ABBREVIATIONS

CR colonization resistance
HMOs human milk oligosaccharides
NCDs noncommunicable diseases and conditions
INTRODUCTION

Study of the human microbiome, the trillions of microbial organisms and their genes found in humans, is revolutionizing our preventive and therapeutic approaches to human health. (1) Modern medicine is gradually transitioning from a mid-20th century paradigm in which the healthiest infant was thought to be one largely free of microbes to a new era in which increasing numbers of obstetricians/gynecologists, neonatologists, and pediatricians understand that an infant must be heavily microbially laden to develop into a healthy child. This microbial requirement for health and well-being is not exclusive to humans but is a fundamental characteristic of most plants, insects, and animals on earth. (2)(3)

Humans are not a single-species mammal and should no longer be medically treated as such. Instead, we are a holobiont or superorganism with both microbial and human eukaryotic cells and genes. In fact, by most estimates, the human body is mostly composed of microbes (based on the number of both cells and genes). In many ways, we are most analogous to a coral reef or a tropical rain forest, each of which contains myriad species and is healthiest when all of the species are present, cooperating, and, themselves healthy. The collection of human microbiota (organisms composed of bacteria, archaea, viruses, fungi) residing on and in the infant, along with their genes, represents the infant’s microbiome. Different species of microbes have different environmental requirements (eg, nutrients, oxygen levels) for growth, and those able to thrive in the colon are not the same as the ones adapted to the mouth or skin.

The challenge for obstetricians/gynecologists, neonatologists, and pediatricians is to fully embrace the new role, that of microbial managers, which is an inherent part of supporting the health of the human holobiont. The first 1,000 days after birth have been recognized as a critical developmental window during which the neonatal microbiome sets the template for subsequent development. (4) For this reason, medical management of the microbiome takes on added importance in the newborn and infant. An ecological management approach to the newborn and infant microbiome can reduce the risk of not only conditions such as obesity, diabetes, and inflammatory diseases, but also the risk of life-threatening infections. This review will describe how the microbiome becomes an integral part of the infant and why proactive management of the infant microbiome is a critical factor for both childhood and adult health. An emphasis will be placed on the perinatal microbiome and risk reduction for infectious diseases through a microbial process known as colonization resistance (CR). (5)

THE MATERNAL MICROBIOME AND PREGNANCY OUTCOMES

Pregnancy and birth are the greatest environmental and microbial transitions that an infant will face across life. During this time, the infant emerges from the protected environment of the womb to the world of environmental challenges including the battle against pathogens capable of producing life-threatening infectious diseases. Planning for a healthy, microbially complete infant begins with the pregnant woman. This is because her microbiome composition determines her donation of a majority of cells and genes to the infant. If the maternal microbiome has been compromised through associated diseases and conditions (eg, obesity, diabetes), dysbiosis-producing infections, environmental factors (eg, urban-associated pollutants, processed foods), or drug treatments (eg, antibiotics), the infant will begin life with a degraded, dysbiotic microbiome. When the pregnant woman’s microbiome has been previously compromised, even efficient seeding of the newborn, as occurs with vaginal delivery, can increase neonatal health risks because of impaired adaptive perinatal immune development. (6) Interrupting the cycle of disease epidemics by ensuring a healthy infant microbiome should be a major health goal along with developing microbial-based strategies to address what is increasingly becoming an intergenerational problem. (7)

Exposure to microbes begins early in life because the placenta carries its own microbiome. (8) Recent studies are examining whether any seeding of an infant’s gut occurs prenatally. (9) Beyond the effects of the placental microbiome, the maternal microbiome appears to affect the course of the pregnancy. Composition of the microbiome, including both biomass and diversity, in various maternal body sites (oral, vaginal, gut, cervical, placental) can drive the course of the pregnancy, affecting such factors as risk of preterm delivery. (10)

Newborns literally become filled with trillions of bacteria, viruses, archaea, and fungi during the developmental period surrounding birth. The acquisition of these microbes in newborns (largely from the mother and surrounding environment) is both necessary and required for normal physiologic development and function. (4)(11)(12)

The infant microbiome takes up residence in numerous body sites including the skin, but barrier-protected mucosal tissues (eg, gut, respiratory, urogenital tract) are particularly rich in microbiota. Not surprisingly, these body sites are where the infant is exposed to the external environment, and also, where a majority of immune cells reside. (13) The traditional routes of external environment (inhalation, oral,
and dermal exposures) are rich repositories of microbiota. Because of this, the infant’s microbiota serves as both a gatekeeper and a filter for the infant’s experience with the external environment including exposure to environmental chemicals, drugs, food, food additives, and pathogens. (14) The infant’s internal body (effectively the internal doses) only sees these chemical and microbial factors after the microbiome has interacted with and acted on them.

**INFANT METABOLISM OF FOOD AND DRUGS**

From the standpoint of risk-benefit, it is impossible to know precisely how much nutrient, drug, active drug metabolite, or environmental chemicals will reach a given patient’s cells and tissues without knowing the status of the microbiome and its metabolic contributions. This uncertainty arises because the composition of the microbiota (eg, bacterial species, strains, communities, and genes) determines how chemicals and drugs are handled. This is both bad news and good news.

The bad news is that, in the absence of information on an infant’s microbiome, physicians may be flying blind when administering drug therapies to patients. (15) Different microbes carry different genes and have different metabolic capabilities. (16) These can affect the delivered dose of many prescribed drugs including 5-fluorocytosine, digoxin, indo-methacin, insulin, ketoprofen, loperamide oxide, methotrexate, metronidazole, nitrazepam, nizatidine, olsalazine, paracetamol, prontosil, and sulfasalazine. (17)(18)(19) Because of the wide range of enzyme activities within the human microbiome and the extensive variation in microbiome status among patients, the delivered internal dose of an active drug can be very imprecise in the absence of a patient’s microbiome profile. Take, for example, the case of β-glucuronidase activity within the microbiome. Glucuronidases are enzymes that can convert certain prodrugs to active compounds, and in other cases, remove potentially toxic drug metabolites. Hence, glucuronidase activity within a patient can affect both deposition and concentration. (20) A recent atlas of β-glucuronidase activity within the human intestinal microbiome reflects the extent to which the microbiome determines the outcome of administered drugs. In addition, comprehensive approaches to predicting microbiome-driven drug metabolism is under way. (21) The human gut microbiome has more than 3,000 drug-metabolizing glucuronidase enzymes, with more than 100 unique to the microbiome to understand the extent of the problem. The good news is that future drug therapies will be working by tapping the microbiome as a way to produce useful “internal drugs.”

**COMATURATION OF MICROBES AND PHYSIOLOGIC SYSTEMS**

One of the most significant changes in our paradigm of 21st century human biology and medicine is the recognition that our mutualistic and commensal microbes shape, if not direct, the infant’s physiologic development and function. (23)(24) Physiologic systems such as immune, neurologic, endocrine, and gastrointestinal are undergoing benchmark developmental changes during the perinatal period, and these critical developmental changes can affect lifelong health. In particular, the immune system must quickly adapt from a pregnancy-skewed environment in which certain immune response capacities (eg, Th1 responses) are dampened versus the postnatal world where discernment of pathogenic threats and the capacity to mount a more complete repertoire of host defenses is needed. The perinatal period of development is arguably the most significant window of development affecting the health, not only of the child, but also of the later-life adult. In the absence of an adequate microbiome, the immune system is imbalanced and deficient in certain specialized populations. For example, in the extreme case of germ-free mice, there is significant underdevelopment of the specialized gut mucosal tissues such as the Peyer patches, lymphoid follicles, and mesenteric lymph nodes. (25)

Postnatal immune development is critically dependent on various interactions with mutualistic and commensal microbes, and immune dysfunction-mediated disease is an expected outcome of an inadequate microbiome. (26)(27) Infants with depleted microbiomes, such as occurs after exposure to antibiotics, are biologically and functionally incomplete. Because the microbiome has been described functionally as representing a new endocrine organ, a second brain, and a second liver, (2) incompleteness of the newborn’s microbiome is functionally analogous to a birth defect, though in this case, it is a preventable birth defect. (28)(29)

Even minor alterations in the early-life comaturation of an infant’s microbiome and immune system can have significant health consequences. As reviewed by Amenyogbe et al, (30) microbial and immune homeostasis in the infant are completely interconnected, with the former temporally preceding the latter during early development. For example, infants with increased fungal species *Rhodotorula* and *Candida* and reduced levels of *Bifidobacterium, Lactobacillus*, and *Akkermansia* bacteria were at increased risk for atopy and also possessed a gut environment that overpromoted interleukin-4 production, an immunoglobulin E–promoting cytokine. In contrast, the presence of specific bacterial
species in newborns such as *Bacteroides fragilis* appears to dampen both immune inflammatory cell signaling and the production of inflammatory cytokines in the infant. (30) *B. fragilis* is also important for altering the balance of 2 critical populations of immune regulatory cells: natural T-regulatory cells and invariant natural killer T cells. The former are increased while the latter are decreased in the intestine when the bacterium is sufficiently present in early life. (30) The Figure illustrates T-regulatory cells as an important immune population that needs to become established in the infant.

Entire fields of study are devoted to the developmental origins of adult health and disease and for good reason. (31) Interruption of key developmental events can epigenetically and physiologically program the child for a life course filled with health issues. (32) Various factors can affect microbiome seeding, feeding, and homeostasis, including mode of delivery, intrapartum and infant antibiotics, maternal diet, maternal and infant probiotics, and infant feeding (eg, extent and duration of breastfeeding).

Because of the 1,000-day critical window for the human microbiome, (4) clinicians working in neonatology are in a unique position to support proper childhood physiologic development. But not just any collection of microbiota will suffice when it comes to adequate support of immune, neuronal, respiratory, and endocrine development and establishment of a healthy pathway for later life.

**HEALTH CONSEQUENCES OF MICROBIAL INCOMPLETENESS (DYSBIOSIS)**

**Noncommunicable Diseases and Conditions**

Considerable research has gone into the associations between microbiome imbalances and the risk of both noncommunicable diseases and conditions (NCDs) and communicable (ie, infectious) diseases. Disruption of the infant microbiome

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**Case Scenario for Cumulative Environmental Health Risks**

**Childhood Incompleteness of the Microbiome**

- **Bereavement**
  - Maternal stress
- **Cesarean delivery**
  - Antibiotics administered
  - Newborn immune programming
  - Reduced microbial seeding
  - Elevated risk of allergy, autoimmunity, obesity, CVD
- **Formula feeding**
  - Missing prebiotics and microbes
  - Altered metabolites & Immune development
- **BPA, heavy metals, certain food additives**
  - Reduced colonization resistance
  - Increased risk of infection
  - Improper control of inflammation
  - Inadequate innate immune training
  - Inflammatory promotion of metabolic syndrome & carcinogenesis

**Figure.** A timeline of environmental factors that affect the microbiome from pregnancy through infancy is illustrated. Cumulative factors are shown that can adversely affect 1) infant microbiota status/level of microbiome completeness, 2) immune development, 3) regulation of inflammation, 4) the level of colonization resistance, and 5) the risk of both noncommunicable and infectious disease. Maternal microbial status, infant microbial seeding and feeding, and risk of exposure to microbially toxic drugs, chemicals, foods, and food additives all contribute to the overall risk for the child. BPA=bisphenol A; CVD=cardiovascular disease; PAH=polycyclic aromatic hydrocarbons; Treg=T-regulatory cells.
elevates the risk of both childhood- and adult-onset NCDs (eg, obesity, asthma, diabetes, cardiovascular disease, cancer, autoimmune conditions) as well as the contribution of microbiome degradation to the ongoing NCD epidemic. (2) In some cases, cause-effect relationships are unknown between changes in microbiota and changes in risk of disease. However, in other cases, there is significant mechanistic information detailing precisely how changes in gut microbiota, cellular signaling, and microbial metabolism drive changes in the risk of disease (supported by shifts in host barrier function, physiology, mucosal immune status, and inflammation-driven tissue pathology.) For example, evidence of a causal relationship between microbiome dysbiosis and specific diseases include the observation in mice that Crohn disease–like ileitis is fully transmissible. Transplantation of the dysbiotic gut microbiota results in the failure of protective Paneth cell function and production of disease. (33) But the risk of a dysbiotic microbiome resulting in NCDs is frequently delayed when compared with the more immediate danger of infant infectious diseases.

A pivotal factor in the relationship between microbiome degradation and elevated risk of disease is the fact that not every species of bacteria is equal in importance. Certain bacteria are redundant in that multiple species can carry out overlapping functions, and the presence of any 1 of these species may be sufficient to meet our needs. In contrast, several functionally unique bacterial species, termed key-stone species, carry out critical biological and/or metabolic functions that are not duplicated elsewhere across the microbiome. When keystone species are lost due to drug, chemical, dietary, psychosocial, or other environmental damage, pathology is likely. (2)

**Infectious Diseases**

If the path from microbiota to NCDs is often lengthy and potentially delayed in emergence, gaps in microbiome communities and their functions can give pathogens a straightforward and rapid foothold. Both microbiota and our immune cells are significantly enriched at mucosal barriers. These barriers developed to separate us from the external environment and its threats and a wide range of pathogens that gain access to the host via these mucosal barriers. (34) Therefore, the status of host defenses at these barriers is a significant factor in vulnerability to infections. At the center of infant risk of infectious diseases are 3 primary superorganism-based factors: 1) status of the developing immune system as conditioned largely by the microbiota, 2) integrity of the mucosal barrier itself, and 3) CR, or mechanisms to protect against the incursion of and/or overgrowth of pathogens.

Infant immune development begins with the maternal diet and microbiome (24) and continues to be shaped post-natally during critical windows of development. (2) Mucosal barrier integrity is greatly affected by microbiome homeostasis, which affects both mucin regulation and thickness as well as immune signaling and control of inflammation. (35) CR includes not only the capacity of commensal and mutualistic microbes to physically exclude access of pathogens to mucosal surfaces, but also the capacity of the infant microbiome to create cooperation among microbes against pathogens as well as metabolically deficient or antagonistic local environments that deter pathogens.

