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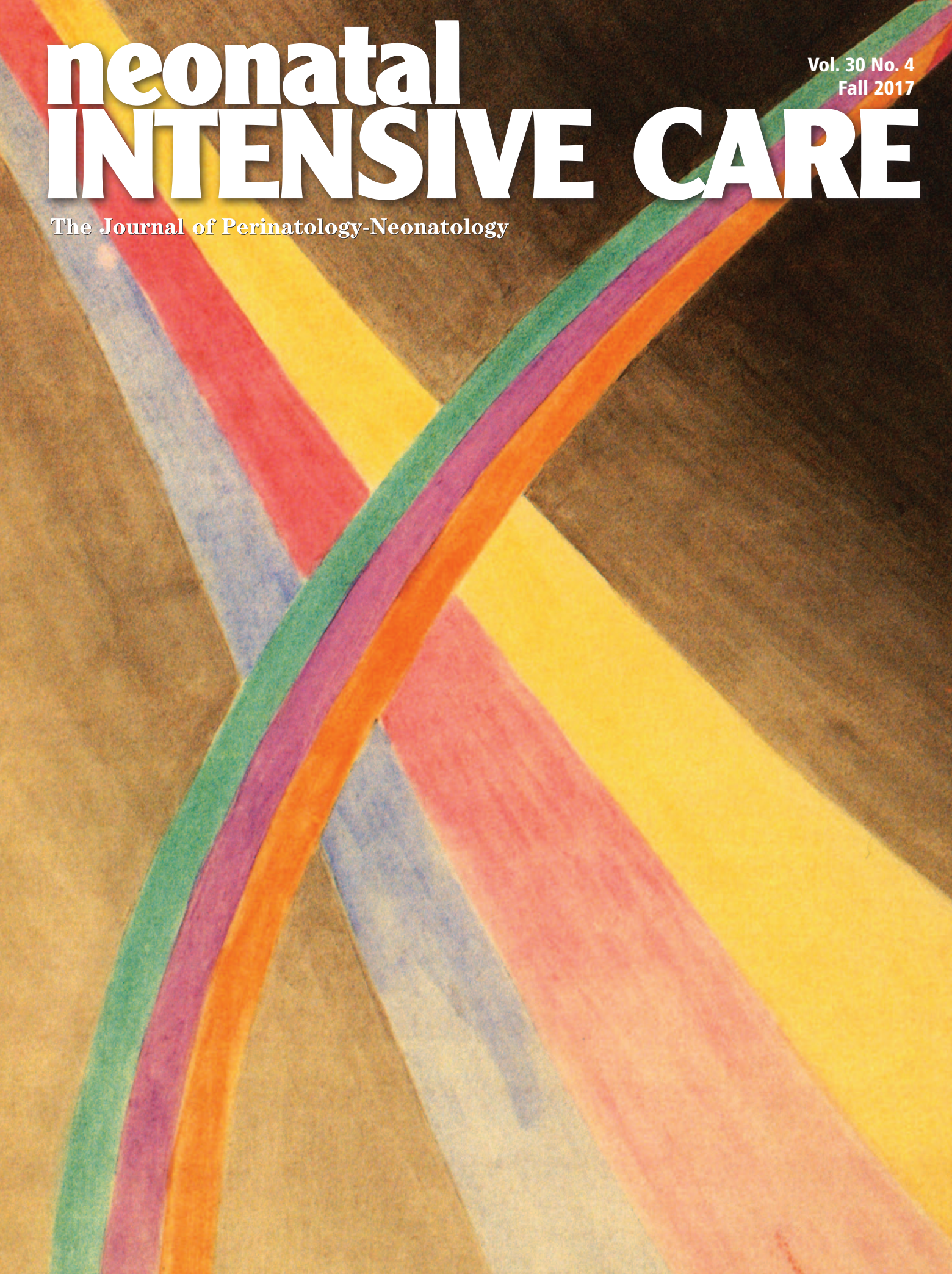

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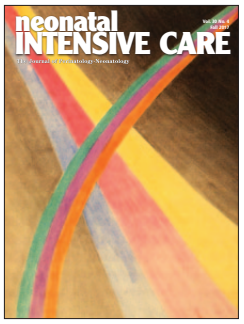

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Human milk makes all the difference

The American Academy of Pediatrics' (AAP) policy recommends the use of human milk for all preterm infants, whether mother's own milk (MOM) or pasteurized donor human milk when mother's own milk is unavailable.[1]

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1. American Academy of Pediatrics. Breastfeeding and the Use of Human Milk. Section on Breastfeeding. [originally published online February 27, 2012]. Pediatrics. DOI: 10.1542/peds.2011-3552

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News

☐ Fall 2017

Save the Date For Bubble CPAP Conferences

These unique programs will share successful bubble nasal CPAP experience, discuss rationale, plus practical aspects and strategies for replicating success with bubble CPAP use. The conferences are intended for the entire neonatal critical care team and other allied health professionals in the neonatal intensive care arena. The 27th Annual Course Respiratory Care of the Newborn — A Practical Approach is on October 7-8, 2017 in New York, NY. Learn more at www.ColumbiaCME.org. And the 7th Annual Bubble CPAP and Non-Invasive Respiratory Management of the Newborn Conference runs December 9-10, 2017 in Washington, DC. Learn more at www.bubblecpap.org.

RT Firm Adds Space

Device manufacturer Hans Rudolph is increasing its manufacturing capabilities by adding two additional CNC (Computer Numerical Control) Mills to its manufacturing floor. The additions of these two machines will help increase its manufacturing capabilities and efficiencies, which will in turn help Hans Rudolph go after new business and help increase its already expansive product line. The line includes masks for pulmonary function/exercise testing, CPAP, BiLevel, NIV, EMS resuscitation, transport ventilation, hi-flow CPAP, Critical Care Ventilation, HBOT, Custom Research applications, Flow, Pressure & Gas Measurements data acquisition and software devices, Linear & Bi-directional Pneumotachometers, Linear Resistors, Volume Calibration Syringes, Volume Validators, DLco Simulators, Flow/Volume Simulators, O2 Conserver Testers, Respiratory Two-Way Non-Rebreathing Valves, Non-Diffusing Gas Bags, Mouthpieces, Nose Clips, Valve Head Supports, Cardiac Output CO2 Rebreathing Valves, Inspiratory Occlusion Valves, Re-Calibration Services on Syringes, Pneumotachs & Resistors, and Custom devices.

Doctor Couple Claims They Toilet Trained Newborn

When two California doctors were expecting their third child, they wanted to stop contributing to the more than 27 billion disposable diapers dumped yearly into US landfills. But washing cloth diapers in water-starved Los Angeles wasn't an environment-friendly alternative, either. Then Dr Rosemary She read about a way to skip diapers altogether. Called elimination communication, the method has parents and caregivers tune into a baby's cues and natural rhythms and bring the child to a toilet when it seems like the right time. Skeptical but determined to find a workable alternative to diapers, Dr She gave it a try as soon as she brought her newborn daughter home from the hospital. "I put her over a potty, gently held her legs, supported her belly, and she went," she said. "It was kind of mind-blowing." Dr She and her husband, Dr Jeffrey Bender, wrote about the benefits of going diaper-free in an editorial published in Pediatrics. Dr She is a pathologist at the Keck School of Medicine at the University of Southern California in Los Angeles. Dr Bender is a pediatric infectious-disease specialist at Children's Hospital in Los Angeles. "For young families interested in protecting the environment for future generations, who want to save some money and keep their kids healthy, this is a good option," he said.

Premature Babies Play Catch Up

A study following more than 1.3 million premature babies born in Florida found that two-thirds of those born at only 23 or 24 weeks were ready for kindergarten on time, and almost 2% of those infants later achieved gifted status in school. Such very prematurely born babies did score lower on standardized tests than full-term infants, but as the length of pregnancy increased, the differences in test scores became negligible, according to the study, conducted by Northwestern University. "What excites

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me about this study is that it changes the focus for the clinician and families at the bedside from just focusing on the medical outcomes of the child to what the future educational outcomes might be for a child born early," Dr Craig Garfield, the first author of the study and an associate professor of pediatrics and medial social sciences at Northwestern Medicine, said in a statement. Researchers analyzed the school performance of 1.3 million infants born in Florida from 1992 to 2002 who had a fetal development term of 23 to 41 weeks and who later entered the state's public schools between 1995 and 2012. Children were assessed for kindergarten readiness and tested in mathematics and reading in grades 3 through 8. The study included 925 babies born between 23 and 24 weeks; these babies tended to have normal cognitive function later in life, with 1.8% even achieving gifted status in school. During the time period the study covered, 9.5% of children statewide were considered gifted. In comparison, 5.8% of all Florida-born public school students were low performing; 33.5% of these children had been born at 23 to 24 weeks' gestation. The study does not account for why extremely premature infants later performed well in school, Garfield said in the statement, and did not look at whether their success could be related to extra support from family or schools, or the children's biological make-up.

Avoiding Empirical Antibiotic Treatment in Infants

Routine empirical antibiotic treatment can be safely avoided in asymptomatic term or late-preterm infants born to mothers with chorioamnionitis, say clinicians from California. This goes against current guidelines, which recommend empirical antibiotic treatment of all infants exposed to maternal chorioamnionitis regardless of clinical symptoms. However, many clinicians are now advocating for change in this management approach in asymptomatic well-appearing infants, according to Dr Amanda Jan from the neonatology department at Huntington Hospital in Pasadena. Researchers tested an alternative protocol for asymptomatic infants born at 35 weeks' gestation or more and exposed to maternal chorioamnionitis. The 240 newborns in their cohort were admitted to a mother-infant unit and underwent initial blood culture and two complete blood counts and C-reactive protein assessment. The babies did not receive empirical antibiotic therapy unless they became symptomatic, had a positive culture, or abnormal laboratory studies, in which case they were admitted to the neonatal ICU and treated. "The rationale for this approach is that well-appearing term newborns are extremely unlikely to have sepsis, regardless of their risk factors," note the authors in a linked editorial. Of the 240 asymptomatic chorioamnionitis-exposed infants in cohort, 162 (67.5%) remained well in the mother-infant unit with a median stay of two days. Seventy-eight infants (32.5%) were transferred to the NICU and given antibiotics due to abnormal laboratory data or development of clinical symptoms. Of those infants admitted to the NICU, 19 (24%) received antibiotics for less than 72 hours, 47 (60%) were treated for culture-negative clinical sepsis, and 12 (15%) were treated for culture-positive sepsis. The median NICU stay was seven days.

Shift in Vaginal Microbiome Associated with Preterm Birth

Changes in the vaginal microbiome occurring between the first and second trimester of pregnancy are associated with preterm birth, new research shows. "There does seem to be a signal for an association between the vaginal bacterial community and preterm birth," Dr Molly Stout of Washington University School of Medicine in St. Louis, Missouri, said. The study is also the first to look at the vaginal microbiome and preterm delivery in a predominately African-American cohort, she noted. While investigators have been studying the relationship between maternal infection and preterm birth for decades, most cases of preterm birth can't be linked to a causative microbe or microbial community, Dr Stout and her team note in their report. "These data likely reflect our incomplete understanding of normal and abnormal vaginal microbial communities during pregnancy," they add. Recent studies have shown that the vaginal microbiome is different in pregnancy, and that there are racial differences in vaginal microbial communities, the researchers write. The one study to date of the vaginal microbiome and preterm birth included mostly white women, they add. Dr Stout and her team used 16S ribosomal RNA (16S rRNA) gene sequencing to characterize the vaginal microbiome in 77 women who

contributed a total of 149 vaginal swabs across pregnancy. Sixty-nine percent were African-American, and 31% delivered preterm. Community richness and Shannon diversity were stable across pregnancy in women who delivered at term. However, women who delivered preterm showed decreased vaginal microbial richness, diversity and evenness, which occurred between the first and second trimesters. Within-subject studies also linked vaginal microbiome instability with preterm birth. There were no specific bacterial taxa associated with preterm birth risk. "The big difference that we see exists between the first and second trimester," Dr Stout said. "It's not an exact specific-enough signal yet to apply clinically but we're dialing in on it." The goal, she added, will be to screen women early in pregnancy and identify those who are at high risk for preterm delivery. Understanding the mechanisms involved in the association between the vaginal microbiome and preterm birth could lead to preventive treatment, Dr Stout said. "We can make smartly designed therapies based on these pathways we know to be abnormal to try and prevent it."

Poor Outcomes and Gestational Weight Gain Link

Gestational weight gain above or below recommended amounts is associated with increased risk for adverse maternal and infant outcomes compared with weight gain within recommended levels, a new systematic review and meta-analysis of more than 1.3 million pregnancies has found. The researchers caution that no causal relationships can be concluded, however. "Compared with recommended gestational weight gain, gain below guidelines was associated with 5% higher risk of both [small for gestational age (SGA)] and preterm birth and 2% lower risk of both [large for gestational age (LGA)] and macrosomia," the researchers write. "Weight gain above guidelines was associated with 3% lower risk of SGA and 2% lower risk of preterm birth and 4%, 6%, and 4% higher risk of LGA, macrosomia, and cesarean delivery, respectively." The researchers also found that 47% of women studied had gestational weight gain greater than 2009 Institute of Medicine recommendations, and 23% had lower-than-recommended gestational weight gain. The researchers included 23 studies, of which 18 were retrospective and five were prospective. Ten studies were conducted in the United States, eight in Asia (four from China, two from Korea, and one each in Taiwan and Japan), and five in Europe (one each in Norway, Belgium, Italy, Denmark, and Sweden). The number of women included in each study ranged from 1034 to 570,672. Eleven studies assessed SGA, 13 studies assessed LGA, 11 studies assessed macrosomia, and eight studies assessed cesarean delivery.

Impact of Arsenic in Drinking Water Studied

Low levels of arsenic naturally found in drinking water in many US states are associated with an increased risk of premature and underweight babies, a study in Ohio suggests. Arsenic is one of the most common elements in the Earth's crust and a natural contaminant in water in many regions of the world. While previous research has found high levels of arsenic in the water associated with a variety of birth complications in places like China, Argentina and Bangladesh, less is known about what happens to babies in places where pregnant women drink water with small amounts of arsenic. For the current study, researchers examined data on 428,804 births in Ohio from 2006 to 2008 to see how birth outcomes differed based on county-level arsenic exposure in the water. Because up to 80 percent of homes in some counties had private well water — for which arsenic data wasn't available - researchers restricted their analysis to counties where less than 10 percent or 20 percent of homes used well water. In counties where less than 10 percent of the population used private wells, arsenic in public drinking water was associated with 14 percent higher odds of very low birth weight babies and 10 percent higher odds of premature deliveries. The study found negative birth outcomes even when women lived in counties where tap water might expose them to arsenic levels below 10 micrograms per liter (10 ug/L), the maximum amount considered safe by the US Environmental Protection Agency (EPA). This suggests EPA regulations may not protect women from reproductive problems linked to arsenic, she said. The study didn't find an association between higher use of tap water at the county level and a risk of very premature infants or infants being small for their gestational age but not very low birth weight. Drinking water with arsenic did appear linked to a higher risk of low birth weight babies, but the added risk was too small to rule out the possibility that it was due to chance. The study wasn't a controlled experiment designed to prove whether or how specific levels of arsenic in drinking water might lead to negative birth outcomes. Other limitations include incomplete or missing arsenic measurements for certain counties at certain points of time during the study, as well as the possibility that pregnant women drank water from sources that weren't measured in the study. Women who are worried about arsenic exposure during pregnancy should get their water tested if they use a private well, said Xindi Hu of the Harvard T.H. Chan School of Public Health in Boston. Municipal water supplies should already get routine testing. They can also take a folate supplement because this can potentially reduce the toxicity of arsenic during pregnancy, Hu, who wasn't involved in the study, said.

Child Asthma Reduction Linked to Vitamin D

Mothers who take a vitamin D supplement during pregnancy could help prevent their newborn baby developing asthma and respiratory infections, say scientists. A study in the Journal of Allergy and Clinical Immunology suggests that taking vitamin D in pregnancy may affect babies' immune systems — a known factor for childhood asthma. The King's College London team say most asthma cases are diagnosed in early childhood, suggesting that the origin of the disease is in early life or when the foetus is still in the womb. Previous studies have been observational, drawing on third-party data to draw conclusions. The latest investigation is based on a study of 51 women who were randomly assigned at between 10 and 18 weeks of pregnancy to take high or low doses of vitamin D supplements. Around half the women were given the recommended daily intake of 400 IU of vitamin D during the second and third trimesters of pregnancy and the remainder took a high dose of 4,400 IU. Samples of all the women's umbilical cord blood were analysed to assess the effectiveness of the newborn babies' immune systems. They found that blood samples from babies born to mothers who had taken the higher doses of vitamin D responded better when exposed to simulations of pathogens. The researchers say future studies should examine the long-term impact on the immunity of the baby.

Twins Joined at Head Separated Successfully

Surgeons at Children's Hospital of Philadelphia (CHOP) successfully completed the separation of 10-month-old conjoined twins Erin and Abby Delaney. The infant girls, from North Carolina, were joined at the top of their heads, a condition called craniopagus, the least common type of conjoined twins. Co-led by neurosurgeon Gregory Heuer, MD, PhD, and plastic surgeon

Jesse Taylor, MD, a multidisciplinary team of approximately 30 members, including physicians, nurses and other medical staff from neurosurgery, plastic and reconstructive surgery, and anesthesiology, participated in the separation, which lasted about 11 hours. "Separating conjoined twins is a very complex surgery followed by a long and complicated recovery, but we are very hopeful for a positive outcome," said Taylor. "Erin and Abby are now recovering in our Pediatric Intensive Care Unit under close monitoring by our expert teams," he added. In addition to their CHOP positions, both surgeons are on the faculty of the Perelman School of Medicine at the University of Pennsylvania. The surgery and reconstruction climaxed months of comprehensive planning and preparation by a large team from many areas of the hospital. It is the 23rd time that surgeons at CHOP have separated a pair of conjoined twins, the first craniopagus pair. "During the separation surgery, our team first meticulously separated the infants' shared blood vessels and dura, the tough protective membrane surrounding both brains, then moved on to separate the sagittal sinus, the most difficult portion of the operation," said Heuer. "Finally, we divided our team into two halves, one for each of the girls, and finished the reconstruction portion of the surgery." On the morning of surgery, a team of anesthesiologists led by Alison Perate, MD, and Matthew Pearsall, MD, managed the surgical preparation for the tightly orchestrated, complex procedure, and then continued to monitor the twins' vital signs and administer their anesthesia throughout the operation. The two sets of monitors and equipment in the room were marked with green or purple tape, one color for each of the girls. Parents Heather and Riley Delaney first learned that Heather was carrying conjoined twins about 11 weeks into her pregnancy, early in 2016. At this point it was too soon to tell whether the twins would be candidates for separation surgery, but on their specialist's recommendation, the family made their initial contact with the Center for Fetal Diagnosis and Treatment at CHOP. After their ultrasound at week 19 of Heather's pregnancy, the family traveled to Philadelphia for evaluation at CHOP, including prenatal imaging: high-resolution fetal ultrasound, fetal MRI and a fetal echocardiogram. After a succession of visits with a multidisciplinary team of CHOP specialists and clinicians, Heather returned home, but travelled back to CHOP every two weeks for prenatal appointments. At 26 weeks, the medical team recommended that Heather stay in Philadelphia for the remainder of her pregnancy. She came to CHOP's Garbose Family Special Delivery Unit (SDU), a dedicated facility where mothers carrying fetuses prenatally diagnosed with birth defects receive state-of-the-art care. Abby and Erin were born in CHOP's SDU by C-section on July 24, 2016, 10 weeks premature, each weighing two pounds and one ounce. They received care in the hospital's Newborn/Infant Intensive Care Unit (N/IICU) for their first seven months, where physical, occupational and speech therapy teams developed innovative treatments and exercises for the infants, still connected at their heads. Surgeons and physicians formulated a comprehensive plan for their full separation. In February of this year, Erin and Abby left the N/IICU for another unit at CHOP where they stayed while awaiting their full separation. For the first time in their lives, Erin and Abby now lie side by side, in separate beds. As the separated infants recover from their surgery, they will be closely followed in the coming months by their surgeons, nutritionists, developmental pediatricians, and other specialists to ensure that they receive the best clinical care to enable them to thrive and grow. They will also likely undergo one or more additional

surgeries. Sometime later this year, Heather and Riley look forward to bringing Erin and Abby home for the first time. "When we go home, it's going to be a big party," says Heather. "Welcome home, baby shower, first birthday."

Docs Urge 'Baby Boxes' For New Moms

Providing new mothers with a "baby box" — a cardboard bassinet with a mattress and fitted cotton sheet - reduces the likelihood that they'll adopt the unsafe habit of sharing a bed with their newborn, new research shows. Bed-sharing is a key risk factor for sudden infant death syndrome (SIDS), according to the American Academy of Pediatrics (AAP). The AAP recommends room sharing without bed-sharing for safer sleeping, as well as using a firm mattress, breastfeeding, placing the baby on his or her back to sleep, keeping the bassinet or crib bare of blankets, pillows and any other soft items, and avoiding exposure to smoking, alcohol and other drugs of abuse. A previous survey of 1,200 new moms, conducted by Dr Megan Heere of Temple University Hospital in Philadelphia and colleagues, found that mothers who received sleep education in the hospital were less likely to bed share, but those who did not have a place for their baby to sleep were at increased risk. To address these issues, Heere and her team developed the Sleep Awareness Family Education at Temple, or SAFE-T, program, which includes face-to-face education from nurses on the AAP sleep safety guidelines, and a baby box packed with diapers, wipes, clothing and other baby supplies. To investigate whether SAFE-T reduced SIDS risk factors, the researchers looked at 2,763 mothers and newborns discharged from the hospital in 2015 and 2016. Within three days of discharge, the mothers were surveyed about their baby's sleep environment. The researchers compared survey responses before and after the introduction of education and baby box distribution in 2016. At the Pediatric Academic Societies Meeting in San Francisco, Heere reported that before introduction of the SAFE-T program, 6.5 percent of mothers reported bed-sharing, compared to 4.7 percent of those who participated in SAFE-T. For mothers who exclusively breast-fed their infants, the rate of co-sleeping was 11.3 percent before the intervention and 5.9 percent afterwards. Fifty-nine percent of the mothers who exclusively breastfed their babies and used the box said it made breastfeeding easier.

Most mothers who received a baby box reported using it as a secondary sleep spot for their infant, while 12 percent relied on the baby box as the baby's main sleeping space.

Temple University Hospital, the WK Kellogg Foundation and Kohl's Cares for Kids provided funding for the baby boxes. Baby boxes have been distributed to all new moms in Finland since the 1930s. In the US, however, the Consumer Product Safety Commission warns on its website that cardboard boxes for babies "are currently not subject to any mandatory safety standards." The Commission says it is working with baby box manufacturers, child safety experts and other interested parties to develop safety requirements for cardboard baby boxes. In the meantime, the Commission urges parents and caregivers to remember: always put the baby to sleep on his or her back, and a bare sleep surface is best.