As discussed by Hand, (36) all infectious diseases are contextual, and are based on combinations of interactions that involve microbes, the host, and the environment. However, most considerations of infectious diseases start and stop with the invading pathogenic organism. Only recently have we realized that it is the microbial ecology of our barriers and immune-microbe interactions that are the tipping point when it comes to risk of infectious diseases. However, to fully engage this broader view of infectious disease ecology, it is necessary to focus on the neonatal microbiome. For example, commensal and mutualistic microbes have extensive cross-talk with the immune system, and in combination, help the host to shape the production of specific antimicrobial peptides at mucosal barriers. These, in turn, also shape microbiota composition, resulting in immune development, and risk of infection. (37) The Figure illustrates examples of factors, conditions, and medical interventions during pregnancy and early childhood that cumulatively contribute to microbiome damage, altered immune development in the infant, and elevated risk of both infectious diseases and NCDs.

**ANTIBIOTICS AND THE INFANT MICROBIOME**

While antibiotics may be necessary to treat both maternal and infant bacterial infections, unintended damage to the infant microbiome from antibiotics can present a serious problem if no complementary therapy such as probiotic and/or prebiotic is used. Antibiotics may be necessary to eliminate life-threatening bacterial infections, but overuse of antibiotics remains a concern. (38) For example, in a recent cohort survey among 5,581 children in New Zealand, 97% of the children had received at least 1 course of antibiotics by age 5 years. These children were prescribed 53,052 courses of antibiotics. (39)

Recently, the oral microbiome is emerging as a sentinel for antibiotic interruption of maternal-infant microbiota.
The earliest oral microbiome in the infant tends to mirror that of the mother. However, because antibiotics are often administered prophylactically during cesarean delivery as a preventive measure against possible postsurgical infections, (40) disruption of the microbiome can occur before birth. Gomez-Arango et al (41) studied 36 mother-infant pairs comparing mother-newborn oral microbes and the impact of intrapartum antibiotic administration. They found that the infant’s oral microbiome most resembled the oral microbiome of the mother. However, the colonizing bacteria were shaped by the maternal exposure to the antibiotic, producing the following 2 key effects: 1) Antibiotic resistance genes were enriched in the infant’s oral microbiome of those with antibiotic-treated mothers. 2) The colonizing microbes were skewed toward a hospital-rich, inflammation-producing bacterial profile (eg, Proteobacteria families such as Bradyrhizobiaceae, Sphingomonadaceae, Comamonadaceae, Neisseriaceae, and Oxalobacteriaceae) with a concomitant reduction in those bacteria (Streptococcaceae, Enterobacteriaceae, and Lactobacillales order) needed to promote a healthy progression of infant microbes as the infant matures.

CR AND PATHOGEN LOADING

Mutualistic and commensal microbes in the gut provide CR against both endogenous and exogenous pathogens. In a recent review, Kim et al (5) detailed 6 mechanisms of CR in the gut that can reduce the risk of pathogen-induced disease. Three direct routes of CR include 1) the capacity of the gut mutualistic and commensal microbiota to deplete the local environment of key nutrients required by the pathogen, 2) the ability of some microbiota to produce bacteriocins (toxic peptides) that act against specific pathogens, and 3) the use of type VI secretion systems by some gram-negative bacteria that result in membrane-associated, cell-cell toxicity against competing pathogens. Three indirect mechanisms of CR also reduced the risk of infectious disease and include 1) receptor-mediated stimulation of innate immune cells to produce antimicrobial peptides against pathogens, 2) metabolism of bile acids to produce metabolites that are toxic for certain pathogens, and 3) maintenance of the mucin layer and protection of the gut barrier by ensuring that epithelial cells are not damaged by pathogens.

The level of CR present in the infant gut can affect the minimum number of pathogenic organisms required to produce disease. Increasing the level of CR also increases the minimum load of pathogens required in the individual to bypass host defenses and produce disease. For this reason, optimizing CR through proactive management of the infant microbiome is a useful preventive strategy. There are key opportunities to institute this approach.

Microbial completeness (establishment of key founding species and richness of microbiota) at birth and microbial feeding and nurturing with human milk oligosaccharides are important steps in shifting the minimal pathogenic load needed to produce disease. But rather than requiring thousands of specific bacterial species to become established in locations such as the gut, there is evidence that gene- and metabolism-directed microbial seeding with a handful of bacterial species may be sufficient for effective CR against significant human pathogens. For example, using microbial loading of germ-free mice to examine resistance against the human enteric pathogen Salmonella enterica serovar Typhimurium, Brugiroux et al (42) reported that as few as 15 bacterial strains afforded the same level of protection against Salmonella infection as did a complete robust microbiome. Even more importantly, the bacterial genome design was used to determine the minimum number of friendly bacteria needed to provide protection.

Both probiotics and prebiotics have the capacity to shift the level of CR. For example, human breast milk contributes not only immune components such as immunoglobulin A to the infant, but also maternally derived microbiota (a natural form of probiotics). (43)(44) Human milk oligosaccharides (HMOs) are the natural component of breast milk that aid the growth and maturation of useful gut bacteria that can compete against pathogens. In addition, HMOs can also directly inhibit the growth of group B Streptococcus in a manner that is independent of their effect on host immunity. (45) The HMOs seem to function as an alternate substrate for group B enzyme activity, which results in reduced growth capacity of the bacterium. As such, HMOs can provide direct antimicrobial activity for these pathogens. In a separate study of group B Streptococcus, both growth and biofilm formation were inhibited by HMOs. (46)

INFANT SELF-COMPLETION FOR HEALTH RISK REDUCTION

NCDs and communicable (infectious) diseases have historically been viewed as completely distinct, with the latter being horizontally transmissible via the transfer of disease-producing microbes. However, emerging fundamental characteristics of the human microbiome are beginning to challenge the idea that NCDs and communicable diseases are necessarily completely separate and distinct. In a recent Lancet Global Health editorial, the authors argued that NCDs share social factors and are, in effect, socially transmitted conditions. (47) Unlike communicable diseases, which
follow the 4 criteria of disease transmissibility based on the Koch postulates, (2) NCDs have been considered to be nontransmissible via the horizontal or vertical transfer of specific microbes (eg, viruses, bacteria, fungi, parasites). But social conditions (diet, lifestyle, air and water pollutants, built environments, elective cesarean deliveries, formula feeding), in part, can shape a human’s microbiomal composition (most of the cells and genes of the microbiome.)

If a dysbiotic microbiome can produce disease, and microbiome elements of the dysbiosis can be transferred vertically (via the pregnant woman) and even horizontally (such as among household members), then there may be a communicable component to the risk of NCDs. The concept is that during periods in which an individual’s microbiome has been degraded (eg, after treatment with antibiotics) and CR is low, acquisition of microbiota from human-human contact and the local environment could predispose the recipient to misregulated inflammation and future NCDs.

Some evidence supports this theory. First, microbiota can be readily horizontally transferred when recipients are depleted in their microbiome diversity. This transfer can occur not only among members of the same species, but also between some species. Howler monkeys fed a highly restrictive diet in US zoos lost microbiome diversity over time. The microbiota of their closest contacts (human zoo handlers) became established in these animals, thereby “humanizing” the microbiomes of the captive animals. (48) Such transfers have the capacity to transfer microbiota-induced physiologic changes that predispose individuals to specific NCDs. Researchers recently transferred the capacity for atopic dermatitis in mice via the transfer of specific gut microbiota. (49) Similarly, Crohn disease–like ileitis can be transferred among mice by transferring dysbiotic, but not healthy, gut microbiota. (33)

Sharing of microbes between the mother and newborn and among household members is not restricted to pathogens. Mutualistic and commensal microbes also can be shared among household members and their pets (eg, dogs). Humans crowded into built environments and detached from the rich diversity of environmental/agrarian microbes will encounter narrow, passively accumulated and largely skin-derived microbiota as they move within the urban landscape. (50) In effect, urban-dwelling humans are more likely to share human-derived microbes that have been tailored by the very social factors that promote microbial dysbiosis and NCDs.

CONCLUSIONS

Active ecological management of the perinatal and infant microbiome offers significant opportunities to reduce health risks not only in the infant but also across later life. Most human genes and key metabolic pathways are present in the microbiota, which are concentrated around both mucosal tissues (eg, the gastrointestinal tract and airways) and additional portals of the infant’s exposure to the external environment (eg, skin). Microbiome assessment should be an annual part of patient personalized medical evaluation. Maternal diet, drugs, environment (eg, urbanization), stress, and birth delivery mode all affect the newborn’s microbiomal composition. Breastfeeding further benefits microbial seeding, feeding, and maturation of the infant microbiome as well as physiologic maturation. Microbial gene- and metabolism-directed optimization of the microbiome via diet, prebiotics, and probiotics—particularly during the first 1,000 days—can aid CR against pathogens. Pathogens not only produce infectious disease, but also can damage barrier function, and predispose the immune system toward inflammation-driven NCDs. For this reason, there is benefit in including optimized CR as a disease management and prevention goal within neonatology. Drug-induced damage to CR should be addressed with complementary therapies to restore this function.

American Board of Pediatrics Neontal-Perinatal Content Specification

- For antibiotics used commonly in the neonate, know indications for their use, clinical effects, pharmacokinetics, side effects, and toxicity.

References

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1. While making rounds in the NICU, you encounter a patient who is receiving antibiotics and several other medications. As you consider how the microbiome may affect the actions of those medications, which of the following enzymes can be found in the infant microbiome that could convert certain prodrugs to active compounds and in other cases, remove potentially toxic drug metabolites?

A. Lysosomal lipase.
B. β-Glucuronidase.
C. Coenzyme Q.
D. Bacterial ribonuclease.
E. Super-β-phosphocarboxymase.

2. A family has a history of atopy, including eczema and asthma, in their children. For a newborn in this family, increased levels of which of the following may increase the risk of atopic disease?

A. *Bifidobacterium*.
B. *Lactobacillus*.
C. Fungal species *Rhodotorula* and *Candida*.
D. *Bacteroides* fragilis.
E. Natural T-regulatory cells.

3. While discussing the use of antibiotics in protocols to prevent early-onset sepsis, a colleague mentions that changing practices may alter the presence of keystone species. Which of the following is an appropriate characterization of keystone species?

A. Mammals in which widespread increase in antibiotic therapies have led to a significant increase in somatic growth both prenatally and postnatally.
B. A group of viruses that provide enteric enzymes that aid in human digestion.
C. Functionally unique bacterial species that carry out critical biological or metabolic functions that are not duplicated across the microbiome.
D. Bacteria and mammal species that work together living in close physical association to the advantage of both.
E. Bacteria that rely on key nutrients and hormones provided by the host organism to proliferate.

4. A pregnant woman at term gestation enters the labor and delivery department, and the fetus is in breech presentation. A cesarean delivery is planned and the woman receives antibiotics for surgical prophylaxis. Which of the following statements regarding maternal receipt of antibiotics and the neonatal microbiome is correct?

A. There is a higher likelihood of antibiotic-resistant genes being enriched in the infant’s oral microbiome.
B. The neonatal microbiome will have increased growth of Streptococcaceae and Lactobacillales.
C. The neonatal microbiome will shift to resembling more of the father’s profile than the mother’s.
D. Colonizing microbes in the infant will have a deficiency of Proteobacteria compared with the mother.
E. The timing of antibiotics so close to the time of delivery will ensure that there will be zero impact on the neonatal microbiome.

5. You are counseling a family regarding the benefits of breastfeeding. They are aware of the potential immune benefit, but ask about other potential benefits. Which of the following statements concerning breastfeeding, immune function, and microbial completeness is correct?
A. The most abundant immunoglobulin found in human milk is immunoglobulin E.
B. Human milk oligosaccharides aid growth and maturation of useful gut bacteria, and can inhibit growth of group B *Streptococcus*.
C. Human milk is the most sterile fluid in the human body, because it contains multiple antimicrobial agents and properties.
D. The most recent evidence points to the key feature of microbial completeness being thousands or millions of specific bacterial species to be established in the gut to "compete" with each other to prevent neonatal disease.
E. Neisseriaceae, Comamonadaceae, and Oxalobacteriaceae are increasingly recognized as the key bacteria that promote healthy progression of a stable neonatal microbiome that prevents infectious disease.
Neonatal Herpes Simplex Virus Infection

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Education Gaps

1. Neonatal herpes simplex virus is a virus capable of infecting the central nervous system of neonates, causing significant morbidity and mortality.

2. Neonatal herpes simplex virus should be considered as a possible etiologic factor when a neonate presents with signs of sepsis.

Abstract

Herpes simplex virus (HSV) is among the most severely debilitating viruses that can infect the neonate, and is associated with significant mortality and morbidity. Neonatal HSV infection generally is acquired in the peripartum period, and can be devastating if not diagnosed appropriately. Studies conducted over several decades have advanced our knowledge of the benefit of antiviral therapy on neonatal HSV disease outcomes. As such, many neonates now are effectively treated and experience no or fewer long-term sequelae of this potentially devastating infection. Clinicians must be astute, because early diagnosis and early treatment are key to a better prognosis.