Immunoprophylaxis Urged to Halt RSV

Respiratory syncytial virus (RSV) illness is often severe, requiring intensive care unit (ICU) admission and invasive

mechanical ventilation, in infants born between 29 and 32 weeks gestational age (wGA) who do not receive immunoprophylaxis (IP), new data show. "The results of our study call for a renewed concern to try to prevent RSV infections in premature babies of 29 weeks gestational age and older," Dr John DeVincenzo of Le Bonheur Children's Hospital, in Memphis, Tennessee, said. Dr DeVincenzo reported the results May 8 at the Pediatric Academic Societies (PAS) annual meeting in San Francisco, California. In the United States, from 1998 through mid-2014, monthly RSV IP was recommended for all preterm infants born at <32 wGA who were younger than 6 months old at the start of RSV season and a subset of infants born at 32 to 35 wGA with additional risk factors. In 2014, the American Academy of Pediatrics recommended against RSV IP administration in otherwise healthy preterm infants born >29 wGA, instead restricting it to those with another qualifying condition such as the presence of chronic lung disease of prematurity or hemodynamically-significant congenital heart disease. In the multicenter SENTINEL 1 study, Dr DeVincenzo's team characterized RSV hospitalizations in preterm infants born at 29 to 35 wGA who did not receive RSV IP in the 2014-2015 and 2015-2016 RSV seasons and who were younger than 12 months of age at the time of their RSV hospital stay. In the 2014-2015 season, among 702 infants admitted to the hospital with community-acquired RSV (CA-RSV), 288 (42%) needed ICU care and 134 (20%) required invasive mechanical ventilation, they report. Of 676 CA-RSV hospitalizations in the 2015-2016 season, 322 (48%) required ICU admission and 131 (19%) required mechanical ventilation, they found. Among all 1,378 CA-RSV cases, 441 (32%) occurred in preemies born at 29 to 32 wGA, 571 (41%) occurred in those born at 33 to 34 wGA, and 366 (27%) were in those born at 35 wGA.

Infants younger than six months accounted for 78% of RSV hospitalizations, 84% of ICU admissions and 91% of those requiring mechanical ventilation. Earlier chronologic age was associated with a higher risk of ICU admission and need for invasive mechanical ventilation. Among infants 29 to 35 wGA in both seasons, ICU admission occurred more often in infants <3 months vs. 3 to <12 month (56% vs. 34%, respectively), as did invasive mechanical ventilation (29% vs. 10%). There were two RSV-related deaths, one in each season.

Impacts of Being Born Underweight Felt Later in Life

Adults born with very low birth weight have lower peak bone mass and a higher incidence of osteopenia/osteoporosis, researchers from Norway report. "Maternal health and diet in pregnancy should be addressed to ensure optimal conditions for both mother and fetus," Dr Unni Syversen from Norwegian University of Science and Technology, in Trondheim, Norway, said. "Mothers should follow the recommendations concerning calcium and vitamin D intake in pregnancy." Increasing evidence suggests that low birth weight is associated with abnormal peak bone mass, and, since nearly 80% of the neonatal body calcium accumulates during week's 36-38 of gestation, preterm infants are especially vulnerable. Some studies have shown that adults born preterm with very low body weight (VLBW) have inferior bone mineral density (BMD). Dr Syversen's team used data on 186 individuals from two cohorts to investigate peak bone mass and bone microarchitecture in adults born preterm with VLBW or small for gestational age at term, compared with term-born controls with normal birth weight. VLBW participants had significantly lower bone mineral concentrations (BMC) and BMD at most sites after adjustment for calcium intake and physical activity. SGA individuals showed minimal differences

in BMC and BMD versus controls, and the difference in BMC lost significance after adjustment for height and weight. After adjustment for height, weight, calcium intake, serum vitamin D, smoking and physical activity, only the VLBW group had a significantly higher risk of osteopenia/osteoporosis. Trabecular bone score, a measure of bone microarchitecture, was similar between the groups and correlated with BMD. The groups did not differ significantly in the number of adults reporting a history of high-energy fractures, the researchers report. In subgroup analyses, results were similar for men and women. Preterm SGA adults had lower BMD at all sites compared with term-born, appropriate-for-gestational-age subjects, and overall, individuals born at earlier weeks of gestation had lower BMD than those born at later weeks of gestation. Weight gain during the first year of life correlated with BMC at all sites in the VLBW and SGA groups, but significant correlation with BMD was only seen among VLBW subjects. In contrast, weight gain up to the fifth year was correlated with BMC at all sites in SGA adults, but not in VLBW adults.

Intelligence Affected by Birthweight

Being born at below-normal weight is associated with a lower intelligence quotient (IQ) not only in childhood and young adulthood, but even at age 50, according to a Danish study. Researchers found IQ differences between underweight and normal-weight babies remained stable into midlife, and even within the normal birth weight range, higher weights equated with slightly higher IQ throughout life. "We found that the association between birth weight and intelligence is stable from young adulthood into midlife," said lead study author Trine Flensborg-Madsen of the University of Copenhagen.

"There are long-term cognitive consequences of birth weight that do not diminish," Flensborg-Madsen said. Birth weights of less than 2.5 kilograms (5 pounds, 8.2 ounces) have long been linked to a variety of health problems including the potential for a lower IQ in youth. Compared to babies born at 2.5 kg or less, infants with a birth weight of 3.5 to 4 kg scored more than five points higher on IQ tests at age 28 and again at age 50, the study found. For the study, researchers examined data on almost 4,700 babies born in Copenhagen from 1959 to 1961, including birth records and results from intelligence assessments done when participants were 19, 28 and 50 years old. They sorted babies into five weight categories: underweight, meaning 2.5 kg or less; 2.5 kg to 3 kg; more than 3 kg up to 3.5 kg; more than 3.5 kg up to 4 kg; and overweight, or more than 4 kg. Average-weight babies in the study fell in the middle category of more than 3 kg up to 3.5 kg. Birth weight was significantly associated with intelligence at all three follow-up assessments, the researchers report. This association remained even after researchers adjusted for other factors that can influence intelligence such as household socioeconomic status and babies' gestational age. A low birth weight can't explain all of this connection because the association also held up among babies born at a range of healthy weights, the authors also note.

Spain Sees Infant Outcomes Improve with Smoking Ban

One year after a nationwide ban on smoking in public took effect in Spain, women had significantly fewer premature or underweight infants, a recent study suggests. Researchers examined data on more than 5 million babies born in Spain from 2000 to 2013. The study included infants born before any restrictions on public tobacco use, after a 2006 ban covering many workplaces with exceptions in the hospitality industry, and after a 2011 law curbing tobacco in nearly all public places. The rate of babies born small for their gestational age declined after the partial smoking ban took effect in 2006, researchers report. With the comprehensive ban in 2011, rates of preterm and low-birthweight babies also dropped. "Second hand smoke exposure during pregnancy is associated with health complications affecting perinatal and neonatal health," said senior study author Dr Inaki Galan of the Autonomous University of Madrid. "The implementation of two Spanish smoking bans (partial and comprehensive) was associated with a risk reduction regarding preterm births and low birth weight infants," Galan said. Overall, the researchers found that during the study period 7.9 percent of infants were premature, 9.2 percent were small for their gestational age and 7.8 percent had low birth weight. The comprehensive smoking ban was associated with an immediate 4.5 percent reduction in the preterm birth rate that was sustained a year after the law took effect. The birth rate of underweight babies fell 2.3 percent immediately and fell a bit more one year after implementation. With the partial ban, the study found an immediate 4.9 percent reduction in the birth rate of babies that were small for their gestational age.

Doctors Suggest Different Look at Opioid Crisis

Every 25 minutes, a drug-addicted baby is born in the US. To try to protect the youngest victims of the nation's opioid epidemic, Tennessee enacted a law that sent new mothers to jail for substance abuse, while other states employ existing child-abuse laws to punish prenatal drug users and remove their children. But sanctions have backfired, serving only to drive pregnant women away from necessary prenatal care and substance-use treatment, pediatricians say in three new papers. In one, the American Academy of Pediatrics exhorts

policymakers to support a public health approach - rather than a criminal justice response - to opioid use in pregnancy. "I don't think these laws are in the best interests of moms or babies," Dr Stephen Patrick, lead author of the report, said in an interview. "Opioid-use disorder is a medical problem and not a moral failing." Patrick is a professor at Vanderbilt University School of Medicine in Nashville, Tennessee, where he treats infants suffering withdrawal from opioids. Instead of jail, he called for improved access to long-term contraceptives and substance-treatment programs designed to care for pregnant women. About 100 substance-using new mothers went to jail in Tennessee between 2014 and 2016 under a fetal-assault law that's no longer in effect, Patrick said. The law incited so much fear in pregnant addicts that some refused to go to the hospital and gave birth at home, in cars or on the side of the road, he said. Meanwhile, the number of pregnant women who use opioids and the number of babies born with withdrawal symptoms continues to rise. Patrick estimated that as many as 440,000 substance-exposed infants are born in the US every year and asserted: "We're not going to arrest 440,000."

Hep C in Infants 'Underreported'

Hepatitis C virus (HCV) infection among infants is vastly underreported, suggesting the need for routine testing in pregnant women, a new study from the Centers for Disease Control and Prevention (CDC) has found. "The data from this study may inform ongoing discussions of HCV screening for all pregnant women to protect their health and that of their offspring," the researchers write. Kathleen N Ly, MPH, from the Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, Georgia, analyzed data from two of the largest population data sets available in the United States: the National Notifiable Diseases Surveillance System (NNDSS) from 2006 to 2014 and the Quest Diagnostics Health Trends national database from 2011 to 2014. The researchers included 171,801 women of reproductive age (15 - 44 years) and 1859 children (aged 2 - 13 years) with HCV infection reported to the NNDSS and 2.1 million reproductive-aged women and 56,684 children who underwent HCV testing by Quest Diagnostics. Between 2006 and 2014, the number of reproductive-aged women with acute HCV infection reported to the NNDSS rose 3.4-fold, from 249 per year to 848. Similarly, the number of past or present cases reported doubled, from 15,301 to 30,191. "[B]y 2012, the total number of cases reported in reproductive-aged women surpassed that of women aged 45 to 64 years," the researchers write. During 2011 to 2014, Quest Diagnostics tested 581,255 pregnant women; of those, 4232 (0.73%; 95% confidence interval [CI], 0.71% - to 0.75%) had HCV infection. "Although no HCV treatments have been approved by the US Food and Drug Administration for use in pregnant women, clinical trials of promising drugs are under way. Pregnancy may be the only time a young woman is seen by a clinician, so some clinicians already are screening pregnant women known or suspected to be at risk for HCV infection according to current guidelines," the researchers explain.

Benefits of Using Functional Scoring System

Use of a functional scoring system dramatically reduced morphine treatment and length of stay among infants with neonatal abstinence syndrome (NAS) compared with the widely used Finnegan Neonatal Abstinence Scoring System (FNASS), a new study has shown. Of 50 infants managed with the functional assessment system, called ESC for eating, sleeping, and consolability, only 6 (12%) received pharmacologic treatment compared with 32 (62%) expected if FNASS had been used, Matthew J. Lipshaw, MD, from the Department of Pediatrics at Yale University School of Medicine, New Haven, Connecticut, said during a presentation here at the Pediatric Academic Societies (PAS) 2017 Annual Meeting. In addition, the length of stay was dramatically reduced after introduction of the new system, with an average of 5.9 days vs 23 days beforehand. Dr Lipshaw noted that several other changes occurred around the same time as part of a larger quality improvement program, such as having infants room with their mothers and caring for the infants on a regular hospital floor instead of the neonatal intensive care unit. Nonetheless, the reduction in morphine treatment has helped shorten inpatient time for these infants.

NANN PREVIEW

Hamilton

Booth 531

What products do you plan to exhibit at NANN?
HAMILTON-C1 neo

What's new this year? Tell us about your latest products or future plans.
The new HAMILTON-C1 neo combines a range of therapy options with maximum mobility in a highly versatile ventilator designed specifically for neonates. The HAMILTON-C1 neo provides tidal volumes as low as 2 ml for effective, safe, and lung-protective ventilation even for the smallest patients.

What educational or training materials will be available?
Hamilton e-college

Why should our readers stop by your display?
The HAMILTON-C1 neo is a versatile neonatal ventilator that combines invasive and noninvasive modes with the additional option of nCPAP. The integrated turbine allows it to be operated independently of a compressed air supply. Due to its compact design, it is an ideal companion for your smallest patients in various environments such as delivery room, the intensive care unit and emergency ward, as well as during intrahospital transport.

Neomed Inc.

Booth 715

What products do you plan to exhibit at NANN?
NeoMed will display our complete NeoConnect family of ENFit NICU/PICU and pharmacy products, as well as our legacy Enteral Safety products. NeoMed is known for innovative designs that support the specialized feeding and medication dosing needs of the low birth weight, neonatal and pediatric patient. Our innovative ENFit low dose tip syringe, designed to administer accurate dosing for low volume administrations, has been accepted as the industry solution for ENFit dose accuracy and has received FDA 510(k) clearance. We are committed to improve patient outcomes through product designs that meet safety, clinical, and regulatory guidelines while supporting cost containment objectives and minimizing process disruption.

What's new this year? Tell us about your latest products or future plans.
Design, development, and launch of our ENFit products have dominated NeoMed's new product portfolio this year. Our new NeoConnect pharmacy and NICU/PICU products support best clinical practices as outlined by leading organizations such as GEDSA, ISMP and ASPEN. Features include open hub designs to help eliminate fluid accumulation while enhancing easy cleanability with our NeoConnect Cleaning Tool, plugged closures that help reduce bacterial accumulation, Low Dose Tip syringes that provide accurate dosing of both enteral and oral medications, pharmacy adapter caps with rapid-fill design, and our NeoSecure™ "click to close" self-righting tip caps.

Over 80 hospitals have completed NeoMed ENFit conversions, with 315 additional hospitals committing to "Go Live" dates. This has already resulted in 4.5 million feeds and doses administered. The transition to ENFit continues to gain global momentum.

NeoMed now offers a line of oral care (swab) and trophic/colostrum collection kits that includes the DoseMate® and DoseMate DL, the 0.5 mL syringe used to draw up colostrum, the hands-free tip cap that provides aseptic handling, individually packaged foam-tipped oral applicators, and a plastic storage bag with label for traceability. This kit was designed to enhance patient comfort and provide directional targeting for colostrum deliveries.

The Tamper Evident Cap [510(k) pending], designed for use with ENFit® Syringes, is indicated to prevent fluid loss and contamination of syringe contents during transport and provides evidence of access.

The 1 oz and 2 oz collection bottles with transfer lids are now available, and were designed to collect and manage precious HBM from home to hospital, helping to meet the specific needs of the neonatal patient.

The NeoMed pharmacy Fill Cap Coupler allows use of Legacy fill caps with oral connectors to be used with an ENFit syringe when no ENFit bottle adapter is accessible. The coupler works with most current EO bottle adapters, including press-in adapters, ADAPTA® caps, and bung adapters.

NeoMed's 100 mL ENFit Syringe delivers larger feeding volumes for gravity feeding applications or through a syringe pump application.

What educational or training materials will be available?
NeoMed recently launched the "NeoConnect Certification Program for ENFit" to provide clinicians "hands on" experience with NeoConnect products in advance of their "Go Live." It highlights minimal change from legacy to NeoConnect and includes medication preparation, transport, and delivery/administration. The NeoMed NeoConnect Workshop Video will be available for viewing at the NANN Conference. Competency skills labs will be demonstrated in our booth.

Tell us about any speakers or in-booth promotions.
Our booth will be staffed by NeoMed certified ENFit Instructors.

Why should our readers stop by your display?
Stop by our booth to see how NeoMed products and Customer Loyalty Program can support your transition to ENFit.

Neotech

Booth 301

What products do you plan to exhibit at NANN?
Neotech will be exhibiting our core product line, including our Little Sucker products, NeoBar, NeoShades, NeoLead, RAM Cannula, and more. Along with our core product line, we will also be featuring some exciting new products!

What's new this year?
Tell us about your latest products or future plans. We're proud to share our new NeoGlo Transilluminator device, NeoPockets Personal Incubator Organizer, the Neotech Ocular Exam Kit, and a couple of fun surprises!

What educational or training materials will be available?
We will have Neotech catalogs, product sell sheets, and our team of Neotech clinical consultants will be available.

Tell us about any speakers or in-booth promotions.
Our team of clinical consultants will be available for any questions or comments anyone may have. This year, we will have some exciting promotions and fun giveaways!

Why should our readers stop by your display?
Neotech loves our nurses and NANN is always the highlight of our year. We're excited to show off our new products and share some fun surprises. Don't forget to stop by and grab some of our giveaways and learn about our exciting promotions!

Owen

Booth 613

What products do you plan to exhibit at NANN?
Owen Mumford will be exhibiting our complete range of Unistik® products, including Unistik® TinyTouch™ Heel Incision Devices, Unistik® 3 Side-Activated Safety Lancets and Unistik® Touch Contact-Activated Safety Lancets. From low flow fingersticks to high flow heelsticks, the Unistik family of products offer multiple activation methods, penetration depths and gauge sizes to meet nearly every capillary testing need. Unistik products are designed with the patient and healthcare professional in mind, engineered to help reduce pain during the sampling process while delivering the results healthcare professionals expect.

What's new this year? Tell us about your latest products or future plans.
Unistik® 3 and Unistik® Touch™ have raised the bar on device traceability. Each device now features individually-marked lot numbers for greater peace of mind.

What educational or training materials will be available?
In addition to IFUs and brochures, we offer tiny foam feet (made to mimic a newborn's foot), that offer an ideal training tool for practicing heel incisions.

Tell us about any speakers or in-booth promotions.
Unistik products offer an opportunity to save versus other leading national brands. Visit our booth to learn about our free cost savings analysis. We will also be offering silipint glasses (one per attendee), free with a badge scan. Promotional items are available while supplies last.

Why should our readers stop by your display?
When it comes to capillary sampling, Unistik® products offer an opportunity to enhance quality of care, while potentially saving your institution money. Besides offering exceptional, high-quality products, we also have the best tradeshow giveaways. Be sure to swing by early—promotional items are only available while supplies last.

RightBio

Booth 707

What products do you plan to exhibit at NANN?
The RightBio Metrics pH indicators which are used to confirm NG/OG tube placement for tubes intended to end in the stomach.

What's new this year? Tell us about your latest products or future plans.
Based on customer feedback, we have created the RightSpot Small (10Fr or less) and RightSpot Large (>10Fr) connections. Additionally we will be exhibiting an ISO Enteral Standard compliant version that has a syringe pre-attached and adapters that fit most major manufacturers configurations.

What educational or training materials will be available?
This year RightBio Metrics will be sponsoring an event at NANN that will educate attendees on the emerging Clinical Practice Statement that will instruct hospitals to use pH first line to confirm NG tube placement and only use X-ray if pH is not conclusive. This statement is currently being drafted and the final-version will be endorsed by the ASPEN Board of Directors. Beth Lyman RN MSN CNSC, Sr. Program Coordinator of the Nutrition Support Team at Children's Mercy Hospital in Kansas City and chairman of the NOVEL project states "the intent of this Clinical Position Statement is to disseminate to every nursing organization in the US evidence based best practices in confirming NG/OG tube placement. NOVEL/ASPEN plans to share the statement with The Joint Commission. Using this document, The Joint Commission can ask hospitals about NG tube placement verification. If the hospital is not using an evidence based method, it can be cited as that is a long standing TJC standard."

Tell us about any speakers or in-booth promotions.
Look for details in your email in August on the speaker program and in-booth promotions.

Why should our readers stop by your display?
With the increasing awareness of mis-placed NG/OG tubes and the US movement to use pH to confirm placement, RightBio Metrics makes the ONLY FDA cleared, CLIA waived product for this application.

EXECUTIVE PROFILE

Neotech

Describe your product(s) and its unique features.
Neotech develops specialized, high quality products for the NICU, PICU, and beyond. We engineer skin friendly products that are designed specifically for tender or fragile skin, both for the benefit of our patients and their caregivers. Neotech is very proud to say that our products are made in the USA! We adopted this business model years ago and what we do is good for America, good for our local economy, good for our vendors, and ensures a steady supply chain.

Tell us about the latest advances in the area your product serves.
Technology in the hospital has changed a lot in the 30 years that Neotech has been in business. In the NICU, especially. Through our clinical consultants, parents, DME companies and other professional contacts, we try to keep abreast of what's new and what's changing, and then provide appropriate products and training to keep up with industry changes.

Discuss your R&D process, including clinical user input.
Most of our products are clinician invented. We welcome new product ideas and our dedicated new product development team is always happy to answer any questions and help bring an idea to reality. We pride ourselves on building positive working relationships and steadily moving through the process, all at no cost to the inventor. We protect their idea, market it, and sell it! Neotech also has a team of over 50 clinicians around the globe who provide valuable clinical input on all our products, new and old. We know that clinicians who work with us will take satisfaction in knowing that they'll make a difference for patients, as well as other clinicians.

Discuss the educational services you offer for use of your product.
Neotech has an experienced team of licensed Clinical Consultants who work with hospitals providing educational support and in servicing. Our highly specialized team is located throughout the United States. Their expertise in the neonatal, pediatric and respiratory fields is utilized to instruct on the safe and proper use of our products. Clinical Consultants can provide onsite in servicing for your staff including nights and weekend shifts.

What new technology do you see as having the greatest impact on your area of expertise?
Our latest acquisitions of two 3D Printers and other engineering equipment have greatly advanced our development process and cut the time required for prototyping and new product design significantly.

Connecting Premature Babies With Life-Saving Technology

In this feature, Neonatal Intensive Care interviews clinicians and healthcare providers about the actual application of specific products and therapies. This interview is with Bert Bunnell, ScD, of Bunnell Incorporated.

Neonatal Intensive Care: After a lifetime dedicated to helping premature babies, what defines your success?
Bert Bunnell: I am most pleased that we have helped save about 150,000 infants with High Frequency Jet Ventilation (HFJV) over the past 30 years. Our Jet is now being used to treat around 10,000 babies a year, which means that some baby somewhere is being connected to one of our ventilators every 53 minutes.

NIC: What is HFJV and after more than 30 years of clinical application, why hasn't it been universally accepted?
BB: HFJV is a gentle and efficient way to facilitate gas exchange in the lungs. It works by squirting tiny spurts of air and oxygen into the patient's endotracheal tube (ETT) through a little jet nozzle built into the ETT adapter at rates up to 11 "breaths" per second. The closest thing to HFJV in the natural world is panting.

HFJV is a "disruptive technology;" it changes the way people ventilate patients. Other examples of disruptive technologies are personal computers, cell phones, the internet, etc. People resist change, so disruptive technologies are typically slow to be adopted, have limited appeal, and take time to demonstrate practical applications. Such is the case with HFJV. However, I am heartened to know that HFJV use has been slowly and steadily rising while overall use of mechanical ventilation has been going down every year for the past several years.

Until HFV came along, every ventilator tried to mimic normal breathing for patients that were anything but normal. While that approach works a great deal better than nothing, it certainly has its drawbacks, which are exemplified by bronchopulmonary dysplasia (BPD) in the case of surviving preterm infants. Despite better prenatal care, surfactant administration, and better understanding of the hazards of mechanical ventilation and excess oxygen, BPD remains the most common severe complication of preterm birth.

NIC: Describe the history of HFJV and how you developed the technology.

Bert Bunnell, ScD, began searching for a better way of breathing for premature babies in 1972. His hard work and dedication led to the development of the Life Pulse® High Frequency Ventilator and Bunnell Incorporated. Input on questions was provided by Neonatal Intensive Care. If you would like to participate in this feature, as a company or healthcare provider, please contact Steve Goldstein at s.gold4@verizon.net.

BB: My personal history of HFJV is illustrative of how sometimes the worst things that happen to you become the best things that happen to you. At age 21 and a senior in engineering school in 1968, I was diagnosed with malignant melanoma, which prompted immediate surgery but also exempted me from military service during the Vietnam war, and enabled me to attend graduate school.

Accepted by MIT's Department of Chemical Engineering, I moved to Boston where my melanoma metastasized, requiring another radical surgery just before school started. At that point, it was obvious that my cancer was tracking the lymph system from my neck toward my lungs, which heightened my interest in medicine in general and lungs in particular.

As luck would have it, once I qualified for the doctoral program a thesis topic related to aerosolizing artificial surfactant for premature infants became available. It immediately caught my interest, because it had something to do with lungs.

I received my doctorate of science degree in 1972 after demonstrating that I could deposit artificial surfactant in newborn lamb alveoli. I then pursued a fellowship in pediatric pulmonology at the Massachusetts General Hospital (MGH) to determine if this therapy would work in human infants.

This aerosol therapy ultimately failed due to the inadequate surfactant then available, but its pursuit, my engineering background, and wonderful mentorship from Dr. Dan Shannon at MGH led us to high-frequency ventilation in 1974.

NIC: What happened with your melanoma?
BB: All I had were the two radical surgeries — no chemo, no radiation. I never even met an oncologist until 1975 when I decided to pursue commercial development of HFJV by taking a job in industry.

One year earlier, five years after my last surgery, I discovered this statistic in Chest: only 10% of patients were alive one year after diagnosis of metastatic malignant melanoma. When the oncologist examined me in 1975, he wanted to start me on chemotherapy immediately. I declined, stating the obvious: I had no signs of cancer at that point.

The doctor said: "But, you should be dead!" I said: "But, I'm not." So, he made me promise to see him every 3 months after that as a condition for him to give me a clean bill of health for my new

job. I had no problem making that pledge, and the melanoma never reappeared.

NIC: So, you went to work for a company?
BB: I pursued commercial development of HFV with two major corporations for five years. The time frame for my project was far longer than their attention spans, so I founded Bunnell Inc. out of desperation in 1980.

NIC: Why would a baby need HFJV?
BB: Two reasons: rescue when babies are dying of complications that conventional therapies can't handle, and prevention of those complications after premature birth.

NIC: Which patient had the biggest impact on your life and why?
BB: Three patients immediately come to mind. The first patient was an infant I unexpectedly resuscitated while left in a room alone with a baby at MGH when I was a graduate student. I just did what I had learned to do with rabbits in cardiac arrest during my early experiments with HFJV, and it worked. The baby survived, it was no big deal, and it gave me confidence that I could save babies.

The second patient was an adult. In 2000, I was recruited to use our prototype Jet for larger patients in extremis to rescue the former dean of nursing at Baylor University in Dallas when her trachea fell apart during the late phase of ALS (amyotrophic lateral sclerosis). She was the only patient I ever treated who told me how it felt to be on HFJV. She said it felt "funny."

After so many people had expressed their concerns that HFJV would somehow hurt the babies we were treating in the early days of its application, I welcomed the feedback that it just felt funny.

The third patient was a heart breaker — a baby girl born full-term, Apgars of 9 and 9, who then turned purple. Kelli had a rare defective surfactant syndrome, which up to then, was always fatal. As was typical in the Phoenix hospital where she was transferred, she was rescued by HFJV. But even though she was fine on the Jet, there was no possibility of weaning. After several weeks, it became apparent that her only chance of survival was to procure a new set of lungs. Thus, the hospital requested our help in transporting this baby on a Jet via jet to St. Louis where she might be able to get a double lung transplant.

The transport went well, and we taught the hospital in St. Louis how to keep her alive on HFJV while the family waited for a donor. It took over three months before another family's disaster became the Arizona family's salvation, and Kelli received a working set of lungs.

Kelli led a short (11 years) but spectacular life that left everyone who met her in awe. She and her family became avid organ donor advocates. She was active in sports, Girl Scouts, and played the piano. She had her own website (www.kelligar.com) that she would eagerly show people as she got old enough to coach them on how to find it on their cell phones. She brought joy to everyone she met.

Kelli's life continues to inspire me to this day, as it demonstrated that we can do more than we know we can do, and that we must constantly push the limits of what we know now to even approach the potential of everything we can do.

NIC: What is the role of small companies in health care, how do they survive, and why is it important that they do?
BB: Most innovation comes from small companies. They take risks, because compared to large companies, they have little to lose and a lot to gain — not just monetary gains, but true advancements in medicine. When an innovation pans out, small companies are typically acquired by big companies who can market new products quickly and effectively. However, it doesn't always work out that way, especially with products that serve small markets like neonatal medical devices.

There are easily 10 times as many adult patients as newborns, and the ratio of adult patients to children age 1-17 is probably 5 times greater than that, because, fortunately, most children are healthy. It takes just as much money to market a product for babies as it does one for adults, so why not go for the product with the biggest financial upside?

Some big companies try to cover the markets for smaller people by designing "cradle-to-grave" products. After all, aren't babies just like adults, only smaller? Some big companies acquire small companies only to discover that their products aren't so easy to sell or don't produce the return on investment that they hoped for, so they just shut them down. (Remember Infrasonics?)

So, small companies matter, and one way to keep them prosperous is to make them employee-owned.

NIC: How does employee ownership improve small company prosperity?
BB: Besides the obvious incentive that employee ownership provides every worker (a stake in the company), employee ownership perpetuates jobs. No large company can acquire an employee-owned corporation without approval of its employees, and most employees want to maintain their jobs until they decide to retire, when their companies have an obligation to buy out their shares of ownership. We converted Bunnell Inc. to an employee-owned company in 2008 after spending the previous five years buying back all outstanding shares from our investors. Since then, we've had several employees retire, everyone still with us is working hard, and it's working great for all.

NIC: So, what does the future hold for Bert Bunnell and Bunnell Inc.?
BB: I'm mostly retired now and living the dream, as the saying goes. Over the past 10 years we've added a collection of bright young people to our 40+ employee firm, and the Company just got FDA approval for a new model of our Jet. So, its future is bright.

I enjoy teaching, so I volunteer my time as an adjunct associate professor of bioengineering at the University of Utah, and I stay involved with Bunnell Inc. as Chairman Emeritus. I'm writing up the 37-year history of the company, and I still accept speaking engagements at hospitals and medical conferences whenever those opportunities arise.

I also love rock climbing and skiing, so it will be interesting to see which goes first, the body or the mind. If it's the body, I'll have to do more reading and writing. If it's the mind, it will make my climbing and skiing even more exciting!

Bacteria in Human Milk: A Review of the Literature

Sandra Sundquist Beauman, MSN, RNC-NIC, CNS

Bacteria exists in human milk. In fact, it is important as it contributes to normal colonization of the intestines of the newborn. Even bacteria that is often thought of as harmful can be harmless or even beneficial to the infant. As the microbiome is studied further, multiple authors present evidence that exposure to bacteria is beneficial as well as their role in developing brain neurotransmitters, competent gut maturity and many other roles they play to improve growth and development, and prevent auto-immune diseases in infants (Torow & Hornef, 2017; Macpherson, Gomez de Aguero & Ganal-Vonarburg, 2017). However, some bacteria that are protective in infants with normal immunity are not in extremely premature infants with compromised immunity. Reports exist of illness or death in the premature infant related to specific bacteria that may be present in human milk (Gastelum et al, 2005; Chen et al, 2016). Large amounts of usually pathogenic bacteria or sometimes even what is considered normal flora can cause illness in these infants. Grovslien and Gronn (2009) reported on practices in Norway where it is usual practice to feed infants, particularly preterm infants, unpasteurized human milk, not from their own mother when mother's own milk is insufficient. They report the screening process for milk prior to using it, which is to test every 500 ml of milk donated by a mother. Milk that contains any pathogen or a high bacterial count defined as >100,000 colony forming units/mL is destroyed. Milk with less than 10,000 colony forming units/mL and no pathogens may be used for the smallest premature infants. They also report the results of a study in which the incidence of late onset sepsis was evaluated in this population. They found that it was actually 4-fold lower in infants who received early enteral feeding with human milk. This included mother's own milk as well as unpasteurized donor milk.

Guidelines for milk handling in the hospital for premature infants are available from the American Dietetic Association (Robbins & Meyers, 2011) and the Human Milk Banking Association of North America (HMBANA) (Jones, 2011). HMBANA also has guidance for human milk storage and processing that is complied with by most human milk banks in the U.S. There is not a lot of recent research into bacterial growth in human milk and the effect on the premature infant. This is of interest because of a perceived high incidence of milk sharing and related to reported incidents of powdered formula contamination that occurred and were reported between 1989 and as late as 2002 specifically contamination with Cronobacter (formerly known as Enterobacter sakazakii) (Lai, 2001; Biering et al, 1989; Simmons et al, 1989; Van Acker et al, 2001; Himelright, et al, 2002). In addition, reports of bacterial growth in feeding tubes increased awareness of the potential for infection related to feeding equipment. In a recent study, the connection between the feeding tube and extension tube was cultured and infant stools were cultured for comparison. This group reported that 15 cases showed presence of bacteria in the feeding tube days (at least 7) before presence in the feces and 16 showed the reverse, demonstrating that bacteria may colonize or infect the infant from the feeding solution at least about half of the time (Gomez, Moles, Melgar, Ureta, Bustos, Fernandez et al, 2016).

Sandra Sundquist Beauman is a research nurse coordinator at the University of New Mexico. She is also an independent consultant with Medela LLC, and provides neonatal consultation and continuing education through CNS Consulting.

Evaluating bacterial growth in human milk

Lemons et al (1983) sampled fresh milk and frozen milk thawed under running tepid water without further warming. These samples were not warmed to room temperature but samples were taken directly from refrigerated or thawed (but still cold) milk. Samples were taken at 0, 5, 6, 7, and 8 hour intervals. Frozen milk samples did not show a significant change in total colony count between 0 and 8 hours but fresh milk samples revealed a significant increase in growth at the 6-hour sample.

Two other studies (Igumbor, Mukura, et al, 2000; Nwankwo et al, 1987) evaluated microbial growth in tropical settings of milk pumped at home for the purpose of feeding term infants whose mothers could not be present to provide direct breast feeding. While multiple organisms were identified, none were considered by the authors to be pathogenic although would be considered so for high-risk preterm infants. These organisms included Candida albicans and Klebsiella in the Africa study while Nwankwo did not list specific species identified. Interestingly, this group reported higher overall bacterial counts in premature human milk when compared to term human milk. Whether this phenomenon is a function of premature milk or the presence of bacteria leads to premature birth is not clear from this report.

Several studies have evaluated how adding fortifier to human milk can effect bacterial growth and antibacterial activity. Several of these are reviewed in the following paragraphs.

Chan, Lee & Rechtman (2007) used milk samples from the same mother, dividied into 2 aliquots. Both were contaminated with Enterobacter sakazaki, Escherichia coli, Clostridium difficile and shigella soneii due to their likelihood to cause sepsis and

necrotizing enterocolitis in the infant. These milk aliquots were then fortified with either a bovine protein-based powdered human milk fortifier or human human milk fortifier. The antibacterial activity of the human milk was almost totally lost in the samples fortified with bovine protein-based fortifier but not effected in those samples to which human human-milk fortifier had been added.

Chan (2003) previously reported the effect of fortifier on preterm human milk antimicrobial activity in one of the largest studies of this kind with 42 different women donating milk for the study. Two commercially available bovine-based human milk fortifiers, both powdered at the time were tested against human milk alone for their antibacterial activity. Iron was found to adversely affect antimicrobial activity specifically against E. coli, Staphylococcus, Enterobacter sakazakii and Group B streptococcus. The effect of additional medium-chain triglycerides (in the form of MCT oil) on antimicrobial activity was also evaluated and there was found to be no effect.

Jocson, Mason, Schanler (1997) evaluated bacterial growth in human milk to which a powdered fortifier had been added. They evaluated total bacterial growth over a 4-hour simulated infusion. During the infusion period, the milk was maintained at room temperature. Bacterial count increased *significantly* in both the previously frozen and fortified milk as well as fresh and fortified milk, although it was significantly greater in the fresh human milk, demonstrating the effect of freezing on bacterial growth.

Telang et al (2005) studied the growth of bacteria in human milk, to which one sample had powdered fortifier added as well as a sample of powdered infant formula. Some samples were inoculated with Enterobacter sakazakii. Bacterial growth was measured while milk was maintained at room temperature (22°C) at 0, 2, 4 and 6 hour intervals. These researchers found that even after 6 hours, neither fresh human milk with fortifier nor powdered formula had a significant increase in bacterial growth.

Ovali et al (2006) reported antimicrobial activity of human milk with the addition of a bovine-protein based human milk fortifier not available in the United States (Eoprotin). In addition to adding this fortifier, one sample contained additional iron. The iron served to reduce the antimicrobial activity of the milk resulting in increased bacterial multiplication. The antimicrobial activity was observed in these samples in spite of having been frozen at -20 for an unspecified amount of time.

Quan et al (1994) reported the effect of adding formula, fortifier and Poly-vi-sol (not specified to contain iron) were added. Some formulas added were bovine-based and others were soy-based. They found that all bovine-based formulas enhanced growth of E coli with incubation found in these samples in as little as 3½ hours after mixing when maintained at 37°C while soy-based formulas and/or addition of Poly-vi-sol did not.

Research Opportunities

While several studies reviewed here demonstrate bacterial growth in human milk that would be concerning for the high risk premature infant, the results are variable. In addition, most of these studies are small. Bacterial growth appears to be influenced by addition of iron and fortifiers in most, although not all, cases. Also, infection control principles tell us that bacterial growth can be effected by how milk is stored and handled including pumping, mixing and feeding. Additional research focused on feeding temperatures of milk, effect of newer liquid fortifiers on bacterial growth and linking presence of these bacteria to infant colonization or illness would add to the body of knowledge and be helpful to inform practice.

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A Case of Benign Recurrent Pneumatosis Intestinalis in a Preterm Infant

Surasak Puvabanditsin, Vidya Puthenpura, Suja Vinod, Marissa Botwinick, Charlotte Chen and Rajeev Mehta

A male preterm first-born twin was born by cesarean section at 28 weeks' gestation to a 35 year-old G3 P1 black woman. Prenatal care was complicated with newly diagnosed acute myeloid leukemia at 24 weeks of gestation and gestational diabetes. Physical examination of the infant revealed a birth weight of 1125 grams (50th centile), length 39 centimeters (60th centile), and head circumference of 26 centimeters (40th centile). Since the neonate required some respiratory support, umbilical arterial and venous catheters were placed soon after birth but were removed on the third day of life. At 12 days of age, the infant developed abdominal distension. An abdominal X-ray showed a small area suggestive of pneumatosis intestinalis (PI) but there was no accompanying thrombocytopenia or metabolic acidosis. An X-ray done 12 hours later showed an improved bowel gas pattern. Because of the suspicion of NEC, the infant kept NPO for 3 days and received a 7 day course of antibiotics. At 3½ weeks of age, the neonate developed stage II NEC, with gas in the portal vein, pneumatosis intestinalis (PI), thrombocytopenia, and metabolic acidosis. An exploratory laparotomy and loop jejunostomy were performed. During the laparotomy, there was no evidence of pneumatosis intestinalis nor gangrenous bowel noted. At 3 months of age (42 weeks post menstrual age, weight 2900 grams) he underwent reanastamosis of the jejunostomy. The post-operative course was complicated by feeding intolerance and abdominal distension. Full enteral feeding was accomplished at 5 months of age, and by then, his weight was 4500 grams. Again, extensive PI was noted at 5½ months of age (Figure 1). There was no evidence of free air or portal venous gas, and there were no metabolic derangement or hematologic abnormalities. A C-reactive protein and urinalysis were negative. The infant did not have any clinical evidence of sepsis or other deterioration in his condition. The PI resolved by withholding of enteral feeding and with the administration of intravenous antibiotics. The infant had a recurrence of PI at 6 months of life (Figure 2), and during this episode, feedings were continued and he was treated with a 42-day course of oral metronidazole. His blood, urine and stool cultures were negative for bacteria and enteroviral cultures, Clostridium difficile toxin were also negative. He was discharged home at 6½ months of age. At 1-year follow-up, the infant has not experienced further gastrointestinal problems, and no further investigations regarding PI were made.

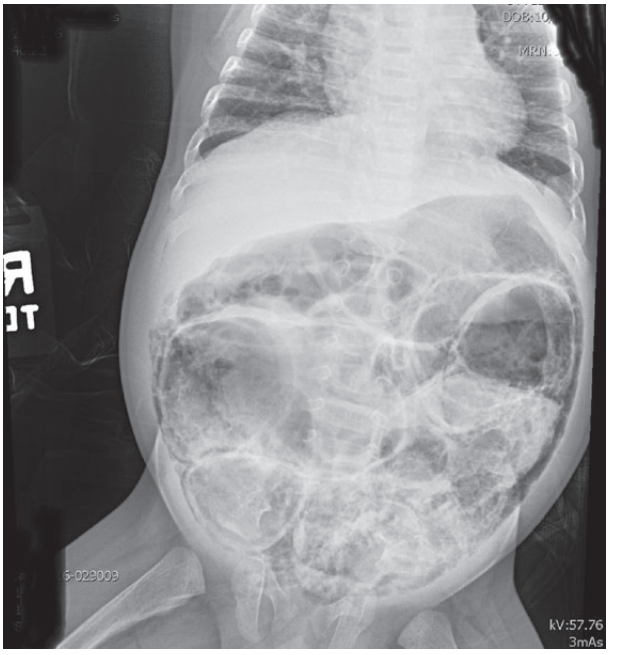

Figure 1. Anteroposterior supine abdominal film showing markedly dilated bowel loops with linear and bubbly pneumatosis intestinalis.

Pneumatosis intestinalis (PI) refers to the presence of gas within the wall of the small or large intestine. PI has been increasingly detected in recent years with the more frequent use of computed tomography for imaging of the intestine.[1] Different terminology used in the literature for describing PI include pneumatosis cystoides intestinalis, intramural gas, pneumatosis coli, pseudolipomatosis, intestinal emphysema, bullous emphysema of the intestine, and lymphopneumatosis.[2,3] PI can be seen in infants (mostly premature), children and adults. The majority of cases in infants are secondary to NEC, a disease associated with a high mortality rate. In premature infants who develop NEC, pneumatosis intestinal is found ~50% of the time. There are numerous conditions in children that may be associated with gas in the bowel wall including obstruction (imperforate anus, meconium ileus, Hirschsprung's disease), carbohydrate intolerance, inflammation, ischemia (intussusception, volvulus, and vascular thrombosis), iatrogenic causes (instrumentation, ventilation, and steroids),

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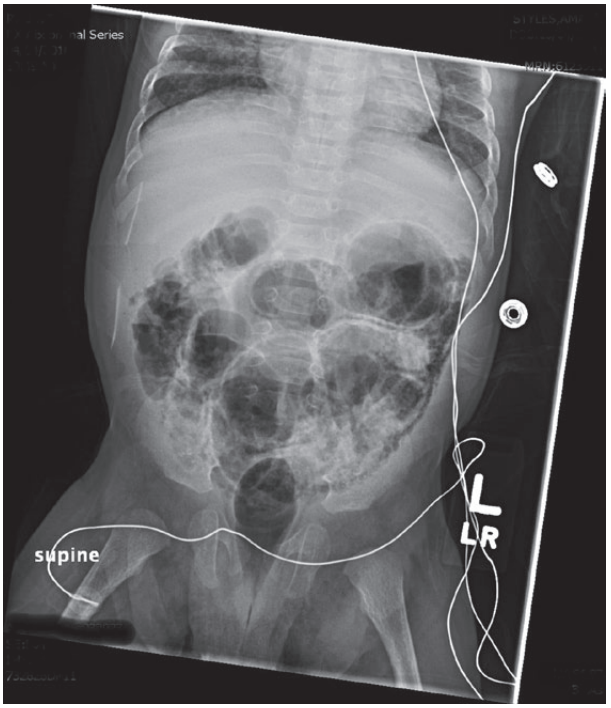

Figure 2. Abdominal x-ray shows dilated bowel loops and intramural air. Both linear and bubbly pneumatosis intestinalis were noted.

and collagen disease (with or without steroid therapy).[4] Different hypotheses have been proposed to explain the pathogenesis of PI, and the presumption is of either mechanical, bacterial, or biochemical causes. For these theories to be considered pertinent, it would be important to associate PI with the underlying conditions, which may in fact be inter-related.[5] In neonates, PI is a well-recognized sign of necrotizing enterocolitis (NEC). The benign form of PI in neonates is very rare and there is inadequate data in the pediatric literature regarding its pathogenesis.

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The Influence of Human Milk on the Preterm Infant Gut Microbiome

Steve Frese, PhD and Tracy Shafizadeh, PhD

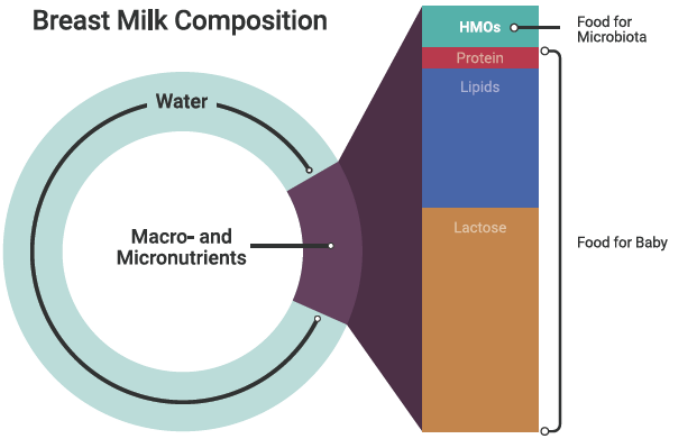
Over 10% of births are considered preterm, or less than 37 weeks gestation, which accounts for over 500,000 births each year in the United States alone.[1] Infants born preterm are challenged with a number of serious issues, including a significantly higher risk for necrotizing enterocolitis (NEC), which affects 2-5% of NICU admissions.[2] In very low birth weight infants with NEC, between 27-63% of these infants require surgical intervention, and overall there is a 20% mortality rate.[3] Although the exact cause of NEC is unknown, the microbiome, or the collection of microorganisms that reside in the intestinal tract, has been implicated.