Objectives

After completing this article, readers should be able to:

1. Discuss the timing and risk factors for neonatal infection.

2. Review the clinical manifestation of neonatal infection and disease.

3. Discuss the diagnostic evaluation of neonatal herpes simplex virus (HSV) disease.

4. Identify the treatment of neonatal HSV.

5. Identify the outcomes of neonatal HSV treatment.

INTRODUCTION

Numerous viruses are capable of infecting the central nervous system (CNS) of neonates, but herpes simplex virus (HSV) is among the most severe, with significant mortality and morbidity. Unlike other viral pathogens, HSV is treatable using a commercially available antiviral drug, acyclovir. Neonatal HSV infection is primarily acquired during the peripartum period, which improves the likelihood of transmission from an infected mother to the neonate.
that antiviral therapy can be beneficial. Viral damage is of a relatively short duration in neonatal HSV disease acquired at birth compared with injury to the developing fetal brain from viruses that are acquired in utero. Studies conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group over the course of 4 decades have advanced our knowledge of the favorable impact that antiviral therapy has on neonatal HSV disease outcomes. Many neonates now are effectively treated and experience no long-term sequelae of this potentially devastating infection. Without treatment, however, neonatal HSV disease can be fatal and extremely devastating. It is important for clinicians and healthcare professionals to recognize signs of neonatal HSV infection. This can lead to prompt diagnosis and treatment, leading to a better prognosis for infants affected by the virus.

**EPIDEMIOLOGY**

The HSV consists of enveloped, double-stranded DNA. The virus establishes latency after a primary infection, and then periodically reactivates and causes recurrent symptomatic disease. It can also cause asymptomatic viral shedding that is clinically unapparent. The incidence of neonatal HSV infection is between 1 in 1,000 and 1 in 20,000 live births. (1) Recent data suggest that this disease incidence may be increasing. (2) Neonatal HSV is acquired in 1 of 3 distinct periods: intrauterine, peripartum, and postpartum. Most infants (~85%) acquire the infection perinatally or in the peripartum period. (3) Approximately 10% of neonates with HSV disease are infected perinatally, and 5% acquire the infection during the intrauterine period. (3) Risk factors that increase the likelihood of HSV transmission from a pregnant woman who is shedding HSV genitally to her infant include:

1. Type of maternal infection (primary vs recurrent) (4)(5)(6)(7)(8)
3. Longer duration of rupture of membranes (7)
4. Integrity of mucocutaneous barriers (using fetal scalp probe, incisions, etc) (8)(12)(13)
5. Mode of delivery (cesarean vs vaginal delivery) (8)

Infants born to women with primary (ie, first episode) genital HSV infection near the time of delivery are known to be at much greater risk of developing neonatal herpes than are infants who are born to women with recurrent genital HSV infection near the time of delivery (25%–60% vs <2%, respectively). (4)(5)(6)(7)(8) This increased risk is because of 2 main factors. First, there is a lower concentration of transplacentally passed HSV-specific antibodies in infants born to women with primary infections, because the pregnant woman has not had time to generate anti-HSV immunoglobulin G. (10) In addition, newly developed antibodies tend to be less effective in binding viral peptides. Second, a larger burden of the virus is shed vaginally and for a longer period in the genital tract of women with primary infection compared with those with recurrent HSV infection. (14) This was demonstrated in a landmark study of approximately 60,000 women in labor who did not have any symptoms of genital HSV infection at the time of delivery. In approximately 40,000 of these women, a vaginal swab was obtained for HSV detection within 48 hours of delivery (Fig 1). (8) Of these, 40,000 women, 121 were identified to have asymptomatic shedding of HSV and had serum specimens available for HSV serologic testing, thereby allowing for the determination of first episode versus recurrent maternal infection. The trial found that 57% of infants born to women with primary infection who were shedding the virus in their genital tracts at delivery developed neonatal HSV; 25% of infants born to women with first episode nonprimary infection (had preexisting HSV-1 antibody and acquired HSV-2 or vice versa) developed neonatal HSV; and only 2% of infants born to women with recurrent HSV developed neonatal HSV (Fig 1). (8) This same large study also confirmed that cesarean delivery effectively decreased transmission of HSV to the neonate when women are shedding this virus in their genital tracts. (7) Despite this degree of protection, the risk of HSV transmission is not eliminated by cesarean delivery, and there are still cases of infants delivered via cesarean section who are found to have HSV. (15)(16)(17)

**CLINICAL MANIFESTATIONS OF NEONATAL INFECTION AND DISEASE**

Based on the extent of involvement, neonatal HSV infection is classified into 1 of 3 categories: disseminated disease; CNS infection; or skin, eyes, and mouth (SEM) infection. Disseminated disease involves multiple organs including, but not limited to, lung, liver, adrenal glands, brain, and skin. CNS disease involves the brain, with or without skin involvement, but no visceral organ dysfunction. SEM disease is limited only to these areas of the body. This classification system is predictive of morbidity and mortality, with disseminated disease having the most significant mortality and CNS disease having the most significant morbidity. (18)(19)(20)(21)(22)(23)(24)

Disseminated infection can manifest as severe hepatitis, disseminated intravascular coagulation, pneumonitis, and possibly CNS involvement (found in 60%–75% of cases).
The mean age at presentation of disseminated infection is approximately 11 days after birth. Interestingly, more than 40% of cases of disseminated HSV disease do not develop skin findings, which can complicate the ability to make the diagnosis promptly. (16)(19)(24)(25)

Neonatal HSV CNS disease can present as seizures (focal or generalized), lethargy, poor feeding, irritability, tremors, temperature instability, and bulging fontanelle. The mean age at presentation for CNS disease is approximately 16 days after birth. (19) Approximately 60% to 70% of infants with CNS disease will also have skin manifestations at some point in the disease course. (19)(24) Mortality is usually due to devastating brain destruction and atrophy, causing neurologic and autonomic dysfunction.

SEM disease is associated with the best outcomes, with virtually no mortality and with morbidity associated solely with cutaneous recurrences but no neurologic sequelae. In addition, infants with SEM disease are most likely to have skin lesions (in >80% of patients), which facilitates diagnosis and allows prompt initiation of antiviral treatment before the disease progresses to involve other organs. Presenting signs and symptoms of SEM disease include skin vesicles, fever, lethargy, and conjunctivitis. (19) The mean age at presentation for SEM disease is approximately 12 days after birth. (16)

**DIAGNOSIS OF NEONATAL HSV DISEASE**

The diagnosis of neonatal HSV infections requires sampling of multiple sites (1):

1. Swabs of mouth, nasopharynx, conjunctivae, and rectum should be tested for HSV surface cultures (if available) or polymerase chain reaction (PCR).
2. Specimens of skin vesicles should be tested for culture (if available) or PCR.
3. Cerebrospinal fluid (CSF) specimens should be tested for HSV PCR.
4. Whole blood samples should be tested for HSV PCR.
5. Alanine aminotransferase should be measured as an indicator of hepatic involvement (1)

In the past, the presence of red blood cells in CSF was suggestive of HSV CNS infection, likely as a result of relatively advanced disease due to diagnostic limitations; however, with the development of more advanced imaging and diagnostic capabilities, hemorrhagic HSV encephalitis is less commonly seen now, and as such, most HSV CNS
CSF indices do not have significant numbers of red blood cells. Performance of whole blood PCR adds to the other diagnostic tools (surface and CSF cultures and CSF PCR), but should not be used as the sole test for ruling in or ruling out neonatal HSV infection. Furthermore, viremia and DNAemia can occur in any of the 3 types of neonatal HSV disease, so a positive whole blood PCR simply rules in neonatal HSV infection but does not assist in disease classification. HSV isolates from culture or HSV DNA detected on PCR can be typed to determine whether it is HSV type 1 or HSV type 2. Chest radiographs and liver function tests can aid in the diagnosis of disseminated infection. Histologic testing is of low yield because it has low sensitivity and should not be used for diagnosis. All infants with HSV disease, regardless of classification, need to have an ophthalmologic examination to look for ocular involvement such as uveitis, conjunctivitis, and keratitis. Infected neonates with any extent of disease manifestations should undergo neuroimaging studies (magnetic resonance imaging preferably, but head computed tomography or ultrasonography are acceptable) to establish baseline brain anatomy. Later findings can include brain abscesses (particularly in the temporal lobe) or severe encephalomalacia.

**TREATMENT OF NEONATAL HSV DISEASE**

Before antiviral therapies were developed and used, disseminated HSV disease caused death by 1 year of age in 85% of patients. In infants with CNS disease, mortality was 50% (Table 1). In a series of research studies conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group between 1974 and 1997, 295 infants with neonatal herpes simplex virus infection were evaluated. Mortality and morbidity outcomes among these infants are summarized in Table 1.

**TABLE 1. Mortality and Morbidity Outcomes Among Infants with Neonatal Herpes Simplex Virus Infection**

<table>
<thead>
<tr>
<th>EXTENT OF DISEASE</th>
<th>PLACEBO (22)</th>
<th>VIDARABINE (20)</th>
<th>ACYCLOVIR (20) 30 MG/KG PER DAY</th>
<th>ACYCLOVIR (18) 60 MG/KG PER DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disseminated disease</td>
<td>n=13</td>
<td>n=28</td>
<td>n=18</td>
<td>n=34</td>
</tr>
<tr>
<td>Dead</td>
<td>11 (85%)</td>
<td>14 (50%)</td>
<td>11 (61%)</td>
<td>10 (29%)</td>
</tr>
<tr>
<td>Alive</td>
<td>2 (15%)</td>
<td>14 (50%)</td>
<td>7 (39%)</td>
<td>24 (71%)</td>
</tr>
<tr>
<td>Normal</td>
<td>1 (50%)</td>
<td>7 (50%)</td>
<td>3 (43%)</td>
<td>15 (63%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>1 (50%)</td>
<td>5 (36%)</td>
<td>2 (29%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0%)</td>
<td>2 (14%)</td>
<td>2 (29%)</td>
<td>6 (25%)</td>
</tr>
<tr>
<td>Central nervous system infection</td>
<td>n=6</td>
<td>n=36</td>
<td>n=35</td>
<td>n=23</td>
</tr>
<tr>
<td>Dead</td>
<td>3 (50%)</td>
<td>5 (14%)</td>
<td>5 (14%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Alive</td>
<td>3 (50%)</td>
<td>31 (86%)</td>
<td>30 (86%)</td>
<td>22 (96%)</td>
</tr>
<tr>
<td>Normal</td>
<td>1 (33%)</td>
<td>13 (42%)</td>
<td>8 (27%)</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>2 (67%)</td>
<td>17 (55%)</td>
<td>20 (67%)</td>
<td>9 (41%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0%)</td>
<td>1 (3%)</td>
<td>2 (7%)</td>
<td>9 (41%)</td>
</tr>
<tr>
<td>Skin, eye, and mouth infection</td>
<td>n=8</td>
<td>n=31</td>
<td>n=54</td>
<td>n=9</td>
</tr>
<tr>
<td>Dead</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Alive</td>
<td>8 (100%)</td>
<td>31 (100%)</td>
<td>54 (100%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Normal</td>
<td>5 (62%)</td>
<td>22 (71%)</td>
<td>45 (83%)</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>3 (38%)</td>
<td>3 (10%)</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0%)</td>
<td>6 (19%)</td>
<td>8 (15%)</td>
<td>7 (78%)</td>
</tr>
</tbody>
</table>

*Data are from an evaluation of 295 infants with neonatal herpes simplex virus infection conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group between 1974 and 1997. Adapted from Kimberlin. (26)
1997, parenteral vidarabine, lower-dose acyclovir (30 mg/kg per day), and higher-dose acyclovir (60 mg/kg per day) were evaluated sequentially. (20)(22)(27) In the first of these studies, 10 days of vidarabine decreased mortality rates compared with placebo at 1 year both for patients with disseminated disease (rates decreased from 85% to 50%) and for those with CNS disease (rates decreased from 50% to 14%). Following comparison of lower-dose acyclovir with vidarabine for 10 days, parenteral acyclovir became the primary treatment choice for neonatal HSV disease because of its more favorable safety profile and its relative ease of administration (vidarabine required prolonged infusion times in large volumes of fluid). A subsequent study of higher-dose acyclovir for 21 days produced further reductions in 1-year mortality rates to 29% for disseminated disease (Fig 2) (18) and 4% for CNS disease (Fig 3). (18)

These series of studies determined that infants with neonatal HSV disease should be treated with parenteral acyclovir at a dose of 20 mg/kg per dose administered every 8 hours; the dosing interval may need to be increased in premature infants based on their creatinine clearance. (28) The treatment duration is 21 days for infants with disseminated disease or CNS disease, while infants with SEM disease should be treated for 14 days. (i) All patients with CNS involvement should have a repeat lumbar puncture near the end of the 21-day course of acyclovir to document that the CSF PCR is negative; if the PCR remains positive, another week of parenteral acyclovir should be administered, and lumbar punctures should be repeated in that manner until a negative CSF PCR is achieved. (19)(29)

The primary toxic effect of higher-dose parenteral acyclovir is neutropenia. (18) Thus, absolute neutrophil counts (ANCs) should be monitored twice weekly throughout the course of parenteral therapy. If ANC is less than 500/\mu L, either acyclovir treatment can be withheld or granulocyte colony-stimulating factor can be administered. Parenteral acyclovir dosing can resume when the ANC is higher than 750/\mu L. (18)

Oral acyclovir suppressive therapy for 6 months after acute parenteral treatment improves neurodevelopmental outcomes in infants with CNS disease. (i) It is well-known that HSV establishes latency in the sensory ganglia, and

![Figure 2](image-url) Mortality in patients with disseminated neonatal herpes simplex virus disease. Adapted from Kimberlin et al. (18)

![Figure 3](image-url) Proportion of surviving patients with neonatal herpes simplex virus disease affecting the central nervous system. Adapted from Kimberlin et al. (18)
occasionally reactivates and causes recurrence of disease. However, it is not well-known if the virus subclinically reactivates in the brain after neonatal HSV has been treated, particularly with CNS involvement. If it does reactivate, it could be the cause of poor neurodevelopmental outcomes in patients with CNS involvement. A recent study involving infants with neonatal HSV with CNS involvement compared Bayley mental-developmental scores at 1 year in infants receiving suppressive therapy with acyclovir for 6 months versus infants receiving placebo (Table 2). The study found that the acyclovir group had a significantly higher mean Bayley score than the placebo group (88.24 vs 68.12, \( P = 0.046 \)). Suppressive acyclovir therapy also has been proven to prevent skin recurrences in HSV disease of all types. Thus, infants should receive oral acyclovir at 300 mg/m² per dose 3 times daily as suppressive therapy for 6 months after the initial parenteral treatment course. This dose should be adjusted for growth monthly, and ANCs should be monitored at 2 and 4 weeks after starting therapy and then monthly thereafter while oral acyclovir is administered. (1)

OUTCOMES OF NEONATAL HSV WITH TREATMENT

Until recently, improvement in morbidity outcomes after antiviral treatment was less dramatic than mortality for neonates with disseminated disease or CNS disease. Oral acyclovir suppressive therapy has significantly improved the neurologic outcomes of infants with CNS involvement. Without treatment, 50% of neonates who survived disseminated HSV disease were developing normally at 1 year of age. (22) With the use of higher-dose acyclovir for 21 days,

### TABLE 2. Bayley Mental Scores at 12 months in Patients with CNS Involvement and SEM Disease*

<table>
<thead>
<tr>
<th>CASG 103 (CNS INVOLVEMENT STUDY)</th>
<th>CASG 104 (SEM STUDY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACYCLOVIR (N = 16)</td>
<td>PLACEBO (N = 12)</td>
</tr>
<tr>
<td>Median</td>
<td>90.5</td>
</tr>
<tr>
<td>Adjusted mean</td>
<td>88.24*</td>
</tr>
<tr>
<td>( P ) value by ANCOVA</td>
<td>0.046</td>
</tr>
</tbody>
</table>

*Patients were treated with placebo versus acyclovir suppression for 6 months. ANCOVA was adjusted for covariates at baseline which were unbalanced between treatment groups. ANCOVA = analysis of covariance; CASG = Collaborative Antiviral Study Group; CNS = central nervous system; SEM = skin, eyes, and mouth disease.