Recent work has indicated that blooms of gut microbial populations associated with gut dysbiosis play a role in driving TLR4-driven inflammation that precedes NEC.[4] Probiotics are hypothesized to alleviate this dysbiosis, but the reported results in the prevention of NEC are not consistent across probiotic organisms and despite mechanistic work in animal models, there is a lack of successful translation of this work to humans. A recent meta-analysis review found that administration of probiotics to infants in the NICU is safe and effective, and can reduce incidence of NEC and mortality.[3] The authors also found that probiotic administration leads to shorter time to full feeds and may reduce incidence of sepsis. However, in the four years since the meta-analysis review was completed further clinical research has concluded that not all probiotics are created equal in their ability to offset the risk of NEC in premature infants.

Despite the increased attention placed on understanding the role of the gut microbiome in human health, we are only beginning to understand how the infant gut microbiome is first established, how diet shapes this community, and the effect this critical period has on infant health.[5,6] The organisms that comprise the gut microbiome are initially acquired at birth, and that community is shaped over time by diet, gut physiology and environmental exposure. It is now increasingly recognized that this early composition of the newborn gut microbiome plays a major role in lifelong disease risk, as well as the acute risk of infection by opportunistic or overt pathogens. Historically, it has been observed that the gut of breast-fed infants was uniformly colonized by *Bifidobacterium*, the keystone gut symbiont of infants.[7] Early *Bifidobacterium* colonization has potentially profound and beneficial effects for the infant, including a role in important immunological and metabolic programming events in the first few months of life.[6]

Bifidobacterium longum subsp. *infantis* (*B. infantis*) is a particular type of bifidobacteria that is well adapted to the infant gut, in part due to its ability to consume complex carbohydrates found in human milk.[8] Interestingly, infants born in the Global South today are still colonized by this organism, but it is essentially absent in the Global North.[9] Changes in human lifestyles resulting from generations of antibiotic use, cesarean section delivery of infants, increasing sanitation, and dietary changes (ie formula feeding) have contributed to a break in transmission of this organism between generations, and result in the high inter-individual variation in the infant gut that is observed today. In the absence of *B. infantis*, microbial populations including *Streptococcaceae*, *Staphylococcaceae*, *Clostridiaceae*, and *Enterobacteriaceae* are often found in the infant gut. High populations of *Enterobacteriaceae* are increasingly recognized as having a negative impact on long term health and represent gut community dysbiosis.[6,10]

To better understand this unique and symbiotic relationship between human milk and *B. infantis*, new techniques have been used to characterize the components of mammalian milk, and the specific role they play in supporting the newborn gut microbiome[11]. Of particular interest is a diverse set of carbohydrates called human milk oligosaccharides (HMO), that naturally make up about 15% of nutrients in human breast milk. Remarkably, these complex carbohydrates are not digestible by the newborn. Instead, HMO are consumed by bacteria in the infant large intestine, or otherwise excreted in the infant stool.



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The key to this milk-microbe interaction is that not all bacteria can utilize HMO equally. HMO are preferentially consumed by some bacteria, such as *B. infantis*, which can convert these carbohydrates to short chain fatty acids in the infant intestine. In the scientific literature, intestinal short chain fatty acids have been shown to lower intestinal pH, improve gut barrier function and serve as energy signaling molecules during growth and development. This process allows for maximum nutrient utilization from milk and a symbiotic relationship between microbe and host. However, if beneficial bacteria are not present, other potentially harmful bacteria can partially utilize these milk oligosaccharides for growth. An infant gut microbiome colonized by *B. infantis* and fed by human breast milk will flourish and minimize the growth of pathogenic bacteria. Recently, *B. infantis* supplementation has been shown to be efficacious significantly increasing the levels of intestinal *Bifidobacterium* in term, breastfed infants.[12]

While considerable work is yet to be done to validate the efficacy of probiotics in reducing risk of disease, the data indicate that there must be a thoughtful rationale to choosing the appropriate beneficial bacteria, paired with the appropriate food source, for maximum benefit to the host. As microbiome research continues to mature, a specific focus on establishing, or restoring, the newborn gut microbiome may be key to long term health in both premature and term infants alike.

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Parent's Perspective of Weekly Interdisciplinary Rounds in Neonatal Intensive Care Unit

Shabih Manzar, MD, FAAP and Liaqat Hayat Khan, MD

Parent's involvement in the care of sick neonates has shown to affect their behavior and enhance understanding and satisfaction.[1-4] The concept of family centered care has gained interest in recent years and interventional paradigm is changing. One example is the evolution of single-room neonatal intensive care unit (NICU) model.

We are a community hospital and have an old fashioned bay area model of NICU. As a part of hospital policy and in compliance with Joint commission, every Wednesday afternoon we conduct interdisciplinary round with parents. The team constitutes of attending neonatologist, charge nurse, NICU manager, clinical pharmacist, social worker, case manager, chaplain, speech and occupation therapist. Parents sign up for the meeting ahead of time. The parents meet with the team on individual basis and address their questions and concern.

The Centers for Medicare & Medicaid Services (CMS) changed the way hospitals interact with patients when it implemented a pay-for-performance (P4P) system.[5] Under this system, a financial reward or penalty is based in part on measures of patient experience. There is thus a constant need to evaluate and analyze patient satisfaction. In NICU, parents are surrogates and their satisfaction is an important part in day to day care and ultimately reimbursement.

To follow up and look at the parent's perspective of the interdisciplinary meeting, we developed a questionnaire (Appendix A).

A preliminary review of eleven responses is very encouraging. Most parents show high satisfaction and wrote nice comments (Table 1). It was interesting to note the parent's response to question 2 and 7. Looking at question 2, 72% of the parents preferred one-to-one talk with MD, RN or NNP instead of a group discussion. While in response to question 7, 54% wanted no extended family present in the meeting. These responses points out to the fact that parents preferred privacy.

We concluded that as health care providers a continuous struggle should be carried out to provide better care and improve patient satisfaction. It will be interesting to collect more data and analyze it. We continue to collect more responses and will submit a more detailed version following this preliminary report.

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Table 1. Summary of responses

Question number	Response	Comments
1	11/11 agreed	Staff have been amazing. I am more satisfied with the care of my son and I have received.
2	8/11 disagreed 2 agreed 1 can't answer	This is an excellent opportunity for parents. I am extremely thankful for all of you.
3	11/11 agreed	The care team has been wonderful and has always kept us appraised. They are fantastic.
4	11/11 agreed	
5	11/11 agreed	
6	9/11 agreed 2 can't answer	
7	6 agreed 4 disagreed 1 can't answer	
8	11/11 agreed	
9	11/11 agreed	We, the parents, appreciate all the NICU staff for their hard work and dedication in caring of our baby.

Appendix A

Interdisciplinary Meeting Questionnaire *Name:*

1. I was told/aware about the meeting in advance (at admit or the following days):
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
2. I felt intimidated with so many people in the room:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
3. I would prefer to talk on one-to-one rather than in this group format:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
4. It gave me good opportunity to discuss all my concerns in one session:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
5. The meeting was very informal:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
6. The meeting/participants were able answered most of my questions/ queries/concerns:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
7. The meeting was two way communication:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
8. I would like my extended family (parents or in laws) to be present with me in the meeting:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
9. I feel much comfortable and know more about my baby after the meeting:
   - ☐ Agree
   - ☐ Disagree
   - ☐ Can't answer
10. I would recommend the other NICU parents to attend this meeting:
    - ☐ Agree
    - ☐ Disagree
    - ☐ Can't answer

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Should Urine Culture Be Obtained as a Part of Septic Workup In Hospitalized Preterm Infants?

Shabih Manzar, MD, FAAP and Liaqat Hayat Khan, MD

Prevalence, clinical features and associated factors of urinary tract infection (UTI) differ in preterm infant as compared to term infants. For example, mere presence of peripheral venous catheter has been reported to be significantly associated with UTI in hospitalized preterm infants.[1]

It has been shown that obtaining urine culture in early onset sepsis is unwarranted in hospitalized preterm infants.[2] However, obtaining urine culture in late onset sepsis has been advocated in an earlier study.[3] Interestingly, in a recent study, it was observed that UTI in preterm infants is not associated with urinary tract abnormalities or anomalies.[4] These findings raise the question on the validity of obtaining urine culture as a part of septic work up in hospitalized preterm infant.

To answers these questions we reviewed all positive urine cultures in our community hospital NICU in the last 6 months. There were eight preterm infants with positive urine cultures, details are depicted in Table 1.

All urine cultures were catheterized specimen obtained for non-specific signs of suspected sepsis. The decision of treatment was based on spontaneous improvement in clinical signs, colony count, presence of multiple organisms and presence of yeast diaper rash in one case.

Table 1. Summary of positive urine cultures

Case no.	Organism (s)	Treatment	Follow up culture	Imaging study	Outcome
1	*Group B streptococcus*	Yes	Negative	normal	normal
2	*Escherichia coli* < 100,000 *Enterococcus faecalis* < 10,000	Yes	Colony count decreased	Not done	normal
3	*Staphylococcus epidermidis* *Enterococcus faecalis*	No	Negative	Not done	normal
4	*Escherichia coli* < 10,000	No	Negative	Not done	normal
5	*Staphylococcus epidermidis* > 100,000 *Candida albicans* <100,000	No	Colony count decreased	normal	normal
6	*Klebseilla pnemoniae* > 100,000 *Staphylococcus aureus* *Streptococcus agalactiae*	Yes	Negative	normal	normal
7	*Enterococcus faecalis* < 10,000	No	Mixed flora	No	normal
8	*Enterobacter cloacae* *Enterococcus faecalis*	No	Not done	No	normal

Colony count = organism/ml

Conclusions

The true incidence of UTI in hospitalized preterm infant remains obscure. Obtaining a urine culture as a part of septic work up for non-specific signs and symptom should be viewed critically. More data is needed to confirm the need for this practice and to find the number to treat ratio. The procedure is not only invasive but is utilizes hospital resources that has cost implications. Also unnecessary treatment with broad spectrum antibiotics could be avoided, impacting the resistance pattern on the organism in the NICU environment.

We suggest a judicious clinical observation and conservative approach with limited use of urine culture in hospitalized preterm infants with further studies to follow.

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Benefits of Water Births Still Unclear

B Petrikovsky, MD, PhD

Underwater delivery is a subject of continuing interest and controversy.[1-9] Birthing pools are found in increasing numbers in North America, Western Europe, Australia, and South Africa. Indeed, according to newspaper reports, there were 20,000 yearly underwater deliveries in Great Britain alone.[1]

The prevalence of this practice in the United States is unknown because such data are not collected as part of vital statistics. A 2001 survey found that at least 143 US birthing centers offered immersion in water during labor or delivery, or both.[2]

A 2006 joint statement from the Royal College of Obstetricians and Gynecologists and the Royal College of Midwives, supported immersion in water during labor for healthy women with uncomplicated pregnancies.[3]

A 2005 commentary by the Committee on Fetus and Newborn of the American Academy of Pediatrics did not endorse underwater births.[4] ACOG committee's opinion on water-births put forward major limitations on studies on the topic. Most, if not all, literature that recommend underwater births are retrospective reviews of a single center experience, observational studies using historical controls, or personal opinions and testimonials.[1,5-7] A typical evidence in favor of water birth appears as follows (May 11, 2017, Fox News). "It's soothing! It's natural! It's…just like giving birth in your childhood kiddie pool? Fans say that water birthing offers a relaxing environment and natural pain relief without anesthesia. For Boston's KR, 28, the decision seemed logical. Her mother-in-law had her children at home, and her husband had witnessed three of his siblings being born. Her midwife advised her to get out of the tub from time to time, because "sometimes if you're in the water too long, your labor can stall. My midwife told me, 'There are situations, and there are emergencies. If something goes wrong, we'd get you to the hospital." Since she was young and healthy, she felt confident, and she hopes to do it again with future kids. All told, the procedure cost $4,000 (the midwife wasn't covered by insurance), plus $70 for the tub. It was money well spent, she says. But next time, she'll buy a tub liner." Additionally, there are no basic science studies in animals or humans to confirm the physiological mechanisms proposed to underlie the reported benefits of underwater births.[8]

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Presumed advantages

Proponents of underwater immersion during labor and delivery argue that there are a variety of benefits:

- Decrease in perinatal pain
- A greater sense of well-being
- Decreased rate of perineal trauma

It can also potentially benefit the newborn infant for a "gentler" transition from the in-utero to ex-utero environment.[9-11]

Labor pain

Although most articles contain some data on the effect of underwater birth on labor pain, only Cammu et al.[12] conducted a prospective, randomized trial to address this issue. A visual analogue scale and post-delivery questionnaire were used to assess pain during labor. The authors concluded that bathing provided no pain relief according to the visual analogue scale, and the need for epidural anesthesia was the same in both groups.

Maternal birth trauma

While many authors reported the effect of underwater birthing on maternal trauma, most restrict their observations to case reports.[12-14] Some claim less blood loss in cases of underwater delivery, but provide no documentation.[13] Most authors do not advocate episiotomy in patients who opt for underwater birthing.[14] In Odent's study, no episiotomies were performed in the group of 100 patients who delivered underwater, resulting in a 29% incidence of first-degree vaginal tearing.[14]

Fetal/Neonatal effects

Numerous claims have been made regarding the potential benefit and/or harm of underwater delivery for the developing neonate. Although individual retrospective analyses and case series argue in support of one or more of the benefits listed previously, among RCTs studying immersion in the first stage of labor that were included in the 2009 Cochrane systematic review, results were inconsistent at best.[15] Among the two trials that reported outcomes from immersion in the second stage of labor included in this systematic review, the only difference in maternal outcomes from immersion during the second stage was an improvement in satisfaction among those allocated to immersion in one trial. None of the individual trials or the Cochrane systematic review has reported any benefit to the newborn infant from maternal immersion during labor or delivery.[15]

Complications from immersion during labor and delivery

Among this list of complications, given its potential seriousness, the possibility of a neonate aspirating water during birth while immersed, has been the focus of concern. Because the denominators are not uniformly reported, the exact incidence of complications is difficult to assess. Some of the reported concerns include maternal and neonatal infections, difficulties in neonatal thermoregulation; umbilical cord avulsion and umbilical cord rupture, which leads to serious hemorrhage, respiratory distress, and hyponatremia that results from tub-water aspiration (drowning or near drowning), and seizures and perinatal asphyxia.[17,18]

Conclusions

There is no evidence that immersion in water during the first stage of labor improves perinatal outcomes. The safety and efficacy of immersion in water during the second stage of labor have not been established, and water immersion during the second stage of labor has not been associated with documented maternal or fetal benefit. Given these facts, facilities that offer immersion in the first stage of labor need to establish rigorous protocols for candidate selection, maintenance, and cleaning of tubs and immersion pools, infection control procedures, monitoring mothers and fetuses at appropriate intervals while immersed, and immediately and safely moving mothers out of the tubs if maternal or fetal concerns develop.[8]

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Forceps Deliveries — An update

B Petrikovsky, MD, PhD

The Chamberlain family kept the invention of forceps a secret, which was guarded for almost a century.[1] By the mid-1700s forceps were rediscovered and introduced into obstetrical practice, and have since been used throughout the world. In recent decades, the use of forceps has steadily declined, while cesarean delivery rates have increased. Zahniser et al.[2] analyzed data from the National Hospital Discharge Survey to examine these trends in the United States from 1980 to 1987. The cesarean delivery rate increased by 48% while the rate of forceps procedures declined by 43%. As forceps delivery skills are not widely taught to resident obstetricians, ever fewer numbers of attending obstetricians are able to use and teach the use of forceps to the next generation of residents.[3]

Dildy et al.[4] in a current commentary on forceps also states that the use of obstetric forceps is approaching this tipping point. Many, but not all, neonates previously delivered with forceps can now be safely delivered with a vacuum or by cesarean section.

The same authors presented a classic indication for the forceps delivery: a prolong deceleration at +2 station in a mother with poor pushing efforts in which vacuum may be ineffective and cesarean delivery will take too long. In such circumstances, a clinician skilled in forceps delivery may be the best hope for avoiding fetal neurological injury.

Key point

Classic obstetrical forceps and vacuum extraction have different indications. Forceps are irreplaceable in cases of fetal distress in a mother who is unable or unwilling to push, provided the condition for the procedure (station, dilation, availability of anesthesia, etc.) are fully met.

Residents training

Current requirements for residency training in obstetrics specify that 15 total operative vaginal deliveries be performed; in most cases these will consist exclusively or primarily of vacuum deliveries.[4]

Key point

How dangerous obstetrical forceps really are? Clearly the main reason for declining forceps is fear of litigation. Medico — legal aspects of forceps deliveries deserve a separate editorial and is beyond the scope of this manuscript.

Dr Petrikovsky is a Senior ACOG Fellow, Professor of Obstetrics & Gynecology & an Editorial Board Member. The author would like to express his gratitude to Greg Bitterman, Esq. for proofreading the manuscript.

Figure 1. Soft forceps (used with permission).

Maternal and neonatal effects of forceps

Johnson, et al.[5] conducted a medical record review of 200 forceps deliveries paying special attention to maternal and neonatal effects. Multivariable logistic regression analysis showed that forceps use was associated with an increase in perineal and vaginal tears, an increase in marks and bruising on the baby's head and face, and a decrease in cephalohematomas compared with the vacuum deliveries.

It appears that the risk of maternal and fetal trauma and, chiefly, the fear of law suits, have contributed to a significant decline in rates of forceps-assisted deliveries and an increase in rates of cesarean sections, especially in the United States. Our experience with gas-sterilized forceps blades covered with a soft rubber coating — the "soft" forceps — may remedy some of these problems.

Authors proposal and experience

To create the soft forceps the blade portion of the forceps was dipped 2 or 3 times for 5 seconds in a multipurpose rubber-coating dip (Performix; Circles Pines, Minn) to provide a soft coating. This rubber cover added 1.5 mm to the thickness of each

Figure 2. Author applying obstetrical forceps (late 70's).

blade (Fig. 1). The forceps were gas-sterilized after being coated, and the coating was removed and replaced after each use. Coating and sterilizing procedures were then repeated for future use. We compared regular forceps outcomes (Group 1) with the ones using soft forceps (group 2). The rate of severe facial markings and abrasions (defined as requiring repair by suturing or placement of adhesive strips) was 4.1% in group 1 compared with 1.9% in group 2 ($P<0.05$). The rate of minimal markings was 61% in group 1 compared with 34% in group 2. There was no incidence of sulcal tearing in either group.

Does experience matter?

A prior wisdom as with any other operative procedure, the more cases done by the operator and/or institution, the better the outcome. Is this true for forceps?

Miller, et al.[6] analyzed the association between the forceps volume and adverse maternal and neonatal outcomes. The experiences of 118 attending physicians (2,369 forceps deliveries) were included. After controlling for patient factors, neither attending forceps volume nor physician years in practice was associated with the number of severe perineal lacerations or composite neonatal injury.

Final comments

Operative vaginal delivery rates across the Unites States and Europe have declined.[8] This decrease has already trickled down to affect trainees; many graduating obstetrics residents do not feel comfortable independently performing a forceps-assisted delivery.[9] According to the Accreditation Council for Graduate Medical Education, the median number of forceps deliveries performed before graduation was six.[10] With dwindling procedure numbers, the question arises of how this diminishing volume affect patient outcomes.

Solution

The solutions are proposed by Dildy et al.[6] is to develop a high-fidelity simulation model for forceps deliveries in which both the size and position of the fetal head, the size and shape of the maternal pelvis, and the volume of maternal soft tissue can be manipulated. Such a model would provide a life-like training experience with a wide variety of clinical scenarios in which both technical skill and judgment could be taught and practiced. Similar simulation modes are successfully used to train doctors to respond to shoulder dystocia. The second solution also proposed by Dildy et al.[6] and unfortunately more likely to take place is to cease teaching and ultimately the performance of forceps deliveries.

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Monosomy 13 Syndrome: A Case Report and Review of Literature

Tarik Zahouani, Maria Monica Ossa, Claudia Lopez, Arkar Ye Hlaing, Anyelina De La Cruz, Upma Suneja, Clarissa Reynoso, Virginia Kaldas, Sergey Prokhorov and Benamanahalli Rajegowda

Introduction

Monosomy 13 syndrome is a rare chromosomal disorder where a portion of the long arm of chromosome 13 is partially or totally deleted.[1] It has a wide range of severity depending on the extent and location of the deletion.[2] It is characterized by mental and growth retardation, craniofacial dysmorphism, hand and foot anomalies, and congenital malformations affecting the brain, heart and kidneys.[1,2] We present a case of a neonate with multiple congenital anomalies diagnosed with monosomy 13 syndrome.

Case presentation

We performed an assessment of a newborn girl born at 34 weeks' gestational age with severe intrauterine growth restriction (birth weight of 1335 grams, head circumference 26 cm, chest circumference 24.5 cm, abdominal circumference 24 cm, and length 39 cm). The Apgar scores were 4, 6 and 7 at one, five, and ten minutes of life, respectively. The infant was born to a 15 year-old mother who had here prenatal care at an outreach healthcare clinic. She admitted to prior marijuana use but none during the current pregnancy with documented negative urine toxicology screen. Her quad screen was negative for any chromosomal anomalies. A fetal ultrasound performed at 20 weeks' gestational age was concerning for a suspected facial anomaly with possible cleft lip and palate and she was referred to our center for further work up. She had a repeat fetal ultrasound performed at our center at 23 weeks' gestational age that confirmed unilateral cleft lip and palate with normal amniotic fluid. She was counseled by maternal-fetal medicine and neonatology teams. Mother refused further prenatal testing and decided to continue the pregnancy. The family history was negative for congenital anomalies.

At birth, the baby girl's physical examination was remarkable for a small for gestational age infant who had multiple congenital malformations. She had orofacial anomalies with hypertelorism, low set ears and a unilateral left cleft lip and palate (Figure 1), widely spaced nipples, musculoskeletal anomalies with syndactyly of 3rd and 4th toes bilaterally with overlapping toes, and cranial anomalies with microcephaly and colpocephayly. The infant was admitted in the neonatal intensive care unit (NICU) for 60 days under the care of a multidisciplinary team including neonatologist, neurologist, gastroenterologist, ophthalmologist and clinical geneticist. She required oxygen therapy for 13 days, initially via nasal continuous positive airway pressure (nCPAP) during the first day of life then by face mask for 12 days with a successful transition to room air. A chest X-ray showed abnormalities of the chest wall including a bifid rib and an anomalous T4 and T9 vertebrae. The infant remained hemodynamically stable and the echocardiogram showed a patent foramen ovale and a peripheral pulmonary stenosis. The infant received total parenteral nutrition for 19 days and due to persistent feeding difficulties secondary to the cleft lip and palate, a gastrostomy tube (GT) was placed in a tertiary care facility and an upper gastrointestinal series performed prior to the GT placement showed a gastroesophageal reflux disease (GERD). Renal ultrasound yielded a normal result. Magnetic

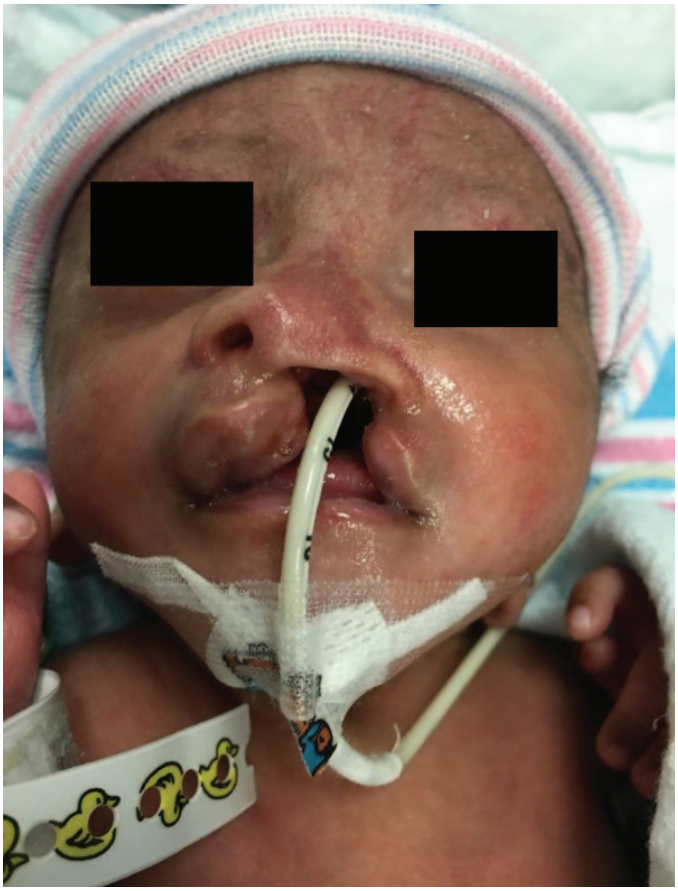

Figure 1. Cleft lip and palate and hypertelorism

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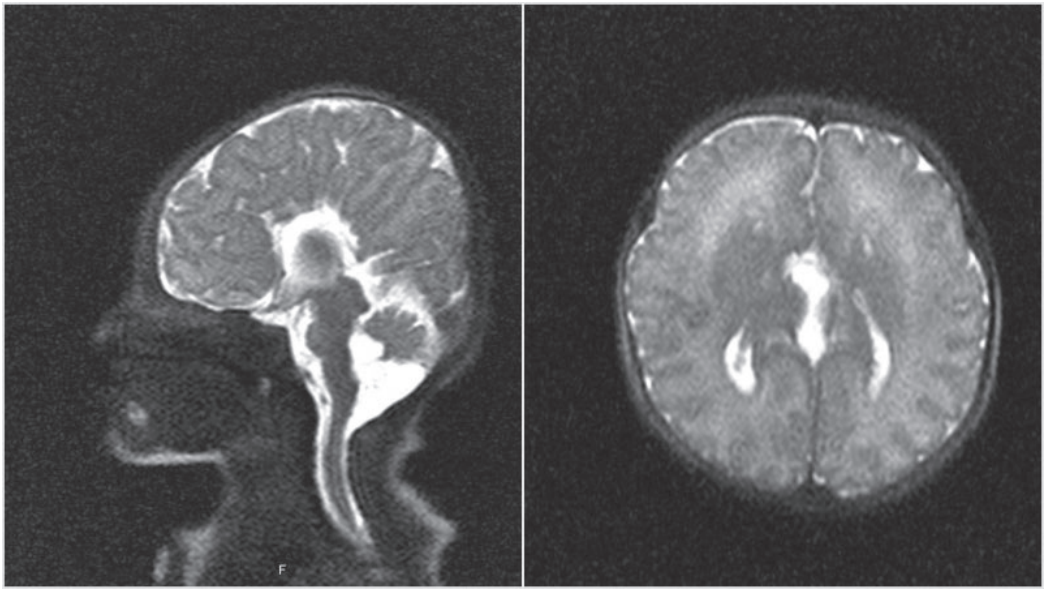

Figure 2. Brain MRI showing colpochephaly, agenesis of the corpus callosum and a posterior fossa cyst

Resonance Imaging (MRI) of the brain showed colpochephaly, agenesis of the corpus callosum and posterior fossa cyst communicating with the fourth ventricle suggesting Dandy-Walker variant (Figure 2). Ophthalmologic evaluation revealed a left microphthalmia and left iris coloboma. The chromosomal banding patterns performed in the tertiary care facility revealed an abnormal female karyotype with loss of chromosome 13 with a very low level mosaicism and the cytogenetic diagnosis of 46, XX,-13,+mar[2]/46,XX[48]. The infant failed the newborn hearing screening test on both ears and also failed the Auditory Brainstem Response (ABR) test. The newborn screening for inborn errors of metabolism was normal. The infant is currently following up with an interdisciplinary team composed of a pediatrician, neurologist, ophthalmologist, gastroenterologist and an endocrinologist. She is feeding through the GT and she has global developmental delay.

Discussion

Monosomy 13 syndrome is a rare chromosomal disorder with more than 125 cases recorded in the medical literature since the syndrome was first described in 1963, with rates slightly higher in females than males.[3] It is caused by the deletion of a segment of the long arm (q) of chromosome 13. The monosomic chromosome is commonly found in all cells but occasionally it is present only in some cells resulting in mosaicism. The deletion usually occurs during the formation of gametes, therefore the condition is not inherited. The syndrome can also be caused by a translocation or inversion in one of the parents and in such cases, there is a risk that siblings will also be affected.[3]

The severity of monosomy 13 syndrome depends on the size and location of the deletion with larger deletion resulting in a more severe form of the syndrome.[3] Three patient groups have been categorized based on the involvement of the band 13q32: proximal deletions with a non-deleted 13q32 band found in patients with mild mental retardation, growth delay and inconstant retinoblastoma; 13q32 band deletion associated with severe congenital malformations; distal deletion without 13q32 deletion in patients with severe mental retardation but without brain malformation or growth delay.[1,2] Additionally, patients with deletion of band 13q14 have a risk of retinoblastoma.[3,4]

Infants with monosomy 13 syndrome usually have a low birth weight, poor sucking and oral motor skills leading to feeding difficulties and failure to thrive.[3] Cleft palate may aggravate the feeding difficulties indicating feeding via GT as in our patient. Craniofacial dysmorphic features include microcephaly, microphthalmia, hypertelorism, large and low set ears, flat nasal bridge, micrognathia and a thick and short neck.[1,3] Iris and choroid coloboma, strabismus, nystagmus, glaucoma and cataracts are other ocular manifestations of the syndrome.[2,3]

Mild-to-moderate mental retardation is a classic feature of the syndrome and, in rare cases, the cognitive development is not affected if the deletion is very small.[3] Frequent brain anomalies include craniosynostosis, holoprosencephaly, agenesis of the corpus callosum, cerebellar hypoplasia, hydrocephalus and Dandy-Walker malformation. Epilepsy can also occur.[1,2,3,5] Our patient had agenesis of the corpus callosum and Dandy-Walker variant.

Cardiac anomalies are common and consist of atrial septal defect, ventricular septal defect, coarctation of the aorta, patent ductus arteriosus and tetralogy of fallot. Renal involvement is rare and include renal agenesis, hydronephrosis and ectopic kidneys.[1,3] Our patient did not have any cardiac or renal anomalies. Gastrointestinal involvement is rare with gastroesophageal reflux, Hirschsprung's disease and imperforate anus being the most common manifestations.[2,3]

Hand and foot abnormalities include hypoplastic thumbs, clinodactyly and syndactyly. Rib and vertebral malformations can also occur as it did in our patient.[3]

Chromosome analysis confirm the diagnosis. Both parents should undergo chromosome analysis to determine if they are carriers of a translocation or inversion.[3]

A comprehensive work up should be performed starting with an echocardiogram to rule out congenital heart defects, a brain MRI to detect brain anomalies. If epilepsy occurs, an electroencephalogram is also indicated. Renal ultrasound to detect any renal anomaly. Regular ophthalmologic examination due to the risk of retinoblastoma.[3,4]

There is no treatment for the monosomy 13 syndrome and the management is mainly a multidisciplinary approach directed towards the control of the different symptoms affecting the patient.[3]

Conclusion

Monosomy 13 syndrome is a rare chromosomal disorder with different degrees of severity depending on the extent of the chromosomal anomaly. It can affect major organs such as the brain, eyes, heart and kidneys and as such, it requires an extensive work up to diagnose and monitor the infant. Management of patient with this condition is challenging and requires a multidisciplinary team approach to assure optimal growth and development.

Acknowledgments

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Neonatal Clinical Trials for Preterm Labor: Takeaways & Insights into Increasing Enrollment of Patient Families

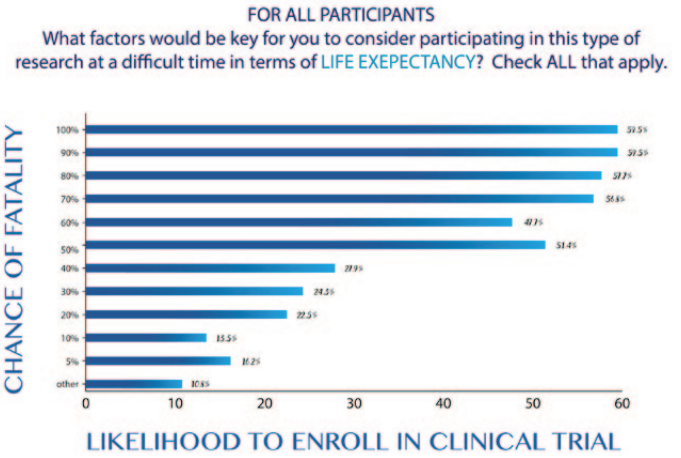
Deb Discenza, Keira Sorrells and Nicole Thiele

In the world of neonatal clinical research, the larger the sample size is, the more likely the study results will garner favorable attention in the medical community for years to come. Yet enrollments can be tricky especially when approaching a patient family in the midst of an extremely difficult situation like preventing preterm labor.

In the spring of 2017 I had the privilege of representing the Preemie Parent Alliance (PPA) in speaking about trial enrollment alongside the European Foundation for the Care of Newborn Infants (EFCNI) at the International Neonatal Consortium (INC). With my portion well received and many attendees urging me to publish the simple findings, it was important to bring PPA and EFCNI into the discussion as together they provide a strong network of parent-led support groups. Detailed below are findings from the research I did as well as an interview with Nicole Thiele, Vice Chair of the Executive Board of EFCNI and Keira Sorrells, President of PPA.

Survey Findings

With a fast turnaround on a short parent survey we received a 112-person (108 mothers, 2 fathers) sampling from every continent. The vast majority of these responders were not approached for a preterm labor trial but they had a clear and consistent view on in what point in life expectancy and in quality of life they would consider enrollment in a clinical trial. The chart regarding life expectancy was ultra clear in how parents viewed research's priority.



Graphic: Parent Voices in Clinical Trial Recruitment presented at the International Neonatal Consortium meeting in Gaithersburg, Maryland, USA on March 28, 2017. Artwork by Cristal Grogan.

The takeaway from the above chart is that the less likely a child were to live, the more likely parents would be positive on a clinical trial enrollment. In fact as one mother commented, "I am a clinical research monitor and a mom of premature triplets. One of my triplets passed away at 4 months in the NICU. I believe more research is needed for preemies." I saw this theme consistently throughout the responses. Parents do want to contribute to research.

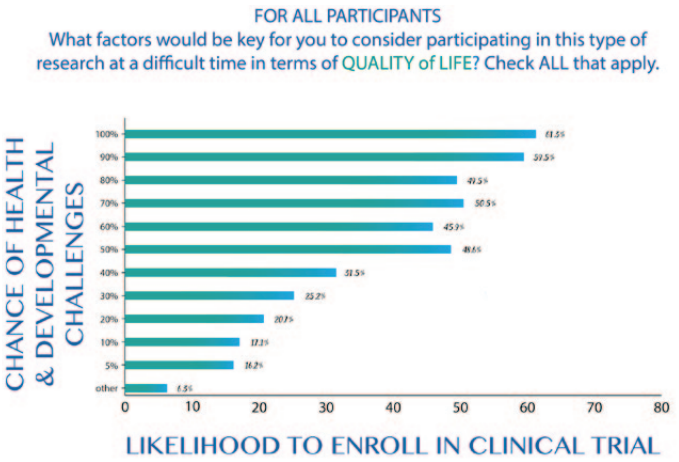
Quality of life was another consideration and that also had clearly defined levels of interest based upon the increasing likelihood of health and developmental challenges as noted in the next chart.

As with life expectancy and quality of life, parents were direct in stating that they wanted clarity into the effects of the study. As one mother put it, "I would like to know what are the possible negative side effects or outcomes for both baby and mother… also pregnancy weeks [added] would make a huge difference." Again this theme rang true throughout each of the many comments received.

Suggestions on Increasing Enrollment Numbers

How do we, then, get those sample sizes higher? Believe it or not, the answers are pretty straightforward:

Deb Discenza is the proud mother of Becky who was born at 30 weeks and is now almost 14 years old. Deb is the head of PreemieWorld LLC (www.PreemieWorld.com), co-author of The Preemie Parent's Survival Guide to the NICU, and the founder and leader of the 37,000+ member Inspire Preemie Community (http://preemie.inspire.com). Keira Sorrells is the mother of triplet daughters, Avery, Lily, and Zoe, who were born at 25 weeks 5 days. Avery and Lily spent 120 days in the NICU and are now 10 years old. Zoe spent 291 days in the NICU and unfortunately died at 14 months old. Keira is the Founder & President of the Preemie Parent Alliance (www.preemieparentalliance.org) as well as the Co-Founder of The Zoe Rose Memorial Foundation (www.zoerose.org). Because of her family experiencing the consequences of an extremely preterm birth at the beginning of modern neonatology nearly 50 years ago, Nicole Thiele became personally committed to improving neonatal care, with a focus on the question about quality of life, parental involvement and sensitive interaction with siblings. Nicole is Vice Chair of the Executive Board of the European Foundation for the Care of Newborn Infants (EFCNI) (www.efcni.org). One of EFCNI's current milestones is the development of European Standards of Care for Newborn Health (www.newborn-health-standards.org).



Graphic: Parent Voices in Clinical Trial Recruitment presented at the International Neonatal Consortium meeting in Gaithersburg, Maryland, USA on March 28, 2017. Artwork by Cristal Grogan.

- **They do want to help.** Understand that they are terrified and are not at their best. But they do want to prevent this happening to another baby, another family. And they do realize that everything that is helping them right now came out of research and the sacrifice of families before them.

- **Clarity Makes A Huge Difference.** Families need clarity in this situation more than ever because again they are stressed and likely sleep-deprived. When presenting them with a research opportunity present everything verbally but also in written form. Do the same in presenting pros and cons as well and do so in a clear format. It is essential that the presentation of this information both verbally and written be done so in a calm and reassuring manner. And at the end of the day, realize that these parents have lost all control and that with you and your request they can finally control something. So presenting it in a manner that not only gives them clarity but also control will up the chance of a trial enrollment.

- **Timing is Everything.** When approaching a parent, make sure the parent is fully aware of what you are asking. Talking to a parent that is under the influence of potent drugs to stop preterm labor will have a hard time grasping the information. If the timing isn't right, leave literature to be read at another point and plan to come back at another time. And it is key to know that in the parent's mind, the ultimate emergency is not the trial request so pressure will not improve your chances of an enrollment. Being calm, empathetic, and being respectful of whatever they choose to do is the ultimate way to approach timing of that request.

- **Building Trust is Key.** Above all, building trust is the ultimate deciding factor in considering a trial. Talking to a family with honesty, empathy and a calm demeanor in a non-clinical manner will make a huge difference. The families are in a clinical, sterile environment and having someone with real humanity connect with them can make a big difference. And when you are talking to that family, see if a nurse or doctor can be on hand to help assist with explanations and answering questions.

Hearing From Organizations That Help Lead Parent-Led Support Groups

Your group is a gateway to a large number of parent-led support groups all over. What do you want researchers to know about your group and its work?

Nicole Thiele: EFCNI (www.EFCNI.org) is the first pan-European organisation and network to represent the interests of preterm and newborn infants and their families. EFCNI is gathering together parents, healthcare experts from different disciplines, and scientists with the common goal of improving long-term health of preterm and newborn children.

We foster research in many ways — from political activities and calls for more research in maternal and newborn or child health over representing the patient's voice in general research questions to being actively involved in individual projects as patient advocate/advisor. EFCNI has extensive experience in

- acting as co-organiser and part of the scientific board of scientific congresses
- developing (and implementing) international guidelines,
- advising on general research questions and priorities,
- advising and representing the patient voice and opinion in individual projects (public and/or private partnership trials)
- serving as work package leader for publicly funded projects
- representing the patient voice in different research project committees or boards, eg steering committees
- providing other patient groups with insights and knowledge about research.

In order to work in a professional way and with the quality we aim for, our contribution, the same as that of other parent organizations, needs to be fairly funded.

Keira Sorrells: The Preemie Parent Alliance (www.preemieparentalliance.org) is a network of 35 NICU parent support organizations from across the United States, and one in Sao Paulo, Brazil, each of which was founded by and/or is run by a parent who had a NICU experience. These Parent Leaders, or "Experts by Experience", are deeply committed to working alongside researchers, clinicians, industry, regulatory, and government to ensure this population of babies and their families have access to the best possible support, education, and resources. They are "in the trenches" day in and day out with NICU families and have a depth of understanding of the unique needs of the parents that only a fellow NICU parent can have.

Your organization and its members see researchers in your space a lot asking for parents to give input and suggestions. What do you feel are the biggest misconceptions of building trust with families when talking about trial enrollment?

Nicole Thiele: In the NICU parents and their babies are first and foremost individuals and families in a very extreme, and for them, frightening life situation. What they need above all is the understanding for their particular situation, for their concerns, worries, and hopes. Researchers should be aware that the last thing these parents have in their mind in these moments is the topic research or yet more unfamiliar people approaching them with even more overwhelming information or requests. The researcher is entering the fragile world of these families, and therefore it is his/her responsibility to work on an atmosphere of trust. Empathy, honesty, true interest in the individual situation

and partnership require the researcher's time and patience. Building trust means respecting the person sitting on the other side- it is not about "interesting research questions", getting quick data or numbers or about experts advising parents. Researchers often feel they are approaching parents in their language and at their level, but we sometimes observe that this is not the same perception the parents have — the language might still be too technical, too frightening, expectations on motives, actions or reactions may differ. Constant exchange and discussions with parent advocates, eg parent associations, may help researchers to better understand, align and communicate with parents.

Keira Sorrells: Building trust in any relationship takes time. Due to the nature of research with this particular patient population, the luxury of time is not often available. Add to that the intense stress and trauma the parents are dealing with, and you have tremendous hurdles to overcome. I think there may be a misconception that parents won't want to participate in research and for many families, as we've seen in the survey, they are willing to participate, even if the particular treatment being studied may not help their own child. I wholeheartedly agree with what Nicole noted, that parent support organizations or peer-to-peer support leaders can certainly play an important role in improving parent/researcher communication.

Your outreach could be very useful to researchers, yes? In what ways?

Nicole Thiele: Organizations such as EFCNI can serve as bridge between theory and practice in many ways and in form of a "two-way-road":

We reach out to large patient networks in different countries which can be beneficial to find out more about the patient's "unmet needs" or getting feedback to specific research questions. We help searching for study participants or patient advisors with disease specific experiences. On our different communication channels, we share information about the importance of research and motivate parents to participate in studies. Additionally, we share study results with the patient networks and translate the technical research language into lay language so that research becomes understandable for more persons.

When an umbrella organization such as EFCNI decides to support a trial, this might signal study participants the importance of the trial topic to the parental community, make them feel more comfortable with the project and may thus help building trust. Also, study participants may wish to turn to a peer organization for general questions or the feeling of receiving support.

Besides, EFCNI's extensive networking with professionals and their societies, with politicians and key opinion leaders ensures that study results can be spread broadly among the target groups.

Keira Sorrells: Our Parent Leaders represent hundreds of thousands of parents of NICU babies and their personal experiences coupled with their working expertise positions them in a way to truly understand the needs of NICU families. They have spent an inordinate amount of time delving into the critical emotional, psychological, and practical needs of these families. This experience of having a critically ill baby in the hospital is one that can not truly be prepared for and it colors the way a family views the world around them, especially in the beginning. Chaos and fear reign supreme and no clinical professional (except of course those who are also NICU parents) can attain this depth or degree of knowledge quite like a parent support leader who has been there themselves and now works daily with others there now. "Experts by Experience" can be and should be integral in all facets of the research process. They can be instrumental in assisting to develop protocols relating to study design, how to approach parents to participate in studies, etc.

Is there anything else related to the survey, to this topic that you would like for researchers to know when connecting with families for trial enrollment?

Nicole Thiele: Parents have the right to receive full, transparent information about the trial—about opportunities but also possible risks involved, their options and how the care for their child would look like. Parents also need to be aware on how the data generated will be used, who will have access to it and who is funding the project.

Many families would wish to stay informed on the outcome of a trial their child is participating in. Milestones, easy-to-understand project information or project results should be available on a specific website and ideally in the native language. Normally, parents cannot easily read scientific texts or publications. Ideally, a lay version should automatically be available.

Keira Sorrells: The survey quite clearly showed that parents ARE in fact interested in participating in research studies and the manner in which parents are approached and educated about the study is a critical factor. This can not be overlooked. An abundance of literature shows how important a compassionate, gentle attitude is when working with these families. It is also extremely important that researchers take time to learn about and understand the trauma these families are experiencing. Only when one takes off the lenses from which they view the world around them, and replaces them with the lenses of a NICU parent can one begin to empathize in a meaningful way. We often ask clinicians to imagine that a family's baby is their own child, and we ask them to think about what they would want to know, how they would want to spoken to and what would given them a sense of comfort and trust.

To learn more:

- European Foundation for the Care of Newborn Infants (EFCNI): www.efcni.org
- Preemie Parent Alliance (PPA): www.PreemieParentAlliance.org

Screening for Glucose-6-Phosphate Dehydrogenase Deficiency in Neonates: a Comparison Between Cord and Peripheral Blood Samples

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Abstract

Background: The use of cord blood in the neonatal screening for glucose-6-phosphate dehydrogenase (G6PD) deficiency is being done with increasing frequency but has yet to be adequately evaluated against the use of peripheral blood sample which is usually employed for confirmation. We sought to determine the incidence and gender distribution of G6PD deficiency, and compare the results of cord against peripheral blood in identifying G6PD DEFICIENCY neonates using quantitative enzyme activity assay.