*Head circumference at birth, birthweight, enrollment weight.

*Enrollment weight.

Adapted from Kimberlin et al. (30)

Figure 4. Morbidity among patients with known outcomes after 12 months of age. CNS = central nervous system; SEM = skin, eyes, and mouth disease. Adapted from Kimberlin et al. (18)(30)
the number of infants developing normally at 1 year of age after disseminated HSV disease has increased to 83% (Fig 4). (18) Similarly, for CNS HSV disease, 33% of patients demonstrate normal neurologic development at 1 year of age after 10 days of lower-dose acyclovir therapy, compared with 31% of children treated with higher-dose acyclovir for 21 days. However, with concomitant use of oral acyclovir therapy for 6 months, this percentage of infants with normal neurodevelopment at 1 year increases to 69% (Fig 4). (30) Morbidity of SEM disease also has dramatically improved since the introduction of antiviral treatment. In the preantiviral era, 38% of patients with SEM disease were developing normally at 1 year of age, but with antiviral therapy, this risk is eliminated completely (due to SEM disease not progressing to CNS or disseminated disease). (19)

CONCLUSION

Neonatal HSV disease is known to have devastating neurologic effects. Fortunately, over the past decades, much has been learned about the natural history, pathogenesis, diagnosis, and treatment of this severe infection. In the 21st century, neonatal HSV disease is treatable, and management recommendations have been standardized and implemented. As more knowledge is obtained, more questions are formed. These questions in turn drive the next series of studies, with further promise of continued advances for the future.

References


Parent Resources from the AAP at HealthyChildren.org

* [https://www.healthychildren.org/English/health-issues/conditions/skin/Pages/Herpes-Simplex-Virus-Cold-Sores.aspx](https://www.healthychildren.org/English/health-issues/conditions/skin/Pages/Herpes-Simplex-Virus-Cold-Sores.aspx)

For a comprehensive library of AAP parent handouts, please go to the *Pediatric Patient Education* site at [http://patiented.aap.org](http://patiented.aap.org).
Human Immunodeficiency Virus Type 1 in the Premature Infant

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Education Gaps

With rapid changes occurring in the field of antiretroviral agents used to prevent and treat human immunodeficiency virus type 1 infection, neonatal clinical providers need to be aware of these advancements and recognize how these agents are used in the preterm infant population.

Abstract

Prematurity and low birthweight are seen frequently in infants with human immunodeficiency virus (HIV) type 1 infection, adding significant comorbidities and complicating the approach to treatment. HIV disease progression accelerates in the setting of an immature immune system. Recent cases have underscored the unique opportunity to not only limit progression, but also limit the establishment of HIV reservoirs that impede viral clearance by starting antiretroviral treatment (ART) early in the neonatal period. Although pediatric ART alternatives are increasing, there are still only few available agents for the treatment of neonates, especially premature and low-birthweight infants. Zidovudine is the only agent for which there is sufficient experience in premature infants, while being an intravenous alternative for infants in whom enteric administration is not possible. Nevirapine has been studied for prophylactic dosing in preterm infants. It is imperative that resources are devoted to the study of the safety and efficacy of ARTs for use during the neonatal period.

Objectives

After completing this article, readers should be able to:

1. Discuss the use of human immunodeficiency virus (HIV) diagnostic tools in the neonate.
2. Describe the strategies to prevent mother-to-child transmission of HIV.
3. Describe the difference between prophylactic and treatment dosing of antiretroviral medications.
4. Identify the available antiretroviral agents for the neonatal age group.

AUTHOR DISCLOSURE Dr Deville has 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.

ABBREVIATIONS

cART combination antiretroviral treatment
CYP cytochrome
HIV human immunodeficiency virus
MTCT mother-to-child transmission
RT reverse transcriptase
ZDV-TP zidovudine triphosphate
INTRODUCTION

The rate of mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) type 1 in the United States has dramatically declined in the past quarter century. HIV MTCT decreased from 22.6% among children whose mothers did not receive antiretroviral therapy (ART) to 7.6% between 1991 and 1994 in children who received neonatal single-agent zidovudine prophylaxis after their mothers received antenatal and intrapartum zidovudine. (1) In 2000, HIV MTCT further decreased to 1% to 2% among children who received neonatal single-agent zidovudine prophylaxis after their mothers received combination ART (cART) regimens. (2) Between 2002 and 2009, a US prospective cohort study found an MTCT of 0.65% when HIV-positive pregnant women received diverse cART regimens and infants were never breastfed. (3) In this study, half of the HIV-infected infants were born prematurely, compared with a prematurity rate of about 12.7% for the general US population. (4)

The preceding 2 decades have harbored many advances in the care of HIV-infected children. In particular, ART has evolved at a rapid pace. However, there is still a significant gap in the introduction of newer, more potent, less toxic, and better tolerated antiretroviral agents in young children, although some have been introduced gradually in recent years. (5)(6)(7) Within the pediatric age group, the gap is accentuated in newborns, most notably in premature infants, the most vulnerable group of HIV-infected individuals. Recent cases of infants treated very early in life who achieved a prolonged period of treatment-free suppression (8)(9) have generated considerable interest in the possibility of modifying the natural disease history by adopting early diagnosis and treatment modalities. (10)

The pathogenesis and virologic principles of HIV infection are similar during the neonatal period and older age groups. Low-birthweight and premature infants, however, constitute the most neglected population in terms of treatment experience, presenting both a challenge and an opportunity. A unique aspect of HIV infection in the neonatal period is the need to rely on virologic methods for establishing the disease diagnosis. In children born to HIV-positive mothers, one must take into account the perinatal route of acquisition, the possible previous exposure to antiretroviral agents during pregnancy, the higher plasma viremia levels observed in infants when compared with adult populations during acute infection, the high level of variability in pharmacokinetic parameters, immunologic immaturity, and the onset of infection in a growing individual.

Virtual all newborns born to an HIV-positive mother will have detectable antibodies against HIV type 1 (HIV-1), irrespective of the testing method used. (11) This situation precludes the use of rapid testing methods that have become widely available and underscores the need for reliable, rapid, and cost-effective nucleic acid diagnostic tools. At the present time, the commonly used virologic methods rely on measuring free plasma virus (HIV RNA) or cell-integrated virus (HIV DNA). Although extremely sensitive, both these methods introduce an element of delay in the diagnosis, usually measured in days, which complicates the early management of HIV-infected newborns.

The medical care of infants and children with HIV infection is continuously evolving, as results of new research are reported, new agents are approved, and new treatment modalities are adopted. Clinical trials guide appropriate dosing regimens in children and allow us to determine the safety and efficacy of these agents as they become available. Early diagnosis and prompt institution of cART can be effective in preventing disease progression. A significant reduction in disease progression and mortality was observed in a study in which cART was started at 6 weeks of age. (12) However, the same study showed that nearly 1 in 5 infants had a significant decline in the percentage of helper T cells (CD4+ cells) during the 3 to 4 preceding weeks. More ominously, 7 HIV-infected infants died and 16 developed signs of advanced HIV during the same pre-cART period. Furthermore, when treatment is delayed for 12 weeks, more than 60% of infants had developed advanced disease. Therefore, diagnosis needs to be attempted as soon as feasible.

Another important aspect is to recognize the increased risk of transmission experienced by women who acquire the infection during pregnancy. In particular, women infected during the third trimester have both a high risk of delivering prematurely and transmitting the virus to their offspring. A South African program identified seroconversion in 3.3% of pregnant women who tested negative earlier in pregnancy, accounting for 26% of HIV-infected infants, including a significant proportion of low-birthweight and preterm infants. (13)

Once diagnosis has been established, the immediate challenge is to identify a safe and effective antiretroviral regimen. Combination regimens using 3 antiretroviral drugs have been shown to enhance survival rates, reduce opportunistic infections, and delay disease progression. In the premature infant, this is of particular importance given the rapid pace of disease progression observed in untreated infants, complicated by the need to avoid short- and long-term toxicities, and development of drug resistance. Due to these complexities and the relative infrequency of this presentation, practitioners should be encouraged to seek consultation with a pediatric HIV specialist when faced with this situation. The Table provides a summary of antiretroviral treatments used for term and preterm infants.
ZIDOVUDINE

Zidovudine is a synthetic nucleoside analogue used in the treatment of infections caused by HIV-1. Intracellularly, zidovudine is phosphorylated to its active 5'-triphosphate metabolite, zidovudine triphosphate (ZDV-TP). The principal mode of action of ZDV-TP is inhibition of reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue. ZDV-TP is a weak inhibitor of the cellular DNA polymerases α and γ and has been reported to be incorporated into the DNA of cells in culture. The finding that an antenatal, intrapartum, and 6-week newborn regimen of zidovudine reduced perinatal transmission of pediatric HIV infection by nearly 70% was a landmark in HIV prevention. (1) The pharmacokinetics of zidovudine were evaluated in infants from birth to 3 months of age. Zidovudine elimination was determined immediately after birth in 8 neonates exposed to zidovudine in utero; the half-life was found to be 13.0–5.8 hours. In neonates 14 days of age or younger, bioavailability was greater, total body clearance was slower, and half-life was longer than in pediatric patients older than 14 days. (14) Zidovudine pharmacokinetics were also studied in preterm infants. (15) A total of 38 HIV-exposed infants born before 35 weeks of gestation who were receiving zidovudine as part of standard prophylaxis to reduce perinatal HIV transmission were enrolled into a pharmacokinetic study during the first 5 days after birth. Infants were given intravenous zidovudine 1.5 mg/kg every 12 hours until 2 weeks of age, then 2.0 mg/kg every 8 hours until 6 weeks of age. Zidovudine was generally well tolerated in this high-risk population. The authors recommended 1.5 mg/kg (intravenous) or 2.0 mg/kg (oral) every 12 hours with an increase in frequency to every 8 hours at 2 weeks of age (for infants with birth gestational age <30 weeks) or at 4 weeks (for infants with birth gestational age ≥30 weeks). To date, zidovudine is the only drug that has been sufficiently studied in premature infants.

LAMIVUDINE

Lamivudine is a cytosine nucleoside analogue RT inhibitor used in the treatment of infections caused by HIV-1 and hepatitis B virus. It undergoes anabolic phosphorylation by intracellular kinases to form lamivudine 5'-triphosphate. The active anabolite prevents HIV-1 and hepatitis B viral replication by competitively inhibiting viral RT and terminating proviral DNA chain extension. Lamivudine is widely distributed into total body fluids. Lamivudine concentrations in maternal serum, amniotic fluid, umbilical cord, and neonatal serum are comparable, indicating that the drug diffuses freely across the placenta. In postpartum women, lamivudine is secreted into breast milk. Lamivudine and zidovudine exhibit synergy in vitro, (16)(17) which provides the rationale for combining these 2 agents in the treatment of HIV-1 infection. More importantly, lamivudine induces a lamivudine-resistant methionine-to-valine substitution mutation at codon 184 (M184V mutation) at the YMDD motif or region in HIV-1 RT. This mutation is associated with a delay in the emergence of zidovudine-resistant mutants in zidovudine-naive patients, restoration of zidovudine sensitivity in some patients already possessing zidovudine-associated resistance mutations, impairment of HIV-1 replication capacity and virulence (“fitness”), and a reduced ability of the HIV-1 env gene to mutate. (18)(19)(20) Lamivudine alone and in combination with zidovudine has been studied in 20 neonates, in whom therapy began 12 hours after birth and was continued for 1 week. (21) The pharmacokinetic parameters of lamivudine were not altered by the coadministration of zidovudine. It was observed that