Methods: We carried out a retrospective and cross-sectional study employing review of primary hospital data of neonates born in a tertiary care center from January to December 2008.

Results: Among the 8139 neonates with cord blood G6PD assays, an overall incidence of 2% for G6PD deficiency was computed. 79% of these were males and 21% were females with significantly more deficient males ($p < .001$). Gender-specific incidence was 3.06% for males and 0.85% for females. A subgroup analysis comparing cord and peripheral blood samples (n = 1253) showed a significantly higher mean G6PD value for peripheral than cord blood (15.12 ± 4.52 U/g and 14.52 ± 4.43 U/g, respectively, $p = 0.0008$). However, the proportion of G6PD deficient neonates did not significantly differ in the two groups ($p = 0.79$). Sensitivity of cord blood in screening for G6PD deficiency, using peripheral G6PD assay as a gold standard was 98.6% with a NPV of 99.5%.

Conclusion: There was no difference between cord and peripheral blood samples in discriminating between G6PD deficient and non-deficient neonates. A significantly higher mean peripheral G6PD assay reinforces the use of cord blood for neonatal screening since it has substantially low false negative results.

Keywords: Glucose-6-phosphate dehydrogenase deficiency, Screening, Neonates, Cord blood

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Background

Despite the wide variability in the assessment methods for glucose-6-phosphate dehydrogenase deficiency, it remains the most prevalent enzyme deficiency in the world. It is estimated that nearly 330 million people may be affected by G6PD deficiency worldwide with a global prevalence of 4.9% [1]. G6PD deficiency in neonates is particularly important because it may have fatal consequences. It could cause severe neonatal jaundice, and if not recognized and managed early, it could lead to kernicterus with permanent neurologic sequelae, if not death [2, 3] In the Middle East, the prevalence estimates of G6PD deficiency is the second highest in the world at 6% (95% CI: 5.7–6.4, $p < 0.001$), the first being Sub Saharan Africa [1, 4]. For males alone, it is estimated to be 7.2% (95% CI: 6.6–7.7, $p < 0.001$). Local studies in Saudi Arabia have shown a wide variability in the region-specific prevalence. Riyadh has a prevalence of 2.0% to 3.8%, Qatif with 30.6%, and AlHasa with 14.7% [3, 5–7]. Most of these studies made use of the cord blood for screening in the neonates. The use of cord blood in the neonatal screening for metabolic diseases including G6PD deficiency is being done with increasing frequency in some centers [6, 8–12]. This method of specimen collection is convenient, easy, and more importantly it spares the neonate of unnecessary pain and stress. The premise is that; coming from the same individual, cord blood should reflect the same glucose-6-phosphate dehydrogenase (G6PD) levels as in the peripheral sample. This may not be entirely accurate; however, considering the tremendous physiologic changes in the newborn period that could affect G6PD activity. In this study, we sought to determine the incidence and gender distribution of G6PD deficiency among neonates using cord blood, and compared cord against peripheral blood in identifying G6PD deficiency in neonates using a quantitative enzyme activity assay.

Methods

We carried out a retrospective, cross-sectional study employing review of primary hospital data of term and near term neonates born (>35 weeks of completed gestation) in a tertiary care center from January to December 2008. King Abdullah International

Table 1 Subgroup analysis of G6PD Quantitative Assay for Neonates with both Cord and Peripheral blood samples

Description		Cord	Peripheral blood	*p*-value
G6PD Quantitative Assay (U/g)				0.0008*
Overall		14.52	15.12	
Mean		4.43	4.52	
Incidence of G6PD % at two cut-off values	5.7 U/g	5.83	5.59	0.796[a]
	8.05 U/g	7.98	7.18	0.450
Mean G6PD Quantitative Assay (U/g Hgb), at 5.7 U/g cut-off	Deficient	1.45 ± 1.16	1.64 ± 1.27	0.3711
		(*n* = 73)	(*n* = 70)	
	Non - Deficient	15.33 ± 3.09	15.92 ± 3.18	<0.001*
		(*n* = 1180)	(*n* = 1183)	
Mean G6PD Quantitative Assay (U/g Hgb), at 8.05 U/g cut-off	Deficient	2.95 ± 2.68	2.85 ± 2.56	0.8001
		(*n* = 100)	(*n* = 90)	
	Non - Deficient	15.52 ± 2.85	16.07 ± 3.01	<0.001*
		(*n* = 1153)	(*n* = 1163)	

*Level of significance: $p = < 0.05$
The cord results are statistically lower than the peripheral results *($p = 0.0008$)*. This also holds true among the non-deficient subgroup of patients (*$p < 0.001$*) but NOT among the deficient neonates (*$p = 0.3711$ and $p = 0.8001$ using cut-off of 5.7 and 8.05 U/g Hb respectively*)
[a]Pearson chi^2 test did not reveal any significant difference in the proportion of G6PD deficient neonates between cord and peripheral blood samples

Medical Research Center (KAIMRC), Ministry of National Guard Health Affairs (MNGHA), International Review Board (IRB) has approved this study protocol (RC09/106), and all patients were provided with written informed consent through their guardian/parent.

Cord blood is defined as a specimen collected from the umbilical artery at the time of delivery and peripheral blood is the blood obtained from any other site of the body within one week of age. Unless otherwise specified, we define G6PD deficiency as a G6PD quantitative assay by spectrophotometric analysis of ≤5.7 U/g Hb (unit of enzyme activity/g hemoglobin), as per laboratory recommendation.

Cord and venous blood samples were collected from each patient using conventional techniques into Vacutainer (BD Plymouth, PL6 7BP, U.K.) or Microtainer (Becton, Dickinson and Co., Franklin Lakes, NJ 07417, USA) tubes with K2EDTA as anticoagulant at a concentration of 1.8 mg/ml. Samples were accessioned into the Laboratory Information System (LIS), then their hemoglobin (Hb) levels were determined on same time and day as the G6PD analyses using Cell-Dyn Sapphire blood analyzers (Abbot Diagnostics Division, Abbot Park, IL 60064, USA). All samples were stored at 2-8C, batched and analyzed for G6PD enzymatic activity within 4–6 h of collection. Sadly, three infants died before the peripheral blood sample could be obtained for measurement of the G6PD.

One hundred microliters of well-mixed whole blood was pipetted in a test-tube containing 400 µl of a proprietary lysing reagent, mixed well and let stand for 5 min. An aliquot of this was poured into a sample cup using the only G6PD assay kit designed for both newborn and adults, which then was placed in the Udilipse Random Access Analyzer (United Diagnostics Industry, P.O. Box 9466, Dammam 31,413, Kingdom of Saudi Arabia). Once hemolysates were made, analysis was carried out immediately and strictly within an hour.

The principle of the test involves the catalysis of glucose-6-phosphate to 6-phosphogluconate by G6PD and reduction of NADP to NADPH in the following reaction [10] (Glucose-6-phosphate + NADP → 6-Phosphogluconate + NADPH + H^+).

The activity of G6PD was proportional to the rate of production of NADPH which possesses a peak Ultraviolet (UV) light absorption at 340 nm. Results from the Analyzer were automatically transmitted to LIS permitting access to patient's previously estimated blood hemoglobin level, computed and reported results in units/g (of hemoglobin).

In carrying out the study, we collected the names and medical record numbers (MRN) of all newborns with G6PD quantitative assays (cord or peripheral) for the specified time interval (January to December 2008). This was the year that our institution began implementing universal G6PD deficiency neonatal screening. We then selected the neonates with cord G6PD assays and from this pool, the overall incidence and the gender distribution of G6PD deficiency was computed. However, due to the unavailability of G6PD molecular genotyping in our institute DNA analysis was not possible. There is a future plan to include DNA genotyping in a forthcoming study.

From among the newborns with cord blood G6PD assays, we picked out those who also had peripheral samples taken (presumably within one week of age). A subgroup analysis was carried out on this particular subset of patients (n=1, 253) comparing the cord and peripheral G6PD values.

Statistical analysis

Statistical analysis was performed using SPSS v.20 (IBM Corp., Armonk, NY, USA). The data were statistically tested by descriptive statistics. Kolmogrov-Smirnov test was used for normality and found that it was normally distributed. The Quantitative G6PD analysis and correlation between deficient and non-deficient groups for both cord and peripheral blood samples were performed using Student T-test and Pearson chi-square. P-value of <0.05 was considered statistically significant. Data was expressed as mean ± SD unless indicated otherwise (see Tables 1, 2 and 3).

Table 2 Gender distributed Differences in G6PD levels

Gender	Cut-off value	Cord		PB		*P*.value
		Mean	Range	Mean	Range	
Male	5.7 U/g	15.29 ± 3.28	(6–27)	15.78 ± 3.35	(5.8–30.5)	
Female		15.36 ± 2.90	(6–29)	16.02 ± 2.87	(5.7–27.7)	$P < 0.001$*
Male	8.5 U/g	15.45 ± 2.77	(8–27)	16.05 ± 2.7	(8.10–30.5)	
Female		15.57 ± 2.95	(8.10–29)	16.08 ± 3.12	(8.10–27.7)	$P < 0.001$*

*Level of significance: $p = < 0.05$, *Cord* Cord Blood, *PB* Peripheral Blood. Normal G6PD level distributed among different gender between the cord blood and peripheral blood samples

Results

There were a total of 8396 neonates admitted to the hospital between January and December 2008 whose G6PD assays were obtained. Cord blood samples for G6PD activity were actually available from 8139 patients. The remainder either had non-optimal cord specimens (clotted or insufficient) or were born outside our Hospital from whom cord bloods could not be taken.

Among the 8139 with cord blood samples successfully taken, the mean result for G6PD assay was 14.6 with a minimum value of 0.2 and a maximum value of 45.4. As we had a standard deviation of 3.28, 2 standard deviations from the mean (~2.5 percentile) give us a cut-off value of 8.05 (at 95% CI) below which defined a G6PD deficient neonate. It was well known that G6PD deficient variants were grouped into different classes corresponding with disease severity [1, 2].

Using a cut-off value of 8.05 Units/g Hemoglobin (U/g Hb), the overall incidence of G6PD deficiency was 3.13% of which 60% were males and only 40% were females. There was a significantly higher proportion of deficient males ($p < 0.001$) with significantly lower values as well ($p < 0.001$).

Presently however, when we applied using normal adult cut-off value of 5.7 U/g Hb, the overall incidence of G6PD deficiency was 2% of which 79% were males and only 21% were females (Fig. 1). There was a significantly higher proportion of deficient males ($p < 0.001$) with significantly lower values as well ($p < 0.001$).

We ran the analysis looking at gender differences in G6PD levels in both cut-off value of 5.7 and 8.05 U/g Hb for cord and peripheral blood samples and we found that there were equally highly significant differences ($p < 0.001$) in the levels between males and females in both samples (Table 2). Pearson chi-square test revealed a significantly higher proportion between gender groups of affected males ($p = 0.001$) consistent with an X-linked pattern of inheritance (M:F ratio) equal to 1.5–3.7:1 respectively. Among males alone, the computed incidence of the disease was 3.06% while female patients had an incidence of only 0.85% female neonates) with a male to female ratio of 3.7:1. The gender-specific incidence for males reinforces the universal screening practice also applied in our institution since it is within the WHO recommendation; that is, to screen for all neonates in populations with prevalence of ≥3% in males [2].

Table 3 Screening Indices for Cord Blood G6PD Assay at two cut-off values

	Cut-off values	
	5.7 U/g Hb	8.05 U/g Hb
Sensitivity	98.6%[a]	90%
	(CI:91.2, 99.9)	(CI:81.4, 95)
Specificity	99.7%	98.4%
	(CI:99.1%, 99.9%)	(CI:97.4%, 99%)
Positive Predictive Value	94.5%	81%
	(CI:85.8%, 98.2%)	(CI:71.7%, 87.9%)
Negative Predictive Value	99.5%	99.2%
	(CI:99.5%, 100%)	(CI:98.5%, 99.6%)
Accuracy	99.6%	97.77%

CI Confidence intervals

[a]98.6% of all the patients with truly deficient peripheral G6PD assays had deficient cord blood results with cut-off 5.7 U/g Hb once compared to 8.05 U/g Hb

To make a comparison between results obtained using cord blood and peripheral blood samples, we used the subset of patients from whom both samples were taken (Fig. 2, Table 1). A total of 1253 observations were analyzed. The mean result of G6PD activity assay was 14.52 (U/g Hb) for cord and 15.12 (U/g Hb) for peripheral blood samples. There was a significant difference between the mean results of the two samples ($p = 0.0008$). The G6PD activity assay was statistically lower in the cord than in the peripheral blood. This was consistent with the result of the concordance correlation coefficient of Lin with Rho $c = 0.774$ ($p < 0.001$, 95% CI: 0.752, 0.796) which only fell under the poor category (Pearson's $p = 0.78$). However, when we dichotomized our patients into deficient and non-deficient, chi-square analysis revealed statistically comparable proportions of G6PD deficiency between the two samples using both cut-off points ($p = 0.796$ and $p = 0.45$ for cut-off 5.7 and 8.05, respectively). It was interesting to note that if we compared the mean G6PD values of the cord and peripheral blood samples in the deficient patients alone, no statistically relevant results were obtained using t-test ($p = 0.3711$) as opposed to the results obtained for the non-deficient group ($p < 0.001$, Table 1). Validity indices of cord blood G6PD assay, with peripheral blood as the gold standard, was confirmed as shown in Table 3.

Discussion

The prevalence of G6PD deficiency is high in the Saudi Arabian population we think because of high consanguinity and the prevalence of endemic malaria. This justifies our thinking that neonatal screening is important for early diagnosis and identification of G6PD deficiency in infants before hospital discharge.

There are important observations that we have arrived at in this study. First, we note the preponderance of the disease in males which is congruent with an X-linked pattern of inheritance of G6PD deficiency. Moreover, the enzyme assays in the affected males are significantly lower than in the affected females which could explain the more severe nature of the disease in the male population.

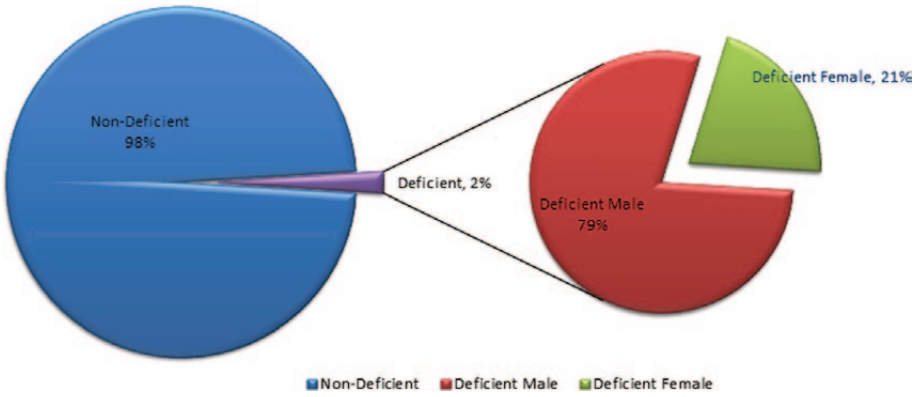

Fig. 1 Incidence and Distribution of G6PD DEFICIENCY among neonates admitted in KAMC, during 2008 (N = 8139).

Second, G6PD activity in the peripheral blood samples appears to be higher than in cord blood especially among non-deficient patients. This could reflect an up-regulation of G6PD activity as a response to oxidative stress in the newborn period by way of enhanced G6PD gene expression (ie transcription) [12–16]. Other possibilities are increased erythropoiesis resulting in normoblastemia, reticulocytosis, or possible other characteristics peculiar to fetal and neonatal erythrocyte metabolism [15, 17]. Cappellini and colleagues cite this as a diagnostic issue among neonates which could lead to false negative results [17]. This even raises doubts as to whether the peripheral neonatal blood is reflective of the true value or it could be a falsely elevated estimate. Therefore, we have used the 5.7 cut-off value as it is much more stringent and is based on the adult values. Using a much higher cut-off value would indeed show more number of G6PD deficient infants some of which could be false positive. Cut-off values used as described in the literature of <2.0 U/g Hb for profound and between 2 U/g to 7 U/g Hb for partial deficiency in the neonate may Indeed be too low.

Among the deficient group of neonates, regardless of the sample, they would have consistently low enzyme assays. We could only speculate that among this group of neonates, the same physiologic changes do not result in increased G6PD activity since the enzyme function is not optimal from the start. These observations reinforce the use of cord blood sample for G6PD deficiency screening among neonates since having a generally lower value than the peripheral blood samples, false negative results are minimized (ie negative predictive value of 99.5%, CI: 99.5%, 100%).

While we observed a statistically substantial discrepancy in the mean of G6PD assay between cord and peripheral blood samples using t-test, a chi-square analysis of the proportion of G6PD deficiency failed to show any meaningful difference. We also noticed that there were different normal G6PD level distributed among gender between cord blood and the peripheral blood samples.

This suggests that in so far as discriminating between deficient and non-deficient patients is concerned; there is no significant difference between the two groups. Again, this underscores the usefulness of cord blood in the universal neonatal G6PD deficiency screening.

Conclusion

In this study, we found no difference between the cord and peripheral blood samples in discriminating between G6PD deficient and non-deficient neonates using quantitative enzyme activity assay. However, peripheral blood appears to have a higher G6PD quantitative assay than the cord blood. This reinforces the practice of using cord blood for neonatal screening since, having a generally lower value, the chances of missing a G6PD deficient neonate will be less (ie substantially low false negative results).

Recommendation

The results of this study are best understood in the light of the following limitations. No randomization was carried out so the recommendations would not be as robust. The subset of patients for whom we analyzed correlation between the cord and peripheral blood for G6PD assay were mostly those for whom jaundice workup was carried out, or those with borderline G6PD values in the cord blood, so that both cord and peripheral G6PD values had to be drawn. This may have imparted a sampling bias in this study.

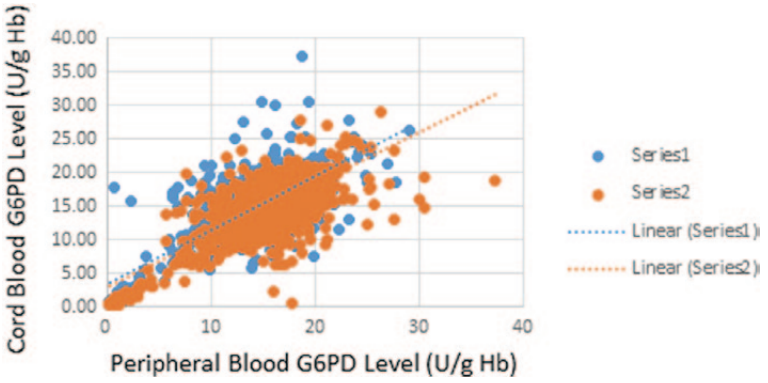

Fig. 2 Concordance of G6PD Quantitative assay between Cord and Peripheral Blood Samples of KAMC neonates

An important question raised in this study which could be worth looking into in the future is establishing the relationship between cord and peripheral neonatal blood with that of older children. Should we be able to prove that the neonatal peripheral blood G6PD assay is indeed an overestimate of the true mature value? It might be plausible to set a higher cut-off point for labeling a newborn as G6PD deficient using a peripheral blood sample?

Abbreviations

G6PD DEFICIENCY: glucose-6-phosphate dehydrogenase deficiency;

G6PD: glucose-6-phosphate dehydrogenase; IRB: International Review Board; KAIMRC: King Abdullah International Medical Research Center; KAMC: King Abdulaziz Medical City; LIS: Laboratory Information System; MNGHA: Ministry of National Guard Health Affairs; MRN: medical record numbers

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Availability of data and materials

The data generated in the current study are available on reasonable request to the corresponding author.

Authors' contributions

SS, BP and KK: drafted the manuscript, and contributed in the preparation of manuscript figures and tables; KT and IA contributed to the gathering and interpretation of data; AS and BB: contributed to the project design; MK, KH and AS conducted the analysis and carried out the interpretation of results; MB: aided in the project design and the overall review, editing and submission of manuscript. All authors read and approved the final manuscript.

Competing Interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The G6PD study was approved by the Institutional Review Board Committee, King Abdullah International Medical Research Center, Ministry of National Guard Health Affair under protocol # RC09/106. A written informed consent was obtained from the parent of each participating neonate.

Consent for publication

Not applicable.

Publisher's Note

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Genome-Resolved Metaproteomic Characterization of Preterm Infant Gut Microbiota Development Reveals Species-Specific Metabolic Shifts and Variabilities During Early Life

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Abstract

Background: Establishment of the human gut microbiota begins at birth. This early-life microbiota development can impact host physiology during infancy and even across an entire life span. However, the functional stability and population structure of the gut microbiota during initial colonization remain poorly understood. Metaproteomics is an emerging technology for the large-scale characterization of metabolic functions in complex microbial communities (gut microbiota).

Results: We applied a metagenome-informed metaproteomic approach to study the temporal and inter-individual differences of metabolic functions during microbial colonization of preterm human infants' gut. By analyzing 30 individual fecal samples, we identified up to 12,568 protein groups for each of four infants, including both human and microbial proteins. With genome-resolved matched metagenomics, proteins were confidently identified at the species/strain level. The maximum percentage of the proteome detected for the abundant organisms was ~45%. A time-dependent increase in the relative abundance of microbial versus human proteins suggested increasing microbial colonization during the first few weeks of early life. We observed remarkable variations and temporal shifts in the relative protein abundances of each organism in these preterm gut communities. Given the dissimilarity of the communities, only 81 microbial EggNOG orthologous groups and 57 human proteins were observed across all samples. These conserved microbial proteins were involved in carbohydrate, energy, amino acid and nucleotide metabolism while conserved human proteins were related to immune response and mucosal maturation. We identified seven proteome clusters for the communities and showed infant gut proteome profiles were unstable across time and not individual-specific. Applying a gut-specific metabolic module (GMM) analysis, we found that gut communities varied primarily in the contribution of nutrient (carbohydrates, lipids, and amino acids) utilization and short-chain fatty acid production.

Conclusions: Overall, this study reports species-specific proteome profiles and metabolic functions of human gut microbiota during early colonization. In particular, our work contributes to reveal microbiota-associated shifts and variations in the metabolism of three major nutrient sources and short-chain fatty acid during colonization of preterm infant gut.

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Background

Microbes colonize most internal and external surfaces of the human body and influence many aspects of human physiology. The largest microbial community is found in the human gastrointestinal tract ("gut"), which is composed of up to 56 trillion microbial cells [1], comprising thousands of different species and five million unique genes [2]. Microbes residing in the gut interact with each other and the host; play important roles in host nutrient availability through the production of vitamins, short-chain fatty acids (SCFA), and amino acids; train the immune system to tolerate commensal bacteria; protect against pathogens; and contribute to intestinal epithelium maturation and integrity [3]. Dysbiosis of the gut microbiota has been linked to many diseases, such as Crohn's disease [4–6], diabetes [7, 8], and autoimmune diseases [9]. The establishment of gut microbiota begins during infancy, and emerging evidence suggests that this initial colonization has lifelong effects on human health [10]. A rapidly increasing number of studies have focused on understanding the establishment of the microbiota at birth, or microbiota associations with infant health and disease.

Although originally thought to be born sterile, the presence of microbes in placental and meconium samples has suggested that infants may be colonized by small populations of microbes prior to birth [11]. Regardless, newborn infants are exposed to large numbers of bacteria from the mother and the environment at birth. Typically, the initial colonizers of the gut are facultative anaerobes and within days or weeks there is a shift from facultative to obligate anaerobes [12, 13]. The establishment of the microbiota is influenced by multiple factors, including gestational age, delivery mode, birth weight, diet and exposure to antibiotics [14–16]. For example, the microbiota of infants born vaginally resembles the mother's vaginal and fecal microbiota, whereas the microbiota of infants born by cesarean section is more similar to the microbiota of skin or other environments [15]. It has also been suggested that C-section-delivered infants have lower diversity gut microbial communities compared to vaginally delivered infants [17]. The infant gut undergoes a rapid increase in the abundance and diversity of microbial communities during the first few weeks [18]. After 2–3 years of life, the gut microbiota become more stable and adult-like

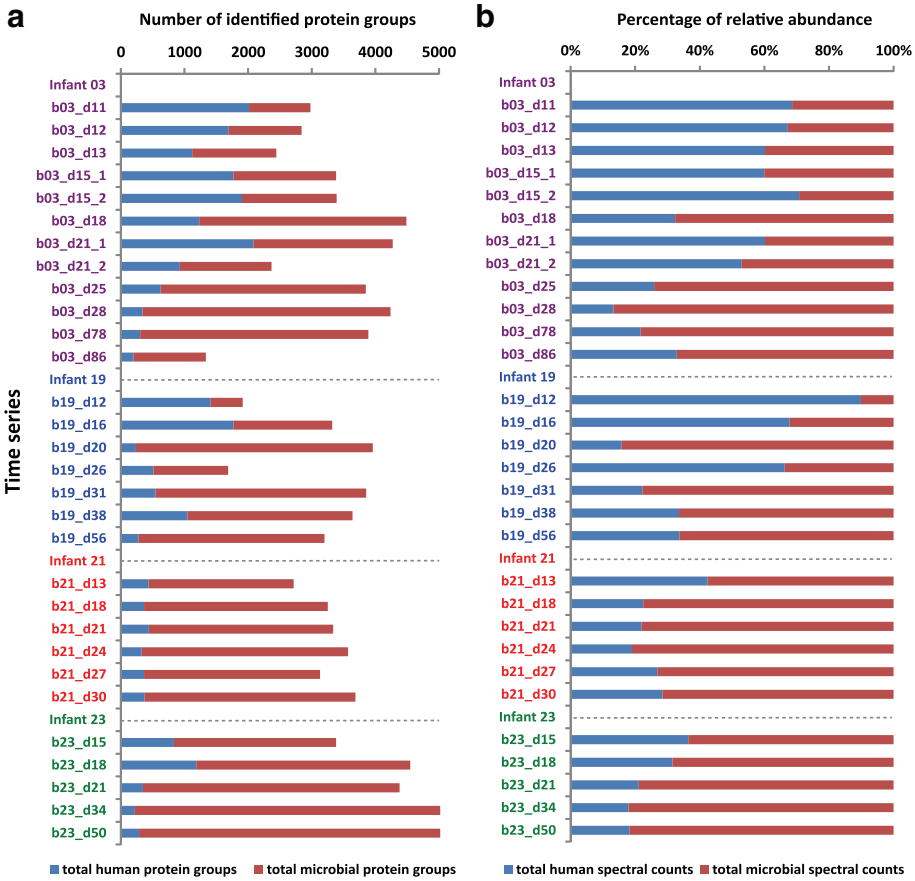

Fig. 1 Number of human and microbial protein groups identified (a) and relative abundance of human/microbial spectra (b) over time. Spectral counts are normalized by the number of total collected spectra and averaged between duplicate runs

[18]. Large variations have been observed among different individuals and also over time within the same individual [19]. It remains to be determined what and how specific factors (eg host genetics, environment, diet, and/or interplay between host and microbiota) determine the path of microbiota development, and how different paths relate to health and disease status. This is particularly critical for premature infants who may have a delayed and aberrant microbiota.

Infants born prematurely are at higher morbidity and mortality risk due to immature organ systems that are not properly adapted to extrauterine life [20]. These infants are susceptible to inflammatory disorders as a result of their poorly developed immune system and prenatal/postnatal events that inappropriately modulate immunity (eg perinatal infection and inflammation) [14, 21]. Among premature infants, the incidence of sepsis and necrotizing enterocolitis (NEC) have remained high and have been associated with aberrant gut microbial colonization during first few weeks of life [22, 23]. The role of bacterial colonization in neonatal NEC has been suggested by a number of observations, including the identification of pneumatosis intestinalis (gas in the bowel wall), which is most likely produced by intestinal bacteria, occurrence of outbreaks in hospital, and resolution of inflammation after treatment with antibiotics [24–26]. Recently, Raveh-Sadka et al. analyzed gut communities in a large number of premature infants during a cluster of NEC infections. Results showed that gut colonization was largely unique among infants and that no single bacterial strain was shared among all infants who developed NEC [27]. This suggests that the disease is not caused by a single bacterial strain, but rather may be associated with multiple deleterious bacteria that disrupt essential activities of mutualistic microbes. Characterization of functional activities and temporal profiles of the gut microbiota during early colonization may further enhance our understanding of the role of gut microbiota in the onset of NEC.

Mass spectrometry-based metaproteomics has been widely used to characterize the proteome of microbial communities and has emerged as a valuable tool in investigating the gut microbiota [12, 28, 29]. In particular, coupling genome-resolved community metagenomics with proteomics allows us not only to explore functional roles of microbial communities in the gut ecosystem, but also to link specific metabolic functions to microbial community members. We have recently described an enhanced metaproteomic approach for the microbiome characterization of infant fecal samples [30]. Here, we employed the approach and expanded our analysis to a total of 30 fecal samples collected during the first three months of life of four premature infants, one of which developed NEC. By integrating metaproteomics with metagenomics, we identified temporal and interindividual variations in community protein abundance, determined conserved metabolic functions of both human host and gut microbiota, and revealed functional differences of gut communities in the metabolism of nutrients and short-chain fatty acids during microbial colonization of the premature infant gut.

Results and discussion

Samples and metaproteomic measurements

Thirty fecal metaproteomes from four preterm infants (03, 19, 21, and 23) collected over the first 3 months after birth were examined by a shotgun metaproteomic approach (each

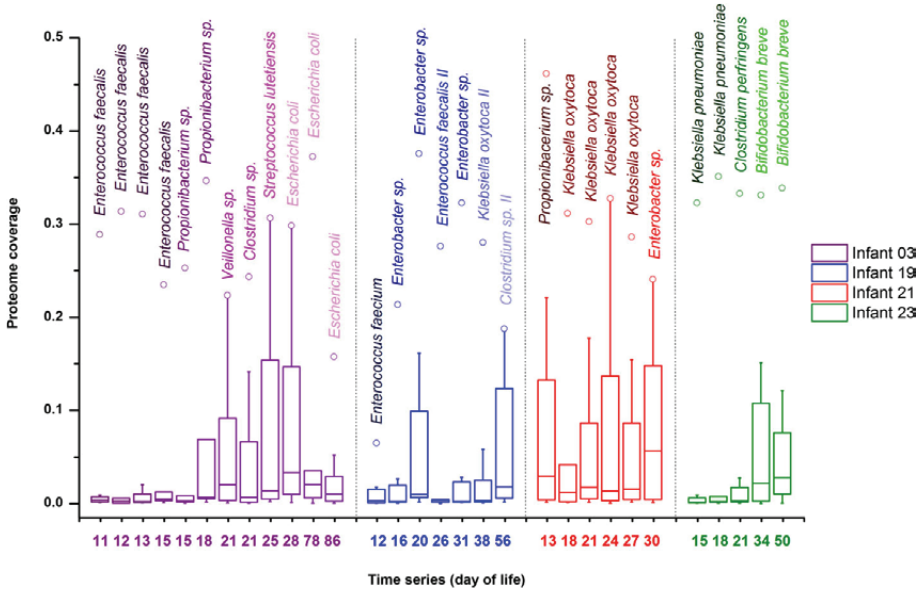

Fig. 2 Boxplot comparing organism-specific proteome coverage across samples. Day of life (DOL) of each infant is shown on the x-axis. For each sample, the organism having the highest proteome coverage is listed and represented as open circles. Only near-complete assembled genomes are included

sample was measured in technical duplicate). One of the four infants (infant 19) developed a case of sepsis and another infant (infant 21) developed necrotizing enterocolitis (NEC) (see Materials and Methods and Additional file 1 for more details). These samples were collected as part of a prior genome-resolved metagenomics study (Raveh-Sadka et al.) [31], and metaproteomic measurement resulted in an average of 108,763 spectra and 17,754 peptides per sample. To alleviate the ambiguity associated with shared peptides, identified proteins were clustered into protein groups (see Materials and Methods for more details). Human and microbial proteins were both monitored, providing a total of 12,568 (9318 microbial and 3250 human), 9665 (7397 microbial and 2268 human), 7091 (6349 microbial and 742 human), and 11,649 (10,330 microbial and 1319 human) protein groups across all time points for infants 03, 19, 21, and 23, respectively (Additional file 2 and Fig. 1). In general, the measuring depth of human proteins decreased with time as microbial proteins became more dominant and abundant, with approximately 200 human protein groups being identified at later time points. Despite the overall trend towards decreased representation of human proteins, variations were observed over time. For example, dramatic decreases of microbial proteins from the second sample collected on day of life (DOL) 21 (21_2) of infant 03, and DOL 26 of infant 19. Interestingly, the decrease observed at DOL 26 of infant 19 coincided with antibiotics use, suggesting that the treatment effectively suppressed the microbiota. However, the decrease noticed at DOL 21_2 of infant 03 might be related to an unmatched database, as a metagenome was only available for the first sample collected on DOL 21. Major microbiota changes occurring between samples would make our constructed database less relevant to the second sample, which could have led to fewer microbial protein identifications.

Varying metaproteome coverage of studied infant gut communities

One of the most important considerations for metaproteomic experiments is the biodiversity and the inherent biological dynamic range within the environment being analyzed. The number of organisms present and their relative abundance directly affect proteome coverage. A typical 24-h LC-MS/MS experiment identifies a few thousand proteins regardless of sample complexity, due to the constrained dynamic range and duty cycle of the mass spectrometer. Therefore, more complex communities yield lower average proteome coverage per organism, and species with higher abundance or activity tend to have a larger percentage of the proteome that can be detected. By integrating strain-resolved metagenomics with deep metaproteomic measurements, we were able to characterize organism-specific proteome coverage across time, as shown in Additional file 3. Since not all predicted proteins are expressed under one condition and the genome size varies among organisms, a typical proteome analysis of a single microbial isolate can identify approximately 50 to 80% of the predicted proteins [32, 33]. Preterm infant gut microbial communities harbor less diversity than other more complex communities (eg human adult gut and soil microbiota) and generally contain a limited number of highly abundant organisms. Here, in total, up to 45% (eg Propionibacterium sp. in DOL 13 of infant 21) of the predicted proteins for an individual organism could be measured and identified. The distribution of different proteome coverage for species/strains in each sample was displayed in Fig. 2, with the species/strain having the highest proteome coverage listed. During early time points, the gut communities were dominated by one or two species (i.e., the outlier with the highest proteome coverage, which is represented as an open circle), and thus proteomes of most other species had low coverage (represented as tight box width). Advancing DOL correlated with a broader distribution of proteome coverage across organisms, indicating that a greater range of bacteria became abundant/active during the colonization process. However, we noticed that distributions of proteome coverage for communities from infant 21 were less skewed and variable as compared to other infants, possibly due to the lower species richness in the gut microbiome of this infant.

Temporal shifts in microbial activity

To further investigate the activity of microbial community members, the proteomic data was matched to genomes previously reconstructed for microbial community members by Raveh-Sadka et al. [31] (Fig. 3). The vast majority of proteins (>99%) were unambiguously assigned to a specific species/strain,

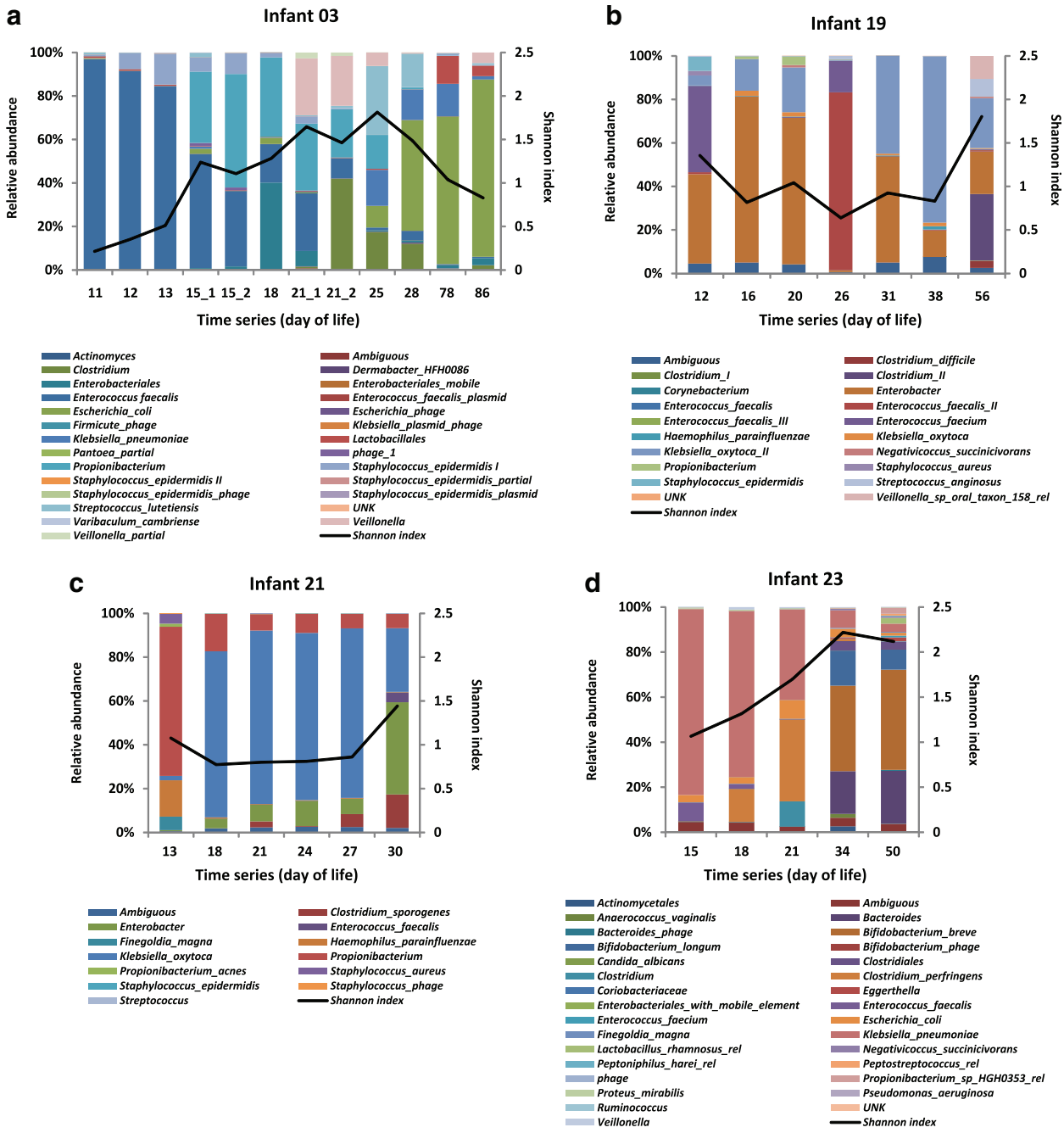

Fig. 3 Taxonomic shifts in infants a 03, b 19, c 21, and d 23 based on protein abundance. Relative abundance of microbial community is based on assigning proteomic data to constructed metagenomes. Shannon diversity index is used to characterize species diversity (both abundance and evenness of the species present) and calculated from the relative abundance of proteins at the species level

but proteins belonging to closely related strains (eg Klebsiella oxytoca and Klebsiella oxytoca II in infant 19; Bifidobacterium longum and Bifidobacterium breve in infant 23), or identical proteins from different species were indistinguishable. Proteins were identified from 25, 18, 12, and 29 different species/strains for infants 03, 19, 21, and 23 respectively, showing that microbiomes of infant 19 and 21 were less complex. We resolved proteins from several phages, identifying a few structural proteins and a majority of unknown-function proteins. In addition, a low abundance of proteins from Candida albicans were identified in infant 23. Microbial composition was largely different between infants, and Enterococcus faecalis was the only species that was shared by all infants. However, a number of species were common between the twins 19 and 21, such as Staphyloccoccus aureus, Enterobacter cloacae, Klebsiella oxytoca, and Haemophilus parainfluenzae, suggesting that the gut microbiome may be impacted by host genetics or exposure to the same environment. The environmental impact might be less relevant because infant 23 was co-hospitalized with the twin infants but was not colonized by the abovementioned species. In addition to the remarkable inter-individual variation, the relative protein abundance and species diversity (Shannon index) of the microbial community within each infant also varied dramatically during the early colonization phase. Intriguingly, apparent differences were observed between the genomic and proteomic patterns on certain DOLs (genomic results have been shown in a prior study [31]), suggesting a few species that are more active in spite of low abundance, such as Streptococcus lutetiensis in DOL 25 of infant 03, Clostridium sp. in DOL 56 of infant 19, and Propionibacterium sp. in DOL 13 of infant 21. In

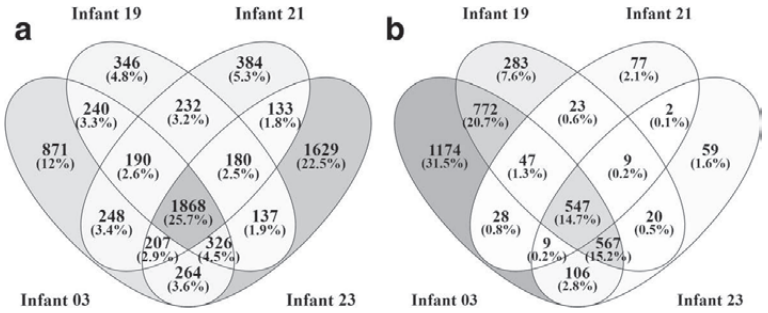

Fig. 4 Venn diagram of microbial EggNOGs (a) and human protein groups (b) in all four infants. A total of 4214, 3519, 3442, and 4744 microbial EggNOGs and 3250, 2268, 742, and 1319 human proteins are identified in infants 03, 19, 21, and 23 respectively

this last sample, Propionibacterium sp. accounted for almost 70% relative abundance in the proteome measurement while its DNA sequence reads only comprised of 15% of the community. These findings could have significant impacts on our understanding of the balance between microbial population structure and dominant metabolic activities.

Moreover, we analyzed different samples collected on the same DOL (pairs 15_1 and 15_2; 21_1 and 21_2 of infant 03, Fig. 3a), to investigate whether the microbiome is stable within a day. Database searching of samples 15_2 and 21_2 was conducted based on metagenomes from different fecal samples collected on the same DOL (15_1 and 21_1, respectively). For both pairs, distributions of microbial protein abundance showed different patterns between the two samples. On DOL 15, the microbiota composition remained almost the same but the most active species shifted from Enterococcus faecalis to Propionibacterium sp. in the later fecal sample. However, on DOL 21, a new dominant colonizer—Clostridium sp. appeared, which greatly changed the community composition in the second sample. A Pearson correlation of r = 0.9 was found between samples on DOL 15 while the correlation between samples on DOL 21 was r = 0.53, indicating that the gut microbiome changed greatly within a day, although the cause of the shift was not apparent. The variance was less likely due to a technical issue, as it was observed that Clostridium sp. stayed relatively abundant until DOL 25. So it was more likely that Clostridium sp. colonized and developed rapidly on late DOL 21, resulting in significant changes in the microbiome. The finding of a shift in community activity is not surprising considering a recent study that also recognized rapid and reproducible alterations of human adult gut microbiome by dietary interventions [34].