### TABLE. Antiretroviral Agents for Term and Preterm Newborns in 2018

<table>
<thead>
<tr>
<th>CLASS</th>
<th>DRUG</th>
<th>USE IN TERM INFANTS</th>
<th>USE IN PRETERM INFANTS</th>
<th>OTHER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside reverse transcriptase inhibitors</td>
<td>Zidovudine</td>
<td>Yes</td>
<td>Yes</td>
<td>Intravenous formulation is available</td>
</tr>
<tr>
<td></td>
<td>Lamivudine</td>
<td>Yes</td>
<td></td>
<td>Dose for first month</td>
</tr>
<tr>
<td></td>
<td>Emtricitabine</td>
<td>Yes</td>
<td></td>
<td>Dose for 0–3 months</td>
</tr>
<tr>
<td></td>
<td>Stavudine</td>
<td>Yes</td>
<td></td>
<td>Frequent toxicities limit long-term use</td>
</tr>
<tr>
<td>Non-nucleoside reverse transcriptase inhibitors</td>
<td>Nevirapine</td>
<td>Yes</td>
<td>Only ≥34 weeks' gestational age</td>
<td></td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Lopinavir/ritonavir</td>
<td>Only after 2 weeks of age and 42 weeks’ postmenstrual age</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Integrase inhibitors</td>
<td>Raltegravir</td>
<td>Yes</td>
<td>No</td>
<td>Competes with bilirubin for protein binding and elimination</td>
</tr>
</tbody>
</table>

![Vol. 19 No. 2 FEBRUARY 2018](image)
lamivudine’s peak serum concentration occurred later, the areas under the plasma drug concentration-time curve and peak concentration were higher, and neonatal lamivudine clearance was about half that in pediatric patients. In view of the lower drug clearance-to-fraction absorbed in neonates at 1 week of age, the dosage of lamivudine in these patients should be reduced from 4 mg/kg twice daily (the recommended dosage for infants and children) to 2 mg/kg twice daily. However, there is insufficient information to establish the time course of changes in clearance between the immediate newborn period and the age ranges greater than 3 months. (22)

NEVIRAPINE

Nevirapine is a non-nucleoside RT inhibitor with activity against HIV-1. This drug remains a mainstay of therapy, especially in resource-limited settings. (23) Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. It is readily absorbed after oral administration, and is highly lipophilic and nonionized at physiologic pH. In vivo trials in humans and in vitro studies with human liver microsomes have shown that nevirapine is extensively biotransformed via cytochrome (CYP) P450 (oxidative) metabolism to several hydroxylated metabolites. (24) Oxidative metabolism of nevirapine is mediated primarily by CYP P450 isozymes from the CYP3A and CYP2B6 families, though other isozymes may have a secondary role. Nevirapine is an inducer of hepatic CYP P450 metabolic enzymes 3A and 2B6 by about 20% to 25%, as indicated by erythromycin breath test results and urine metabolites. In adults, autoinduction of CYP3A- and CYP2B6-mediated metabolism leads to an approximately 1.5- to 2-fold increase in the apparent oral clearance of nevirapine as treatment continues from a single dose to 2 to 4 weeks of dosing with 200 to 400 mg per day. (25) Autoinduction also results in a corresponding decrease in the terminal phase half-life of nevirapine in plasma, from approximately 45 hours (single dose) to approximately 25 to 30 hours following multiple dosing with 200 to 400 mg per day. This resulted in the need for a lead-in dose of 200 mg daily for 2 weeks, followed by 200 mg twice daily. Older pediatric subjects receiving nevirapine at 150 mg/m² twice daily (after a 2-week lead-in of 150 mg/m² daily) produced geometric mean or mean trough nevirapine concentrations between 4 and 6 μg/mL, similar to adult data. (26) Evaluation of pediatric subjects 1 to 3 months of age showed that plasma nevirapine concentrations observed were within the range observed in adults and the remainder of the pediatric population, but were more variable among subjects, particularly in the younger population. (27)

The increased rate of resistance observed with the widespread use of nevirapine for MTCT prophylaxis (28)(29) led to many practitioners opting for treatment dosing of HIV-exposed newborns. This modality was undertaken in an effort to prevent the emergence of resistance. Moreover, results of a randomized study showed that nevirapine was inferior to lopinavir-ritonavir in young infants, but not in older children, questioning the use of the lead-in dose in this population. (30) For the premature infant, only preliminary data are available. In premature infants, most data have been accumulated in prophylaxis studies, where the target trough concentration is above 0.1 μg/mL compared with the more than 3.0 μg/mL dose needed for treatment regimens. These studies showed a decrease in drug clearance inversely proportional to gestational age. (31)(32) To date, treatment doses of nevirapine for premature infants remain investigational, because data are only available for infants of 34 weeks’ or longer gestation who weigh 1.5 kg or more. For these patients, the recommended dose is 4 mg/kg per dose twice daily for the first week, increasing to 6 mg/kg per dose twice daily thereafter. (33)

OTHER ANTIRETROVIRAL AGENTS

Stavudine is a nucleoside RT inhibitor that was widely used in the pediatric population. Stavudine is virologically antagonistic with zidovudine, and the 2 agents should not be used together. Stavudine was studied in full-term neonates, in whom a dose of 0.5 mg/kg twice daily for the first 2 weeks of age was recommended with an increase to 1 mg/kg twice daily subsequently. However, because of the observed increased risk of adverse effects and overlapping toxicities, such as mitochondrial toxicity, insulin resistance, dyslipidemia, lipoatrophy, especially after long-term use, it is no longer recommended.

Emtricitabine and lamivudine have long been considered interchangeable. Both agents share similar resistance profiles and lack additive benefit, precluding their use together. Emtricitabine, similar to lamivudine, has relatively low toxicities, and evidence suggests that emtricitabine and lamivudine have equivalent efficacy and toxicity in antiretroviral-naïve patients. Although the neonatal dose of emtricitabine has been established as 3 mg/kg daily and its dosing frequency is attractive, lamivudine has been generally preferred because of the larger experience accumulated with this drug.

Lopinavir-ritonavir has been for many years one of the most effective antiretrovirals used in children. However, the adverse experience with lopinavir-ritonavir in neonates highlights the risks of using these drugs in neonates without appropriate safety and pharmacokinetic data. Life-threatening cardiovascular, renal, and central nervous system toxicities were reported to the Food and Drug Administration in 10...
neonates

conclusion

CONCLUSIONS

Since the introduction of zidovudine, monotherapy has been invariably followed by the emergence of resistant strains. The high-level viral replication of HIV, in conjunction with the known mutation rate of its RT, results in the emergence of resistance that can be detected as early as 24 hours after monotherapy is started. Modeling of single-drug regimens led to the observation of the emergence of resistance within 24 hours, while dual-drug agents take about 90 days for this to occur. Effective treatment forces the virus to mutate simultaneously at multiple positions in its genome, which is achieved with the use of 3 agents.

The overall goal of ART is to preserve immune function, limit the size of viral reservoirs, and reduce the emergence of antiretroviral resistance. In this setting, the use of zidovudine monotherapy could be seen as counterproductive, and therefore practitioners need to seek dual or, ideally, 3-drug regimens. At the present time, these regimens could only be safely constructed in consultation with a pediatric HIV specialist, because many considerations need to be taken into account, especially comorbidities associated with prematurity. Every patient needs to be carefully and individually evaluated, and a general regimen cannot be safely recommended. In some clinical situations, a 2-drug regimen could be appropriate temporarily until the third agent could be given safely. One important additional factor that needs to be taken into account when treating a premature infant with HIV is the family's socioeconomic situation and support system. In the author's experience, a multidisciplinary approach is essential, because of the presence of multiple comorbidities, and the use of multiple agents given several times a day to a small infant as an outpatient is difficult enough under ideal circumstances. Therefore, high levels of support are essential for a successful outcome when treating this vulnerable population.


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A Rare Case of Antenatal Hemorrhagic Stroke and Recurrent Purpura Fulminans in a Preterm Newborn

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CASE REPORT

A female infant is born prematurely at 33 weeks of gestation to consanguineous parents (parents are first cousins). The mother has had 2 uneventful pregnancies and both children are well. There is no history of recurrent miscarriages, stillbirth or neonatal stroke, or family history of bleeding diathesis or clotting disorders. Antenatal scans had revealed the fetus to be growing well until 30 weeks of gestation, when the weight dropped from the 40th to the 3rd percentile. At that time, the amniotic fluid volume, Doppler scan, and placental blood flow were normal. The mother had been asymptomatic, with normal blood cell count and erythrocyte sedimentation rate.

Now at 33 weeks of gestation, the mother presents to the emergency department with reduced fetal movements over the previous week. She is admitted for observation. Antenatal ultrasonography confirms the earlier findings of intrauterine growth restriction and documented normal amniotic fluid volume and Doppler scan. She remains clinically well. However, cardiotocographic monitoring the next day reveals the presence of repeated decelerations suggestive of nonreassuring fetal status, prompting an urgent cesarean delivery.

The infant is well at birth, and has a good cry. Apgar scores are 6 and 9 at 1 and 5 minutes, respectively. Birthweight is 1,160 g (<3rd percentile), length 39 cm (3rd-10th percentile) and head circumference 27.5 cm (3rd percentile). Soon after birth, the infant develops respiratory distress secondary to partially compensated metabolic acidosis (pH, 7.32; partial pressure of carbon dioxide, 19.5 mm Hg [2.6 kPa]; partial pressure of oxygen, 111 mm Hg [15 kPa]; base excess, −12; and bicarbonate, 14 mEq/L [14 mmol/L]). Clinical examination findings are unremarkable and vital signs are normal. No hypoglycemia or electrolyte abnormality is noted. Laboratory investigations on day 1 reveal thrombocytopenia (platelet count 39 × 10^3/μL [39 × 10^9/L]) and coagulopathy (prothrombin time 60 seconds, activated partial thromboplastin time >180 seconds) in the absence of clinical bruising and external hemorrhage. The hematologic abnormalities are promptly corrected with platelet and fresh frozen plasma (FFP) transfusions. She is empirically treated with intravenous antibiotics because of unexplained thrombocytopenia.

Cranial ultrasonography on day 1 after birth shows bilateral intraventricular hemorrhage with echogenicity in the temporoparietal area. Serial cranial ultrasonography in the ensuing weeks shows progression of hemorrhage, resulting in dilation of both lateral and third ventricles (Fig 1). She appears pale and had tachycardia throughout the first 2 weeks after birth, associated with persistent
decline of hemoglobin from 16.6 g/dL (160 g/L) to 9.2 g/dL (92 g/L) despite receiving red blood cell transfusions.

Brain magnetic resonance imaging (MRI) on day 18 after birth shows multiple hemorrhagic lesions (Fig 2). The widespread distribution of affected areas clearly does not correspond to arterial ischemia and suggests the possibility of venous thromboembolism. Amplitude-integrated electroencephalography demonstrates episodes of electrographic seizures that are difficult to control despite multiple phenobarbital boluses. Seizures are finally controlled with phenobarbital 6 mg/kg per day and levetiracetam 80 mg/kg per day.

In consultation with pediatric hematology, thrombophilia screening is undertaken to investigate the etiology of the stroke. The diagnosis of severe protein C deficiency is rendered after an extremely low protein C level of 1% (age-specific reference range 12%–44%) is found. Protein C level is low in both parents (63% and 49%; adult reference range 70%–140%). Initial management consists of daily FFP transfusions (40 mL/kg per day), which transiently raises the protein C level to 10% but this is poorly sustained. A second MRI/magnetic resonance angiography of the brain on day 44 after birth shows interim development of sagittal sinus thrombosis as well as evolving encephalomalacia (Fig 3). Subcutaneous enoxaparin treatment is commenced on day 50 after birth in view of sagittal sinus thrombosis in the absence of further bleeding. Despite achieving therapeutic anti-Xa levels of 0.5 to 1.0 U/mL, she develops purpura fulminans 10 weeks after birth (Fig 4), which then recurs 20 weeks after birth during an episode of rotavirus gastroenteritis (Fig 5).

Figure 1. Top row: Coronal section of cranial ultrasonographic images obtained on day 1 after birth. Note the focal hyperechoic area in the anterior aspect of the corpus callosum (black arrow), suggestive of a periventricular bleed. A fluid-fluid level in the cavum vergae (white arrow) indicates intraventricular hemorrhage. Bottom row: Coronal section of cranial ultrasonographic images obtained on day 18 after birth. Note the ventricular dilation (white arrow) and multiple areas of echogenicity in the frontal and parieto-occipital regions (black arrow), suggestive of progression of hemorrhage when compared to previous images.

Figure 2. Noncontrast magnetic resonance imaging of the brain on day 18 after birth showing axial T1 (A, B) and T2 (C) sections. Multiple areas of ischemia and hemorrhage can be seen, involving brain parenchyma (thick short arrows), subdural spaces (open arrow), subarachnoid spaces (thin arrow), and intraventricular spaces (arrowheads).
From the third month after birth, there is no further intracranial hemorrhage. Neurologically, the infant has marked head lag and poor truncal tone, with evolving signs of an upper motor neuron impairment involving all 4 limbs. She requires nasogastric tube feeding because of sucking-swallowing-breathing incoordination. Frequent accumulation of oral secretions places her at risk for aspiration. Her parents receive counseling about the underlying etiology of intracranial hemorrhage and definitive management plans, including the need for long-term replacement therapy with protein C concentrate. Taking into consideration the cost of regular protein C concentrate and the severity of encephalomalacia with high likelihood of poor neurodevelopmental outcomes, a joint decision is made to pursue limited medical care. This consisted of intermittent FFP transfusions, subcutaneous enoxaparin, and physiotherapy.

DISCUSSION

Severe congenital protein C deficiency is frequently diagnosed late or underdiagnosed because of its rarity and variable presentation. A frequent mode of presentation is neonatal purpura fulminans or disseminated intravascular coagulation. (1) It may also be associated with massive thrombosis or in-utero death. Case ascertainment often depends on whether mothers with unexplained miscarriage or stillbirth undergo thrombophilia screening. Investigations in cases of neonatal stroke are often not offered because parents are asymptomatic as in the case reported here. Thus, the prevalence of congenital protein C deficiency of 1 in 4 million may be grossly underestimated.