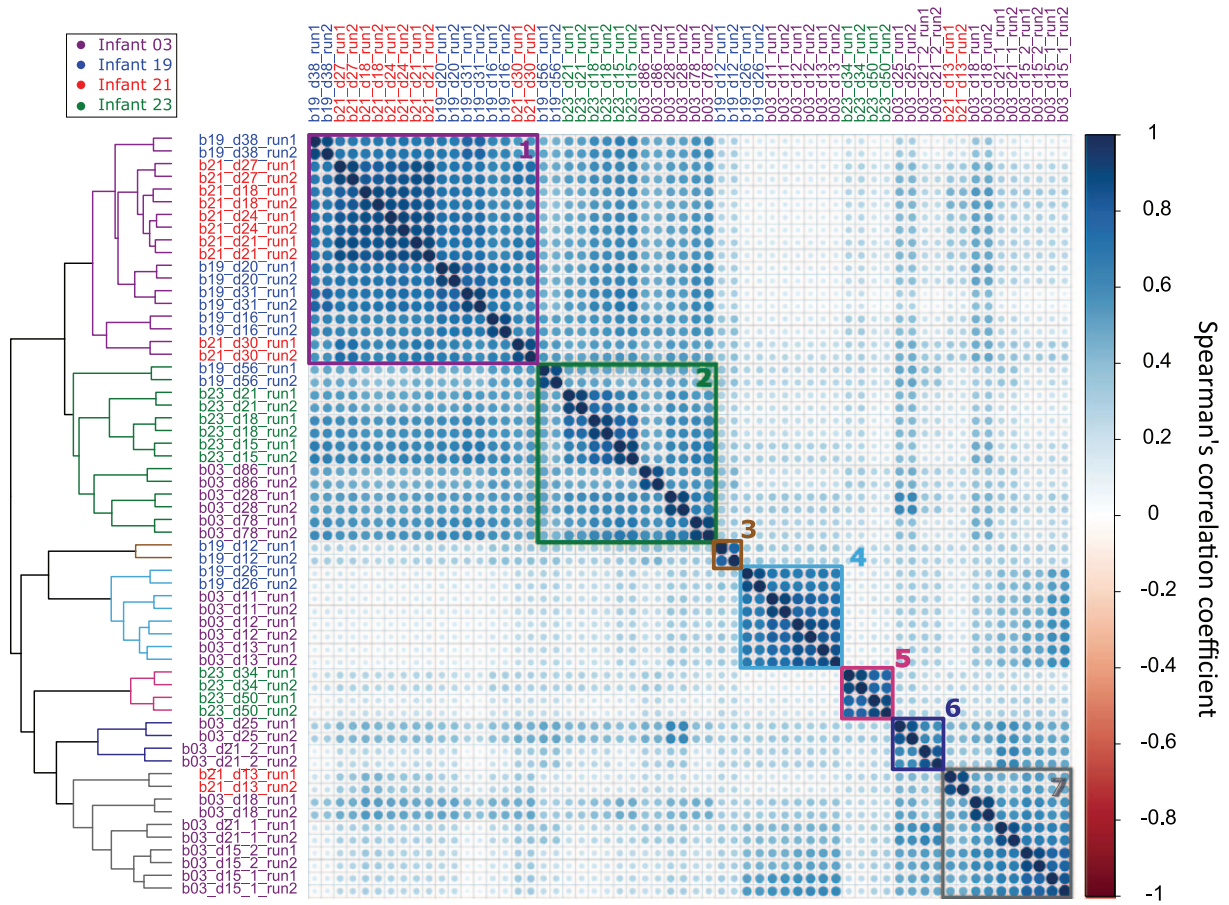

Fig. 5 Correlation plot with hierarchical clustering of microbial EggNOGs. Shown is a correlation matrix plot showing the spearman correlation of each mass spectrometric measurement. Sample names are composed of the infant number (b), day of life (d) and the measurement number (run). The color of sample names indicates different infants. Rows are hierarchically clustered using complete linkage clustering and the seven clusters are highlighted by colored boxes

Temporal and individual variabilities of infant gut metaproteome profiles

As discussed above, the microbial composition and proportions not only vary dramatically during the early colonization phase but also can be remarkably different among infants, and therefore we employed the strategy of annotating identified proteins by orthologous groups to determine the presence and abundance of different proteins across samples. By using the recently updated EggNOG orthologous database [35], annotations were obtained for over of 85% microbial genes and 98% of identified microbial proteins. In total, 4214, 3519, 3442, and 4744 non-redundant microbial EggNOGs were assigned for metaproteomes of infant 03, 19, 21, and 23 respectively (Fig. 4a). Among all identified microbial EggNOGs, 1868 (26%) were commonly identified in all four infants. The highest number of unique EggNOGs (1629, 22.5%) was found in infant 23. When considering samples collected at multiple time points within an infant, 1249 EggNOGs were found in at least half of the samples, but only 81 were present across all 30 samples (Additional files 4 and 5), showing large dissimilarity of these proteome profiles. The common 81 EggNOGs mainly participate in carbohydrate transport using bacteria-specific phosphotransferase system (PTS), carbohydrate metabolism including glycolysis, pentose phosphate pathway and galactose degradation, energy production involving ethanol production and glycerol degradation, amino acid (eg glutamate, arginine and glycine) metabolism, nucleotide metabolism, transcription, translation and chaperonin-assisted protein folding, revealing a conserved functional profile of gut microbiota. In addition, 547 human proteins (15%) were commonly identified in four infants, but only 57 proteins were detected across all samples (Fig. 4b and Additional file 4). Unlike essential microbiome functions which mainly support cell growth and maintenance, these common host proteins in the gut included proteins related to lipid and protein digestion, antibacterial activity, innate immune response, and gut mucosal barrier development and protection (Additional file 5). Notably, as essential components in the infant innate immunity, intestinal barrier and immune factors were present at all times. These factors might constantly fine-tune the activities of the microbial community in order to maintain the homeostatic balance between the developing gut microbiota and the host environment [36].

To further assess the correlation of studied metaproteome profiles, Spearman's rank correlations with hierarchical clustering were applied, and seven clusters were identified (Fig. 5). Typically, samples collected from adjacent time points of the same infant were highly correlated, but not all samples clustered by individual. Intriguingly, samples from different infants (DOL 11 of infant 03 and DOL 26 of infant 19) collected after antibiotics treatment clustered together (cluster 4). This might be related to an abundance of E. faecalis occurring in both samples after antibiotics use. Potential antibiotic resistance proteins identified in E. faecalis were analyzed by CARD [37] and shown in Additional file 6. Additionally, we found that the microbiota seemed to be restored after antibiotic treatment in infant 19, as samples taken before and after antibiotics treatment clustered together (DOL 20 and 31), but not with the sample from the administration period (DOL 26). As mentioned above, a number of bacterial species were shared by twin infants 19 and 21. Microbial proteomes from the two infants were also closely related (cluster 1). A recent study has revealed a subject-specific and stable gut metaproteome in human adults [38]. However, our

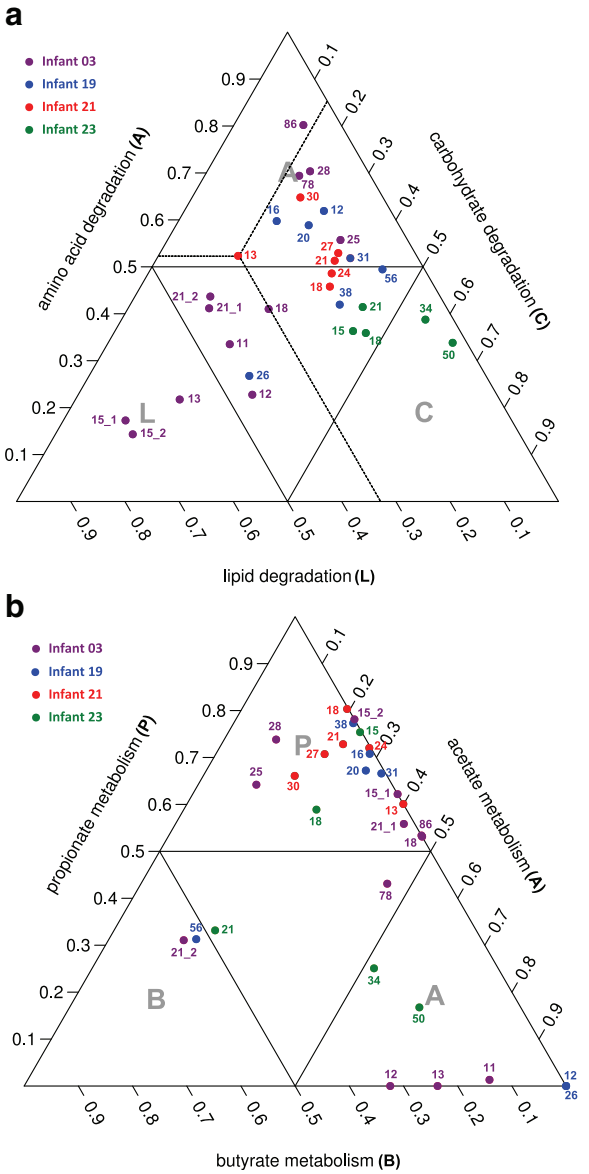

Fig. 6 Tri-plot representation of the gut communities in the exploitation of three major energy sources (a) and the metabolism of short-chain fatty acids (b). For each metabolic category/trait (eg carbohydrate/lipid/ amino acid degradation and propionate/butyrate/acetate metabolism), the abundance was defined as summed protein abundances of GMMs associated with the category divided by the number of GMMs in that category. Shown tri-plot representation is based on the relative abundance of the three defined categories. The dashed lines give an example of the proportions of carbohydrate, lipid and amino acid degradation for the sample DOL13 of infant 21. Each corner triangle labeled with one-letter category name (eg amino acid (A); lipid (L); carbohydrate (C); propionate (P); butyrate (B); acetate (A)) represents dominant proteomic investment in that particular metabolic category. Day of life (DOL) is shown on each sample/dot

observations showed that during infancy, the proteome was less individualized and more unstable as compared with that observed in human adults. Infant age, genetic background and antibiotics use are likely to be major determinants for the microbiota development in neonates. Among conserved functional groups identified in adults from the previous study, formate acetyltransferase, glutamate dehydrogenase, and three glycolytic enzymes were also conserved in infants studied in our study. However, enzymes involved in the production of butyrate (butyryl-CoA dehydrogenase) and vitamin B12 (sirohydrochlorin cobaltochelatase) were conserved in human adults but only found in a portion of

infant samples, suggesting that the infant intestinal microbiota is variable and has not stabilized for these functions during the early colonization.

In order to further investigate proteins that distinguish metaproteome clusters, proteins with significantly different abundances between clusters were identified (Additional file 7). Notably, cluster 5 was the most distinguishable cluster, having the longest distance from it to other clusters. An abundance of proteins involved in carbohydrate metabolism were identified in cluster 5, for example, ABC transporter proteins (likely for sugars), beta-galactosidase and galactose-1-phosphate uridylyltransferase participating in the degradation of lactose, and xylulose-5-phosphate/ fructose-6-phosphate phosphoketolase acting as a key enzyme in the "Bifid shunt" pathway. The "Bifid shunt" in which carbohydrates are fermented via phosphoketolase is a unique process in Bifidobacteria. Samples (DOL 34 and 50 of infant 23) clustered in cluster 5 had a dominance of Bifidobacterium breve in both communities. Interestingly, this infant 23 is the only infant with dominant obligate anaerobic Bifidobacterium. Therefore, cluster 5 was clearly distinct from other metaproteome clusters, possibly due to the distinct carbohydrate utilization of Bifidobacterium.

Metabolic profiles in association with shifts in microbial communities

To assess the diversity and stability of metabolic functions across these communities, a recently developed gut-specific framework was applied to infer species-associated GMMs (gut metabolic modules) for our dataset [39]. A total of 104 GMMs were inferred for all infants, with module coverage higher than 0.556 (55.6%) (the optimal coverage cutoff is inferred during module prediction), and the temporal abundances of all microbial members that participate in the module were determined (Additional file 8).

Modules associated with carbohydrate, amino acid, and lipid metabolism

We assessed infant gut communities in the exploitation of three major energy sources (carbohydrate, amino acid, and lipid) across time. All measured communities were found to use all three types of nutrients, but the relative abundance of saccharolytic (carbohydrate degradation), proteolytic (amino acid degradation), and lipolytic (lipid degradation) fermentation modules varied among communities (Fig. 6a, Additional file 8). Among four infants, a larger separation in the utilization of nutrients was identified in infant 03 over time, while samples from the same infant were closely clustered for the other three infants. This is likely due to the longer sampling period covered for infant 03.

Intriguingly, gut communities associated with infant 03 exhibit high levels of lipolytic fermentation at early time points and shifted towards dominant proteolytic fermentation over time. The availability of genome sequences reconstructed from matched metagenomes enabled us to determine the contribution of specific species to these functions. Enterococcus faecalis was dominant in the community over DOL 11 to 15 and contributed significant protein abundance to glycerol degradation through expression of glycerol dehydrogenase and dihydroxyacetone kinase. The source of glycerol may have been the complete digestion of breast milk triglycerides via host pancreatic lipase and breast milk-derived bile salt-stimulated lipase [40], which were both identified in the samples. Thereby, free glycerol can be utilized by gut bacteria and converted to the glycolysis intermediate dihydroxyacetone phosphate, potentially for ATP production. On DOL 18, the proportional contribution of bacteria to amino acid degradation began to increase, mainly contributed by Enterbacteriales sp. For example, aspartate aminotransferase and aspartate ammonia-lyase were both identified, allowing the conversion of L-aspartate to oxaloacetate and fumarate, respectively. Both products can be further consumed via entering the TCA cycle. Starting from DOL 21, glutamate degradation was observed and became relatively abundant, which was contributed by Clostridium sp. and Escherichia coli. We identified glutamate decarboxylase A and B in E. coli, which were required for the degradation of L-glutamate to 4-aminobutanoate. 4-Aminobutanoate can be further degraded by 4-aminobutyrate aminotransferase and succinate-semialdehyde dehydrogenase (both were also identified in E. coli) to TCA intermediate succinate. The action of all above four proteins, known as "GABA shunt," channels glutamate into the TCA cycle [41]. Increased tryptophan degradation via tryptophanase was also identified in E. coli during the time after DOL 25, which can produce indole, pyruvate, and ammonium. While ammonium can be used as a nitrogen source and indole can act as a signal molecule, pyruvate can be redirected into the TCA cycle.

The trend towards increased protein utilization observed in infant 03 was not identified in other infants. Microbes from infants 19 and 21 remained relatively stable in the fermentation of these three substrates (carbohydrates, amino acids, and lipids), except slightly increased carbohydrate degradation observed during late time points in infant 19. This was mostly driven by Klebsiella oxytoca II on DOLs 31 and 38, and Clostridium sp. II on DOL 56. A high abundance of beta-galactosidase and alpha-galactosidase were identified in Klebsiella oxytoca II, hydrolyzing lactose and melibiose into galactose and glucose. While glucose can directly be utilized via glycolysis, galactose requires five enzymes to be converted into the more metabolically versatile D-glucopyranose 6-phosphate: galactose-1-epimerase, galactokinase, galactose-1-phosphate uridy-lyltransferase, UDP-glucose 4-epimerase, and phospho-glucomutase, which were all detected in Klebsiella oxytoca II. All proteins mentioned above except alpha-galactosidase were also identified in Clostridium sp. II. Among the four infants, the gut microbiota of infant 23 presented the highest proportion of carbohydrate fermentation proteins, primarily related to lactose, galactose, and melibiose degradation. These functions were mostly contributed by Klebsiella pneumoniae on DOLs 15 to 21 and Bifidobacterium breve on DOLs 34 and 50.

However, we did not observe clear associations between macronutrient utilization and clinical outcome, mode of delivery, feeding regimen, or gestational age at delivery. It is important to note that the composition of human milk can vary substantially between mothers and between time points [42]. Since each infant in the study received breast milk, it is possible that this factor could have sharply impacted the variability of gut microbial metabolism.

Modules associated with short-chain fatty acid (SCFA) metabolism

SCFAs, primarily acetate, propionate, and butyrate are major end-products of human milk oligosaccharide (HMO) fermentation by intestinal microbiota. We further explored acetate, propionate, and butyrate metabolism in all measured samples (Fig. 6b, Additional file 8) and found a majority of

communities predominantly invested in the metabolism of propionate.

Two different pathways were inferred for propionate production (Additional file 9): the succinate pathway and the propanediol pathway. The succinate pathway utilizes succinate as a substrate and employs succinyl-CoA synthetase, methylmalonyl-CoA mutase, methylmalonyl-CoA epimerase, and methylmalonyl-CoA carboxytransferase to convert succinate to propionyl-CoA. All these enzymes were identified and mainly found in species of Propionibacterium for all infants. The propanediol pathway is characterized by the conversion of propionaldehyde to propionyl-CoA via CoA-dependent propionaldehyde dehydrogenase as a marker enzyme. This pathway also involves lactaldehyde reductase and propanediol dehydratase, responsible for conversion of L-lactaldehyde to propionaldehyde. The propanediol pathway was primarily found in Klebsiella pneumoniae of infants 03 and 23 and in Klebsiella oxytoca of infants 19 and 21. The production of propionate in these organisms was further supported by the identification of fucose degradation pathway in the same organism. L-fucose is a major component of glycosylated mucin proteins in the intestinal epithelium and oligosaccharides in human milk [43, 44]. L-fucose isomerase, L-fuculokinase, and L-fuculose phosphate aldolase, enzymes needed to degrade L-fucose to L-lactaldehyde, were all detected in above Klebsiella genus that were able to further convert L-lactal-dehyde to propionate.

Although most communities exhibited dominant propionate metabolism, samples from DOL 21 of infant 03, DOL 56 of infant 19, and DOL 21 of infant 23 showed relatively high levels of butyrate metabolism. Two different pathways are possible for butyrate production in gut bacteria, but the butyrate kinase pathway was the major one observed in these communities, which employed crotonyl-CoA reductase, phosphotransbutyrylase and butyrate kinase to convert crotonoyl-CoA to butyrate (Additional file 9). This pathway was primarily found in Clostridium species, including Clostridium sp. of infant 03, Clostridium difficile and Clostridium II sp. of infant 19, Clostridium sporogenes of infant 21, and Clostridium sp. and Clostridium perfringens of infant 23.

Acetate can be produced by converting acetyl-CoA to acetate via phosphate acetyltransferase and acetate kinase. As opposed to propionate and butyrate production that were mainly controlled by a few organisms in these communities, many organisms were able to produce acetate (Additional file 9). We noticed that samples with very low complexity, either collected from early time points (DOL 11, 12, and 13 of infant 03; DOL 12 of infant 19) or after antibiotics treatment (DOL 26 of infant 19), showed dominant acetate metabolism, possibly because microbes that have the capacity to produce butyrate and propionate have not colonized or have been removed during the time and thus acetate production became relatively predominant. Two samples (DOL 34 and 56) from infant 23 also had relatively high abundance of acetate metabolism enzymes, mainly contributed by Bifidobacterium breve. In premature infants, high level of fecal acetate has been associated with increased Bifidobacteria [45].

Conclusions

In this study, we conducted a metagenome-informed metaproteomic analysis of 30 gut communities from four human preterm infants, allowing us to characterize species-specific gut microbial functional variations between infants during the critical first few weeks of life. The use of sample-matched, genome-resolved metagenomics databases enabled us to identify an average of 2606 microbial protein groups with up to 45% proteome coverage obtained for the most dominant species in each community, and further to reconstruct species-specific metabolic functions and pathways. We found that the pattern of community relative protein abundance varied substantially among individual infants and over the time course, but generally the community began with colonization of facultative anaerobes, such as Enterococcus and Klebsiella, followed by the emergence of some obligate anaerobes, for example, Clostridium, Bifidobacterium, and Bacteroides. While rapid shifts in the infant gut microbiome were observed, conserved metabolic pathways were identified, largely associated with microbial cell growth and maintenance. The roles of environmental factors in the early life gut microbiota development are still poorly understood. As opposed to human adults, the infant gut exhibited unstable and individual-unspecific gut metaproteomes, possibly as a result of infant gut microbiota ecosystem being immature/underdeveloped and thus susceptible to disruption by environmental factors. Our data showed a few observations where antibiotics altered the gut metaproteome, but future research is needed to describe the effect of antibiotics on the infant gut microbiota. Given different patterns in the gut communities, our results further revealed species-related metabolic shifts and variations of the infant gut microbiota, particularly in the nutrient exploitation and SCFAs metabolism.

Methods

Sample collection

Fecal samples were collected from four preterm infants (03, 19, 21, and 23) over the first 3 months after birth. All samples were collected as part of a prospective cohort study of premature infants with and without necrotizing enterocolitis. The four infants in this study represent four of the first infants within the overall cohort to undergo metagenomics sequencing that also had remaining fecal samples with enough biomass to permit proteomic analysis. The specific samples selected for proteomics were selected on the basis of completeness of bacterial genomes assembled by metagenomics (Raveh-Sadka et al.) [31] in order to enable genome-resolved proteomic analysis. Infant 03 was a healthy preterm infant. Infants 19 and 21 were two infants from triplets, among which, 19 developed severe sepsis, but not NEC, while 21 developed NEC and died from NEC totalis. Infant 23 was co-hospitalized with infants 19 and 21, who was healthy aside from mild lung disease. Stool samples were collected on day of life (DOL) 11–86 as available. For the infant 03, samples were collected twice on days 15 (15_1 and 15_2) and 21 (21_1 and 21_2). Additional medical details of four infants were described in a prior study [31] and Additional file 1. Subjects were enrolled and samples were collected according to a research protocol approved by The University of Pittsburgh Institutional Review Board (PRO10090089).

Sample preparation

~0.3 g raw fecal material was prepared by the indirect enrichment method, as previously detailed with modifications [30]. In brief, raw fecal material was passed through a 20-μm vacuum filter followed by centrifugation to enrich microbial cells. Collected microbial cells were lysed by sodium dodecyl sulfate (SDS) with sonication. One milligram of crude protein extract was then precipitated by trichloroacetic acid (TCA) and washed with ice-cold acetone. Pelleted proteins were resolubilized in 8 M urea and sonically disrupted to fully

solubilize the protein pellet. Denatured proteins were reduced with 5 mM dithiothreitol (DTT), cysteines blocked with 20 mM iodoacetamide (IAA) and digested into peptides with sequencing grade trypsin. The digested samples were then adjusted to 200 mM NaCl, 0.1% formic acid (FA), and filtered through a 10-kDa cutoff spin column filter to collect the tryptic peptides.

2D LC-MS/MS measurement

For each sample, obtained peptide samples (50 µg) were analyzed via 22-h MudPIT two-dimensional (2D) nanospray LC-MS/MS system on LTQ-Orbitrap Elite (ThermoFisher Scientific, San Jose, CA). As previously described [46], peptides were separated/eluted in 11 steps (each lasting ~2 h) with an increasing amount of salts (ammonium acetate) followed by organic gradients in each step. Mass spectra were acquired in data-dependent mode: full scans were acquired at 30-k resolution (1 microscan) in the Orbitrap, followed by CID fragmentation of the 20 most abundant ions (1 microscan). Monoisotopic precursor selection was enabled. Unassigned charge and charge state +1 were rejected. Dynamic exclusion was enabled with a mass exclusion width 10 ppm and exclusion duration 30 s. Technical replicates (duplicates) were performed for each sample.

Peptide and protein identification

Protein databases were constructed for each individual infant by combining proteins predicted from sequenced metagenome (see Raveh-Sadka et al. [31] for more details) collected on multiple days, human protein sequences (NCBI RefSeq_2011), and common contaminants. All MS/MS spectra were searched with the Myrimatch version 2.1 algorithm against the constructed protein database and filtered with IDPicker. Peptide modifications including a static cysteine modification (+57.02 Da), an N-terminal dynamic carbamylation modification (+43.00 Da), and a dynamic oxidation modification (+15.99) were included in all searches. A decoy database consisting of reverse protein sequences was appended to the target database to calculate false discovery rates (FDR). Peptide identifications were filtered by maintaining at least two distinct peptides per protein and a 2% peptide spectrum match-level FDR to achieve confident peptide identifications (FDR <1%). For protein inference, proteins were grouped base on 90% amino acid sequence identity for human proteins and 100% identity for microbial proteins, as previously described [47]. Spectral counts were balanced between shared proteins, and normalized by total numbers of all collected MS/MS in each run.

Data analysis

EggNOG annotations were obtained using EggNOG database v4.5 via eggNOG-mapper with HMM search mode [35]. Venn diagram was generated using an online tool VENNY 2.1 (http://bioinfogp.cnb.csic.es/tools/ venny/index.html). The EdgeR package [48] was used to calculate significantly different protein abundances between clusters via quasi-likelihood negative binomial generalized log-linear model (glmQLFTest). The dataset was normalized based on scaling factors for library sizes, which were determined using a trimmed mean of M values (TMM) between samples. The correlation plot was built using the corrplot package after sorting based on hierarchical clustering. KEGG Orthology (KO) annotations for each protein sequence were assigned by KEGG Automatic Annotation Server (KASS) using GHOSTX search and the SBH (single-directional best hit) method [49]. Gut metabolic modules (GMMs) and tri-plot representations were inferred and visualized from an online tool GOmixer, specifically for gut meta-omics data analysis [39, 50].

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