Interpretation of protein C levels in a newborn is fraught with difficulty. Physiologically, newborn infants have low levels of protein C—a term neonate has protein C levels equivalent to 35% of normal adult levels, whereas a preterm neonate has 10% to 15% of normal adult levels, but this has not been universally studied in a preterm neonatal population. (2) Levels are further reduced in the setting of acute thrombosis and hence, thrombophilia screening is usually not advised during such an episode. However, there may be value in testing to exclude severe deficiencies.

In our patient, the declining fetal growth at the 30-week antenatal scan and the paucity of fetal movement from the...
32nd week of gestation were red flag features of fetal compromise. Given the severity of the protein C deficiency, it was surprising that she did not initially present with purpura fulminans. We conjecture that the predilection of cerebral vessels for hemorrhage was due to disturbances in cerebral blood flow coupled with intrinsic fragility of the germinal matrix of an immature brain. Also, the presentation of purpura fulminans 10 weeks after birth illustrates that it can occur at any point of the disease process.

With the initial presentation of unexplained metabolic acidosis and thrombocytopenia, clinicians are inclined to investigate for neonatal sepsis first. In retrospect, the paucity of signs to support a diagnosis of sepsis and the presence of severe intraventricular hemorrhagic infarct, out of proportion to the degree of prematurity, would suggest a need to consider alternative etiologies for the thrombocytopenia and acidosis.

There are published recommendations based on expert opinions for initial and long-term treatment of patients with severe protein C deficiency. (3) The standard initial treatment is 10 to 20 mL/kg of FFP every 12 hours until clinical symptoms resolve. Human plasma–derived viral-inactivated protein C concentrate given intravenously has now become available and is the treatment of choice for patients with severe deficiency. (4) In severe protein C deficiency, it is notable that the reported complications are not only thrombotic but also hemorrhagic, reflecting the delicate balance between pro- and antithrombotic forces and the need to approach this with great care. In our patient who developed initial intracranial hemorrhage and later MRI evidence of sagittal sinus thrombosis, the decision to institute procoagulation therapy initially but anticoagulation later was made in consultation with a pediatric hematologist.

The twice-daily FFP transfusions (40 mL/kg per day) produced only a transient rise in protein C levels for our patient. We speculate that the later lack of efficacy might have been related to the highly variable protein C content of each FFP transfusion. Although protein C concentrate is the most reliable source of protein C delivery, this was not started because of the parents’ decision to provide limited medical care after having considered the cost and poor neurologic prognosis.

The most widely used long-term treatment is oral anticoagulation to maintain an international normalized ratio in the range of 2.5 to 3.5. Other options such as low-molecular-weight heparin and protein C concentrate administration have been tried with excellent results. Warfarin is the preferred anticoagulant because of its lower cost and ease of administration over the long-term. (5) However, it requires close monitoring and dose adjustment in the initial period, issues that may be problematic for a premature infant with limited blood volume. In consultation with the pediatric hematologist, the patient was given subcutaneous enoxaparin from 2 months after birth when the intracranial hemorrhage had stabilized, achieving an anti-factor Xa level of 0.5 to 1 U/mL. Enoxaparin was favored over warfarin because

1) protein C levels cannot be reliably measured in patients receiving warfarin,
2) enoxaparin minimizes the need for routine blood tests,
3) there is a risk of warfarin-induced skin necrosis, (6)
4) there are reports of drug-drug interactions between antiepileptic drugs and warfarin, and
5) there are concerns of future interaction with food.

Monitoring D-dimer levels was said to be useful to confirm the adequacy of anticoagulation. (4)(7)

Although the predominant pattern of inheritance is autosomal dominant, autosomal recessive forms have been reported. The patient in this report was likely to be of the latter type because of parental consanguinity and the assumption that they had 12.5% genes in common. Genetic testing is vital for risk counseling in future pregnancies. Although the parents declined this on the basis of having completed their family, there remains a role for screening siblings of the proband to provide opportunity for genetic counseling.

In conclusion, we recommend that newborns with hemorrhagic cerebral infarcts or thrombosis be screened for hereditary thrombophilias including protein C deficiency, especially in cases of parental consanguinity or when the severity of hemorrhage is out of proportion to the degree of prematurity. It is difficult to achieve adequate protein C levels without protein C concentrates, which is expensive and not easily available in many countries. The challenges in decision making regarding anticoagulation in the presence of hemorrhage and the complexity of care required are best managed by a multidisciplinary team for optimal outcomes.

Acknowledgments
A special thank you to Dr Wendy Liew Kien Ming for giving neurology input for this case report, and also to Dr Sarat Kumar Sanamandra for his radiology input on the imaging studies conducted for this patient.

American Board of Pediatrics Neonatal-Perinatal Content Specifications

- Know the causes and pathophysiology of congenital and acquired thrombotic disorders.
- Know the clinical and laboratory features, management, and potential adverse effects of treatment of congenital and acquired thrombotic disorders.
References


A female infant with a birthweight of 2,925 g is delivered at 38 6/7 weeks of gestation by a 32-year-old gravida 1, para 1 mother. The pregnancy is uncomplicated, and the mother’s evaluation for infection before delivery is negative. The birth occurs via precipitous vaginal delivery and is uneventful except for terminal meconium. At delivery, the infant is initially vigorous with an appropriate heart rate and tone, but she subsequently requires resuscitation because of increased work of breathing and oxygen saturation in the low 60s. She is treated with positive pressure ventilation, and the fraction of inspired oxygen is increased to 100%. Despite positive pressure ventilation, her physical examination findings and oxygen saturation do not improve. Her 1- and 5-minute Apgar scores are 7 each. Initial chest radiography shows a right-sided pneumothorax and pneumomediastinum. Her first capillary blood gas is notable for a pH of 6.9, a partial pressure of carbon dioxide (PCO₂) of 101 mm Hg (13.4 kPa), and a base deficit of 13.

Needle decompression of the right chest is attempted, after which the infant is intubated and mechanical ventilation is started. Screening infectious laboratory tests are performed and empirical broad-spectrum antibiotics are started. The infant is then transferred to a tertiary care facility for further care. Upon arrival, the infant is switched to a high-frequency oscillator. At this point, repeat chest radiography shows bilateral pneumothoraces and pneumomediastinum, so bilateral chest tubes are placed.

DISCUSSION

Despite oscillatory ventilation, 100% fraction of inspired oxygen, and surfactant, the infant’s oxygen saturation remained in the 80s. An echocardiogram was unremarkable. The capillary gas at 2.5 hours after birth improved slightly, with a pH of 7.03, PCO₂ of 90 mm Hg (12 kPa), and base deficit of 7. Inhaled nitric oxide and inhaled epoprostenol were empirically initiated. Because of persistent hypotension and hemodynamic instability, the infant started treatment with epinephrine and vasopressin, as well as milrinone and hydrocortisone. She had brief clinical improvement, and 8 hours after birth, her capillary gas pH was 7.20, with a PCO₂ of 55 mm Hg (7.3 kPa) and base deficit of 6. However, she progressively deteriorated over the next 24 hours with prolonged oxygen desaturation and hypotension, leading to the initiation of extracorporeal membrane oxygenation (ECMO).
Differential Diagnosis
The infant’s initial presentation was concerning for meconium aspiration syndrome with persistent pulmonary hypertension. However, the continued clinical deterioration despite increasing support made this diagnosis less probable. Sepsis was also considered, and the infant was treated with empirical antibiotics, but the lack of improvement despite intervention made this diagnosis less likely as well. Echocardiography ruled out cardiac etiologies such as total anomalous pulmonary vein return, leaving primary pulmonary pathology as the most likely cause for the patient’s disease. Persistent respiratory failure requiring continuation of ECMO led to consideration of the diffuse developmental disorders of the lung, including acinar dysplasia (AD), congenital alveolar dysplasia (CAD), and alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV). The differential also included congenital surfactant deficiencies (surfactant protein B deficiency, surfactant protein C deficiency, ABCA3 deficiency).

The Condition
Normal lung development occurs in 5 phases: the embryonic (3–7 weeks after conception), pseudoglandular (5–17 weeks after conception), canalicular (16–25 weeks after conception), saccular (24–36 weeks after conception), and alveolar (36 weeks after conception through adolescence) phases. (1) Lung development can be arrested during any phase, and the timing of the arrest determines the severity of disease. (1) Developmental lung dysplasia was noted as early as 1948 by MacMahon, (2) but because of the rarity of the developmental lung dysplasias, research on the topic is limited, leading to an incomplete understanding of the disease. Incidence and prevalence of the neonatal developmental lung diseases remain unknown, (3) and it remains unclear whether AD, CAD, and ACD/MPV are individual diseases or part of a spectrum of the same disease. (3)

Lung growth arrest during the pseudoglandular or early canalicular phase leads to the diffuse impaired pulmonary acinar development seen in AD. The saccular or alveolar spaces necessary for gas exchange are completely absent, and the lungs may be smaller than expected for the patient’s gestational age. Radiologic findings may include hyperinflation, interstitial prominence, and diffuse increased pulmonary density. These patients are typically born at term gestation, and death occurs within hours of delivery. (4) In patients with CAD, lung development is arrested in the late canalicular or early saccular phases. These patients have incomplete alveolarization and absence of secondary septation, though the lungs are normal in size. Patients with CAD are typically born at term gestation, and death typically occurs in the neonatal period but later than in AD. (5) Disorganized pulmonary maldevelopment during the saccular and alveolar phases leads to ACD/MPV. This condition is associated with defects in the FOXF1 gene. (6) More than 90% of patients are born at term, and acute hypoxemic respiratory failure develops within 48 hours. (6) Chest radiograph is usually normal but there may be a diffuse ground glass pattern. Although ACD/MPV is also fatal in the neonatal period, 4 known cases presenting as late as 7 months of age are noted in the literature. However, all symptomatic patients have died. (6)

Treatment
Initial therapy for patients with this presentation is supportive. Patients require intubation and potentially ECMO. However, as noted here, these diseases are fatal and to date, there have been no effective life-saving therapies. High-resolution computed tomography may be useful for demonstrating the type of abnormality, extension, and distribution of disease. (4) If a patient is stable with supportive care, a biopsy and genetic testing for the FOXF1, NKX2.1, SFTPB, SFTPC, and ABCA3 genes may be beneficial if lung transplantation is a consideration. (4) In one case, prenatal testing helped avoid escalation to ECMO in a symptomatic patient. (7)

Progression
The infant was given maintenance ECMO for 19 days, during which she developed seizures, and left-sided
parenchymal bleeding was noted. Following ECMO decan-
nulation, the infant had persistent respiratory decompensa-
tion for 36 hours, and she died shortly after withdrawal
of support with elective extubation on day 24 after birth.

The infant’s autopsy findings were consistent with CAD. The
lungs were normal in size and weight, but on histopathologic
review (Fig), alveolar development was limited and patchy, and
intrapulmonary surface area for gas exchange was also limited.
This was because of the markedly reduced airspace and persis-
tence of immature interalveolar mesenchyme.

Lessons for the Clinician

- This case highlights the importance of suspecting dis-
orders of lung development and congenital surfactant
deficiency in infants with severe and refractory respira-
tory failure.

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A late preterm female neonate is born at 34 weeks, 6 days of gestation, with a birthweight of 2.1 kg. The mother is a gravida 2 woman and the infant is delivered via cesarean section in view of scar dehiscence. The infant cries immediately after birth and does not require any resuscitation. She develops respiratory distress in the delivery room, is transferred to the NICU, and given continuous positive airway pressure (CPAP). She is comfortable with CPAP and is gradually weaned to room air 48 hours after birth. On the third day after birth, tube feeding is commenced at 20 mL/kg per day. However, the next day she develops abdominal distention, gastrointestinal bleeding, and melena for which feeding is stopped. There is no history of treatment with steroids or nonsteroidal anti-inflammatory drugs. On the same day, she is transferred to a level IIIB NICU for further management.

On admission to the institute, the infant is lethargic and tachypneic. Blood pressure and capillary refill time are in the normal range. The abdomen is soft without any distention and bowel sounds are present. CPAP (positive end-expiratory pressure, /CO2; fraction of inspired oxygen, 21%) is restarted for respiratory distress.

The infant is given nothing by mouth (nil per os; NPO) and total parenteral nutrition. Empirical antibiotic treatment (intravenous meropenem) is started after performing sepsis screening; laboratory testing for platelet count, prothrombin time, and activated partial thromboplastin time; and cultures of blood, cerebrospinal fluid, and urine. Arterial blood gas shows a pH of 7.21, bicarbonate of 14.4 mEq/L (14.4 mmol/L), and anion gap of 22, suggesting increased anion gap metabolic acidosis. Radiograph of the chest is normal but that of the abdomen suggests the presence of gas in the wall of the stomach (Fig 1). The total leukocyte count is 3,800/μL (3.8 x 10^9/L), C-reactive protein is 68.5 mg/L (652.4 mmol/L), and prothrombin time, activated partial thromboplastin time, and platelet counts are in the normal range. Blood culture yields gram-negative bacteria, Achromobacter xylosoxidans, for which modified antibiotics are administered. The opinion of pediatric surgery is obtained, and the treating team and radiologist agree to treat her for isolated gastric pneumatosis without any bowel involvement. She is weaned to room air 36 hours after admission. Repeat radiography performed on days 7 and 10 after admission shows the resolution of pneumatosis (Figs 2 and 3). The infant continues to remain NPO for 10 days and starts receiving small
feedings of 20 mL/kg per day on day 11 of admission. Feedings are gradually increased and she reaches a full feed of 150 mL/kg per day on day 18. For gram-negative bacterial sepsis, she is treated with antibiotics for 14 days and discharged 20 days after birth.

**DISCUSSION**

Isolated gastric pneumatosis in a neonate is very rare and only a few cases have been reported. Pneumatosis is defined as the presence of gas in the bowel wall. (1) However, it can involve any part of the gastrointestinal tract. Most cases of gastric pneumatosis are caused by obstruction of the proximal bowel because of pyloric stenosis, pyloric atresia, or duodenal web. (2)(3)

Gastric pneumatosis is of 2 types—gastric emphysema or emphysematous gastritis. Whenever intraluminal pressure is increased because of proximal bowel obstruction, the intraluminal gas dissects through the intact gastric mucosa and produces linear or cystic pneumatosis. (4) This is known as *gastric emphysema*. It has a good prognosis and these patients improve quickly once the obstruction is relieved. (5) Emphysematous gastritis has a more indolent course because of an infection or inflammation that causes a breach in the gastric mucosa, which is followed by gas entering the mucosa. It has a poorer prognosis and these patients usually take longer to recover. Management is usually conservative with the treatment of underlying infection/inflammation, but surgical exploration may be needed in the presence of pneumoperitonium or suspicion of gangrene. (6) In the current case, there was no proximal bowel obstruction. Presence of gastrointestinal bleeding, metabolic acidosis, classic radiographic findings, normal coagulation profile, and positive blood culture favors the diagnosis of isolated gastric pneumatosis, with late prematurity and sepsis being the predisposing factors.
Lessons for the Clinician

- Usually gastric pneumatosis is associated with fulminating necrotizing enterocolitis or benign etiologies like upper gastrointestinal obstruction or drug exposure.
- Uncommonly gastric pneumatosis can occur in isolation and can present early, with prematurity and sepsis being the predisposing factors.

References


American Board of Pediatrics
Neonatal-Perinatal Content Specifications

- Know the clinical manifestations and differential diagnosis of gastrointestinal (GI) bleeding in newborn infants, including the various coagulation disorders that cause GI hemorrhage.

- Know the laboratory and radiographic findings and management of GI bleeding in newborn infants.
Swallow Studies in Preterm Infants: Indications and Interpretation

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CASE

Please view the video clip (Video 1) showing the results of a videofluoroscopic swallow study (also known as a modified barium swallow study).

![Video Clip]

Video 1. Click here to view the video.

The most likely clinical scenario of the infant in this video is a:
A. Former 29-week preterm infant with a postmenstrual age of 41 weeks who is currently receiving continuous positive airway pressure
B. Former 33-week preterm infant with a postmenstrual age of 42 weeks with desaturations with feeding and requiring supplemental oxygen
C. Late preterm infant with Prader-Willi syndrome now at 43 weeks who is not yet rooting or maintaining an awake state for feeding
D. Term infant who sputters with a fast flow nipple

CRITIQUE

The videofluoroscopic swallow study (VFSS), also known as a modified barium swallow study, is a form of instrumental assessment of swallow function. It is conducted in the radiology department by a speech-language pathologist in collaboration with a radiologist; thus, the patient needs to be stable to allow transfer to this location. The infant’s caregivers and nurse may also be present.

AUTHOR DISCLOSURE Mss Davidson and Hernandez have disclosed no financial relationships relevant to this article. Mss Davidson and Hernandez’s current affiliation is Department of Otolaryngology and Communication Enhancement, Boston Children’s Hospital, Boston, MA. This commentary does not contain a discussion of an unapproved/investigative use of a commercial product/device.
and often participate in the study by feeding the infant. The following video clip (Video 2) is an example of a “normal” study, demonstrating adequate integration of the suck-swallow-breathe pattern, with 1 suck per swallow, timely swallow onset, and intact airway protection from above; anatomical markers are shown in Fig 1.

There are 3 primary purposes for performing a VFSS:

1. Defining the nature and pathophysiology of a swallowing impairment
2. Provoking the swallowing system to try to demonstrate the dysphagia complaint (eg, ensuring that the end of a feeding is documented by fluoroscopy in a patient who consistently exhibits difficulty near the end of a feeding)

<table>
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<th>TABLE. Considerations for Determining Appropriateness of Videofluoroscopic Swallow Study (VFSS) in a Neonate</th>
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<td><strong>Postmenstrual age</strong></td>
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<td><strong>Physiologic stability and respiratory status</strong></td>
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<td><strong>Comorbidities</strong></td>
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| **Response to interventions at the bedside**                 | The following therapeutic interventions improve coordination of the suck-swallow-breathe pattern and may alleviate need for instrumental assessment with radiation exposure:

- **Positioning:** Swaddling an infant in a flexed body position optimizes his/her organization and neural alignment for overall stability during feeding (Clark et al, 2007; Thoyre et al, 2012). Transitioning the infant to an elevated side-lying position promotes greater physiologic stability with improved bolus management for self-regulated breathing and less variability in oxygen saturation, heart rate, and respiratory rate (Thoyre et al, 2012; Clark et al, 2007).
- **Flow Rate:** Slower flow nipples improve physiologic stability during feeding and optimize integration of respiration and swallowing (Chang et al, 2007; Ross and Brown, 2002).
- **External Pacing:** Pacing refers to either tilting the nipple toward the palate to cease the flow of liquid or removing the nipple from the infant’s mouth entirely. This provides external support for integration of respiration and improves the infant’s endurance and physiologic stability (Law-Morstatt et al, 2003). |
3. Identifying compensatory strategies to facilitate the patient’s best performance so that a management protocol can be developed (Benson and Tuchmann D, 1994).

Although aspiration may be captured by a VFSS, it is important to understand that the presence or absence of aspiration is not the sole purpose of a VFSS. There are many considerations for determining whether a patient is an appropriate candidate for a VFSS including, but not limited to, postmenstrual age, physiologic stability, respiratory status, and comorbidities (Table). In addition, many feeding interventions made at the bedside can successfully improve coordination of the suck-swallow-breathe pattern and should generally be tried first before seeking VFSS.

The patient in the case (Video 1) presents with an immature suck-swallow-breathe pattern, resulting in eventual aspiration of thin liquid via a standard flow nipple; this aspiration is shown in Fig 2. This patient’s coordination improved significantly with a slower flow rate (ie, Dr. Brown’s preemie flow), with no further episodes of laryngeal penetration or aspiration. This case demonstrates the need to assess for change in swallow function over time, because most preterm infants exhibit worsening coordination as the feeding progresses, resulting in higher risk of bolus misdirection. Use of a slower flow nipple in this case eliminates the need for thickening, which comes with associated risk factors including gastrointestinal issues (eg, increased risk for necrotizing enterocolitis for some types of thickening agents, constipation, changing nutritional composition), poor endurance, and variability in viscosity. This infant can achieve full oral feedings using the preemie flow nipple and may be appropriate for clinical advancement as he matures, with consideration of repeat VFSS should any signs or symptoms concerning for aspiration emerge.

Video 3 shows an infant with more severe oropharyngeal dysphagia characterized by discoordination of the nutritive sucking pattern and gross, silent aspiration with both thin and thickened liquids. Interventions completed at the bedside, including positional adjustments, slower flow rates, and external pacing, did not resolve the infant’s apneic and bradycardic events. Therefore, a trial of thickening was also attempted during the study.

Thickened liquids are often tried when other strategies prove unsuccessful because thickening slows the rate of liquid flow and increases sensory input. However, this case emphasizes the importance of gathering instrumental information of swallow function in at-risk neonates rather than implementing empirical trials of thickening. Although this strategy of foregoing a VFSS can be beneficial later in infancy and throughout childhood, most preterm and term newborns with dysphagia aspirate silently (ie, no protective cough response). Empirical thickening would have led to silent aspiration of thickened liquids while also introducing the risks associated with thickening. This patient will require nonoral means of nutrition and would likely benefit from oral stimulation in the interim to maintain interest and prevent oral aversion, which might include use of a dry pacifier or pacifier dips during bolus nasogastric feedings.

CORRECT RESPONSE

B. Former 33-week preterm infant with a postmenstrual age of 42 weeks with desaturations with feeding and requiring supplemental oxygen.

Suggested Readings

Benson J, Tuchmann D. Other diagnostic tests used for evaluation of swallowing disorders. In: Tuchmann D, Walter E, eds. Disorders of


Preeclampsia with Severe Features and Acute Abruption

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ELECTRONIC FETAL MONITORING CASE REVIEW SERIES

Electronic fetal monitoring (EFM) is a popular technology used to establish fetal well-being. Despite its widespread use, the 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 (FHR) 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 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 (eg. 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 FHR do not occur alone and generally evolve over time

DEFINITIONS

Baseline FHR

- 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

AUTHOR DISCLOSURE Dr. Grable has 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.
Baseline Variability
- Fluctuations in the baseline FHR of ≥2 cycles per minute, 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 6–25 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 FHR Pattern
- Visually apparent, smooth sine wave-like undulating pattern in the baseline with a cycle frequency of 3 to 5 per 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 3-tier FHR 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

### Table: Arterial Umbilical Cord Gas Values

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Pco₂ (mm Hg)</th>
<th>Pao₂ (mm Hg)</th>
<th>BASE EXCESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal*</td>
<td>≥7.20</td>
<td>&lt;60 (35 to 70)</td>
<td>≥20</td>
<td>≤-10 (-2.0 to -9.0)</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>
</tbody>
</table>

– Absence of induced accelerations after fetal stimulation
– Recurrent variable decelerations with minimal or moderate variability
– Prolonged decelerations
– Recurrent late decelerations with moderate variability
– 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**

A 40-year-old gravida 1, para 0 woman presented to the labor and delivery (L&D) department at 28 1/7 weeks of gestation (dated by a 7-week ultrasound scan) with uterine cramping. The cramping began 3 days earlier and had become more painful. She denied vaginal bleeding or leaking fluid. She had no headache, visual changes, or epigastric pain.

She had a medical history significant for a uterine fibroid. Her surgical history was significant only for a breast excisional biopsy without complication. She was active and worked as a physical therapist. She denied smoking, drinking, and recreational drugs. Her medications included only prenatal vitamins.

Her pregnancy was significant for a large lower uterine segment fibroid measuring 16 cm at 18 weeks’ gestation. She had an episode of vaginal bleeding at 14 weeks. She underwent level II ultrasonography, which demonstrated normal growth and an echogenic focus in the left ventricle. She is Filipino and because this is a common finding in Asian populations, the echogenic focus was considered a normal variant. However, the patient was offered a noninvasive prenatal screen (cell-free fetal DNA) and amniocentesis and declined both. At the time of the level II ultrasonography, the fibroid measured 17 cm and appeared to be obstructing the lower uterine segment. At 21 weeks’ gestation, she presented to L&D feeling pressure and contractions every 2 minutes. She was dehydrated and had a closed cervix. She was treated with intravenous fluids and discharged from the hospital. She failed her 1-hour glucose challenge test, but underwent a 3-hour glucose tolerance test, findings of which were normal (serum glucose, 78 mg/dL [4.3 mmol/L], 180 mg/dL [10 mmol/L], 119 mg/dL [6.6 mmol/L], and 121 mg/dL [6.7 mmol/L]).

On arrival at L&D at 28 1/7 weeks’ gestation, her initial blood pressure was 184/94 mm Hg with a heart rate of 68 beats/min, and a temperature of 98.9ºF (37.2ºC). A repeat blood pressure 7 minutes later was 170/89 mm Hg. She was complaining of sharp uterine cramping, progressively worsening to an intolerable level, with a current pain score of 9/10. At the time of presentation, the fetal heart tones appeared to be recovering from a deceleration. The baseline heart rate was 165 beats/min. The initial FHR tracing is shown in Fig 1.

![Figure 1. Electronic fetal monitoring strip 1.](image-url)
Findings on EFM strip 1 are as follows:

- Variability: Minimal
- Baseline rate: 165 beats/min
- Episodic pattern: Recurrent variable decelerations
- Periodic pattern: None
- Uterine contractions: Irritability
- Interpretation: Category II, however difficult to interpret given areas of discontinuous tracing (i)
- Differential diagnosis: Placental abruption, cord prolapse, placental insufficiency, preeclampsia, intrauterine growth restriction, oligohydramnios, vasa previa/velamentous cord insertion

- Action: Obtain intravenous access, perform bedside ultrasonography and internal examination, evaluate for preeclampsia, notify maternal-fetal medicine

On examination, her cervix was 2-cm dilated, 50% effaced, and high. There was no evidence of ruptured membranes or cord prolapse. Bedside ultrasonography demonstrated the fetus to be in breech presentation with normal amniotic fluid. The fibroid was again identified. The scan was limited because of the size of the fibroid. Intravenous access was obtained. She had laboratory screening to rule out preeclampsia as a result of the severe range blood pressures and new-onset hypertension. Anesthesia and maternal-fetal medicine were notified. The continued FHR tracing is shown in Fig 2.
Findings on EFM strip 2 are as follows:

- Variability: Minimal
- Baseline rate: 160 beats/min
- Episodic pattern: Recurrent variable decelerations
- Periodic pattern: Prolonged decelerations
- Uterine contractions: Irritability
- Interpretation: Category II. Although the tracing is interpreted as a category II tracing, areas of discontinuous tracing are audible as prolonged decelerations
- Differential diagnosis: Placental abruption, preeclampsia, placental insufficiency
- Action: Consider urgent delivery

Given a likely diagnosis of placental abruption in the setting of contractions, severe range hypertension/possible preeclampsia, and recurrent FHR decelerations, the decision was made to proceed with an urgent cesarean section. The benefit of waiting for laboratory results and observing the FHR for improvement was outweighed by the risks of continuing the pregnancy. Intravenous medication was not provided for blood pressure control at this time because of the expected need for general anesthesia and risk of intraoperative hypotension. The patient was transferred to the operating room. Before being prepared for surgery, a final brief FHR tracing was obtained, which is shown in Fig 3.
Findings on EFM strip 3 are as follows:

- Variability: Minimal
- Baseline rate: Likely 155 beats/min, but unable to be determined given the short FHR tracing
- Episodic pattern: Recurrent variable decelerations
- Period pattern: None
- Uterine contractions: Not measured
- Interpretation: Category II with areas of discontinuous tracing
- Differential diagnosis: Placental abruption, preeclampsia
- Action: Due to continued recurrent decelerations, proceed with urgent cesarean delivery

OUTCOME

The patient initially presented to L&D at 11:20 pm. The initial external fetal monitor was placed at 11:26 pm. At 11:43 pm, the patient was transferred to the operating room for urgent delivery. After reevaluation of the FHR in the operating room, the patient was prepared for surgery. General anesthesia was initiated at 11:55 pm and a 600-g viable female infant was delivered at 11:56 pm through an uncomplicated low transverse cesarean delivery (13 minutes after transfer to the operating room). A placental abruption was noted with evidence of bloody amniotic fluid and clots adherent to a small-appearing placenta. Quantified blood loss was 1,115 mL. Umbilical cord gases could not be obtained because of insufficient cord blood. Results of the laboratory testing for preeclampsia were available after surgery and were consistent with preeclampsia with severe features based on blood pressure and proteinuria. The protein-creatinine ratio was 14.93 (normal <0.30) and the remainder of the laboratory test results were normal.

She received no antihypertensive medication before delivery. Due to general anesthesia and the delivery itself, there was an initial significant improvement in her blood pressure intraoperatively and after delivery. The patient received magnesium for seizure prophylaxis for 24 hours. However, with increasing blood pressures on postpartum day 1, she received extended-release nifedipine 60 mg daily for blood pressure control. Of note, antihypertensive drugs were discontinued by 4 weeks after delivery.

Placental pathology demonstrated a 120-g placenta, which is small for gestational age (SGA; normal 210–331 g). Findings were consistent with severe maternal vascular malperfusion with villous infarcts (the largest of which measured 5.5 cm in greatest diameter and occupied 30% of the parenchyma), with accelerated diffuse villous maturation and decidual vascular thrombi.

In addition, a small chorangioma (0.6 cm in greatest dimension), focal chronic villitis, and paramarginal insertion of the umbilical cord were noted.

After delivery, the infant’s heart rate was normal, but she had apnea with poor tone. The neonatology team was present and immediately started positive pressure ventilation (PPV). She initially made only intermittent gasps and small crying efforts. Given steady improvement, PPV was discontinued at 4 minutes of age and she made a transition to continuous positive airway pressure +5 mm Hg. During the resuscitation, the fraction of inspired oxygen was initially 0.40, which increased to 0.80, and then decreased to 0.50. The infant’s Apgar scores were 3 at 1 minute, 5 at 5 minutes, and 7 at 10 minutes.
The infant’s birthweight was 600 g, which is the 4th percentile \((Z = -1.78)\) based on Fenton weight-for-age data. The height was 29 cm, which is less than the 1st percentile \((Z = -2.94)\) based on Fenton length-for-age data. The head circumference was 21 cm, which is less than the 1st percentile \((Z = -2.94)\) based on Fenton length-for-age data. A urine cytomegalovirus polymerase chain reaction was negative. Thrombocytopenia was noted, likely due to growth restriction, but resolved without transfusion. Head ultrasound did not show any abnormality. She had respiratory distress, but resolved without transfusion. Head ultrasound did not show any abnormality. She had respiratory restriction, but resolved without transfusion. Head ultrasound did not show any abnormality. She had respiratory restriction, but resolved without transfusion.

**DISCUSSION**

Intrauterine growth restriction (IUGR) and preeclampsia are significant causes of maternal and neonatal morbidity and mortality. Preeclampsia affects approximately 2% to 5% of pregnancies. It is also associated with a 4-fold increase in the risk of IUGR, which may be associated with long-term neonatal complications. (2)(3) Risk factors for preeclampsia include hypertension, diabetes, renal disease, multiple gestations, obesity, and a history of preeclampsia. While the cause of preeclampsia is unknown, both IUGR and preeclampsia are associated with inadequate placental blood perfusion and ischemia resulting in endothelial dysfunction and platelet and clotting system activation. (2) This may result in catastrophic pregnancy complications, such as abruptio and fetal demise.

Stable patients with preeclampsia with severe features are managed expectantly until 34 weeks’ gestation to reduce the complications of prematurity. However, affected women may face many potential complications as they work toward the goal of increasing gestational age. There are increased risks of intensive care unit admission; hemolysis, elevated liver enzymes, and low platelets counts (HELLP) syndrome; pulmonary edema; eclampsia; abruptio; subcapsular liver hematoma; stroke; stillbirth; neonatal death; and perinatal asphyxia. The risk of abruptio in a review of patients of less than 34 weeks’ gestation who received expectant management was 5.1% (2.2%–8.5%). In the same study, more than 30% of infants were SGA. (4)

A placental abruption is defined as a premature separation of the implanted placenta before delivery of the fetus. (5) It occurs on average in 1 in 200 deliveries and contributes significantly to maternal and neonatal morbidity and mortality. Although multiple risk factors exist, the most common cause is maternal hypertensive disorders, which occur in 44% of cases. Maternal trauma, including a motor vehicle accident, falls, and assaults, is associated with 1.5% to 9.4% of cases. Cigarette smoking, drug use, premature rupture of membranes, twin gestation, chorioamnionitis, advanced maternal age, elevated maternal serum α-fetoprotein, bleeding in the second trimester, uterine fibroids, and a history of abruption are all associated risk factors. The risk of recurrence of abruption is 4% to 12% after 1 pregnancy and 25% after 2 consecutive pregnancies complicated by abruptions. (6)

Fetal and neonatal morbidity is caused by the severity of the abruption itself and the potential complications of prematurity. Delivery is required when there is maternal bleeding that is not resolving and causing maternal shock or recurrent FHR decelerations that are a sign of inadequate placental perfusion. Expectant management may be possible in the setting of a marginal or partial abruption. However, with a complete abruption, an expedited delivery is necessary. Potential maternal complications of an emergency cesarean section include disseminated intravascular coagulation, postpartum hemorrhage, and hysterectomy.

In a case of category III FHR tracing that does not resolve, a prompt delivery should be considered. However, with a category II FHR tracing, the recommendation is for surveillance and implementation of intrauterine resuscitative measures as indicated. These may include lateral positioning, oxygen, intravenous fluids, discontinuation of oxytocin, administration of tocolytic medications such as terbutaline, and amnioinfusion. However, when the cause of the category II tracing does not resolve, and the tracing does not improve, a decision to proceed with delivery must be considered. If the FHR tracing is highly likely to proceed to a category III tracing in a short amount of time, and if the cause of the abnormality cannot be resolved, delivery is indicated to maximize the chance of a successful outcome. Both a cord prolapse and a complete abruption are 2 examples of clinical scenarios in which there may be a category II FHR tracing, but an urgent delivery is indicated. (7)

This patient was unaware that her symptoms of cramping could be associated with both preeclampsia with severe features and an FHR tracing with recurrent FHR decelerations. She had no change in fetal movement and no significant symptoms of preeclampsia. However, she had multiple risk factors for preeclampsia including advanced maternal age, primigravida, and SGA growth. In addition to these risk factors, there were also other possible causes of SGA growth including a small placenta, placental infarcts on placental pathology, and a large fibroid. Together, the risk for placental abruption was significant. The FHR tracing was an effective tool in determining the status of the fetus and need for an urgent cesarean delivery.
American Board of Pediatrics
Neonatal-Perinatal Content Specifications

• Know the effects on the fetus and/or newborn infant of severe preeclampsia, including HELLP syndrome, and its management.
• Know the diagnosis and management of maternal/fetal blood loss such as placenta previa, placenta abruption, vasa previa, and maternal-fetal hemorrhage.

References

A Unique Case of Facial Dysmorphism in an Infant

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THE CASE

A preterm infant with a ventricular septal defect and facial dysmorphisms.

Prenatal and Birth Histories
- Born to a 21-year-old gravida 2, para 2 woman with a healthy prior child.
- The pregnancy was complicated by an abnormal quadruple screen (with an elevated α-fetoprotein) and fetal cardiac ultrasonography that showed concerns for tetralogy of Fallot and persistent oligohydramnios of uncertain etiology. There was no evidence of growth restriction.
- Estimated gestational age: 32 6/7 weeks.
- An emergency cesarean delivery was performed because of premature labor and concerns for placental abruption.
- Apgar scores: 8 and 9 at 1 and 5 minutes, respectively.

Presentation
After birth, the infant was admitted to the NICU because of prematurity. He required supplemental oxygen through a nasal cannula for a brief period (<1 week) and was successfully weaned to room air. Echocardiography revealed a large perimembranous ventricular septal defect (VSD) with 2 small ventricular muscular defects. The team identified facial dysmorphisms and ordered a micro-array analysis.

Physical Examination (After Birth)
- Birthweight: 1.57 kg (27th percentile), length: 41 cm (34th percentile)
- Head circumference: 28 cm (17th percentile)
- Heart rate: 150 beats/min
- Respiratory rate: 76 breaths/min
- Blood pressure: 55/33 mm Hg (mean arterial pressure 40 mm Hg)
- Oxygen saturation: 89% (in room air)
- Head: Small head circumference; normal, open, flat fontanelles; no cranial molding; almond-shaped eyes, epicanthal folds, hypertelorism, flat nasal bridge, low-set ears, thin upper lip, and micrognathia (Figs 1 and 2); red reflex was present bilaterally; intact palate
- Lungs: Coarse breath sounds bilaterally; tachypnea; no other signs of respiratory distress
- Cardiovascular: Normal S1 and S2 with 3/6 harsh holosystolic murmur loudest at the left parasternal border

AUTHOR DISCLOSURE Drs Samuel, Karpawich, and Scheurer-Monaghan 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.
Abdomen: Soft, nontender; no organomegaly; no masses

Genitourinary: Bilateral hydroceles, patent anus

Skin: No jaundice, no rash

Musculoskeletal: Spine appears normal; no limb abnormalities

Neurologic: Increased tone in upper extremities; symmetric Moro reflex, normal suck reflex

PROGRESSION

The infant had a 2-month NICU hospitalization complicated by congestive heart failure secondary to the large VSD, which was associated with poor weight gain. At 2½ months of age, the VSD was surgically repaired with a complicated postoperative course as a result of a methicillin-sensitive Staphylococcus aureus infection, which was treated with antibiotic therapy for 6 weeks. The infant had a postoperative pericardial effusion that required drainage. Following the VSD repair, his growth improved. He developed bilateral inguinal hernias that were surgically repaired at 3 months of age. During the hospital course, the infant was found to have increased tone in the upper extremities and magnetic resonance imaging was performed, which showed delayed myelination consistent with prematurity. At 4 months of age, the infant exhibited mild gross motor delay.

DIFFERENTIAL DIAGNOSIS

- Cri du chat syndrome (chromosome 5p deletion syndrome)
- Noonan syndrome (multiple gene mutation)
- Prader-Willi syndrome (chromosome 15 segment deletion)
- Rubinstein-Taybi syndrome (chromosome 16p13.3 syndrome)
- Trisomy 18 syndrome
- Trisomy 21 syndrome

ACTUAL DIAGNOSIS

The microarray analysis revealed a duplication of chromosome 16q23.1, which has not been previously reported in the literature.

The duplicated interval involved 3 known genes (CNTNAP4, LOC101928203, MIR4719). The assay did not rule out balanced chromosomal abnormalities; imbalances of chromosomal regions that were not represented by probes on the array; or mosaicism. Further genetic testing with fluorescence in situ hybridization and genetic counseling were recommended. However, the parents were non-compliant with that request. Family history did not reveal any other inherited genetic defects.
WHAT THE EXPERTS SAY

To our knowledge, this is the first reported case of chromosome 16q23.1 duplication. The unique feature of chromosome 16 that differentiates it from other chromosomes is its larger than average fraction of repetitive sequences and mutations in which certain pathogenic characteristics may develop. (i) Although chromosome 16 abnormalities are relatively rare in the population, some of the known abnormalities include complete trisomy 16, mosaic trisomy 16, and deletions and duplications of certain segments of the long and short arm of chromosome 16. Trisomy 16 is not compatible with life and is a common cause of miscarriages. 

(2) An interesting study by Neusser et al concluded that paternal meiotic nondisjunction errors are associated with an increased rate of spontaneous abortion similar to maternal chromosomal disomy. (3)

The features of mosaic trisomy 16 vary greatly but the more common characteristics include intrauterine growth restriction, congenital heart defects, delayed development, intellectual disability, obesity, seizures, and an association with autism. (4)(5) It was initially thought that this mutation is a variant of uncertain clinical significance but later studies found the pathogenic nature of the mutation. Deletion of chromosome 16p11.2 is associated with autism, poor weight gain, microcephaly, and developmental delay, with speech delay being most prominent. (6) Deletion of the 16q24.1 region can lead to alveolar capillary dysplasia, misalignment of the pulmonary veins, and pulmonary hypertension. (7) Translocation of certain segments of chromosome 16 with other chromosomes has been linked to hematologic abnormalities including acute myelogenous leukemia. (8) Rubinstein-Taybi syndrome (also known as chromosome 16p13.3 syndrome) is associated with failure to thrive, increased risk of life-threatening infections, intellectual disability, and an association with leukemia and lymphoma. (9) Inversion of chromosome 16 (p13q22) is also thought to be associated with a unique cytogenic subtype of acute myelomonocytic leukemia with a favorable prognosis. (10)

CONCLUSIONS

- Information on chromosome 16 mutations and associated clinical defects is limited. Although certain mutations of chromosome 16 (full trisomy, mosaic trisomy) have been described, in general, the amount of available information is scant.
- This report presents the first case of an infant with chromosome 16q23.1 duplication and describes the unique phenotypic features, congenital heart abnormality, and complications associated with his clinical course. A follow-up evaluation 3 months after birth demonstrated abnormalities of motor development.
- Whether or not this specific mutation is clinically relevant remains a question. The associated facial dysmorphism is most likely the strongest factor that evokes concern of any clinical significance associated with this duplication.

American Board of Pediatrics Neonatal-Perinatal Content Specification

- Know the concepts of insertion, deletion, inversion, and translocation.

References