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neonatal INTENSIVE CARE

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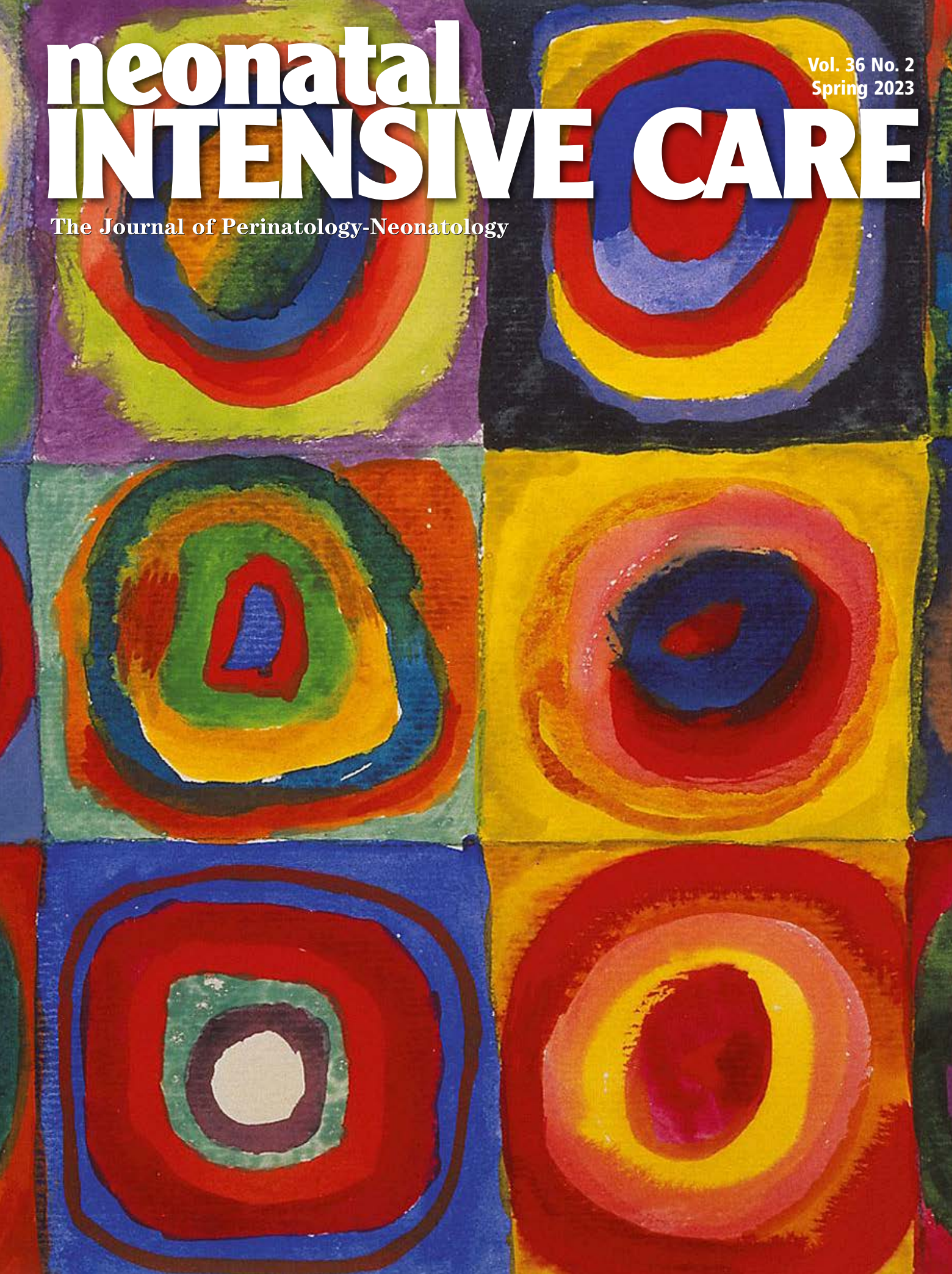

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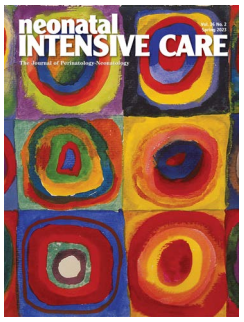

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☐ Spring 2023

Babies' Movement in the Womb Means Something

The random movements that babies make while in the womb help boost their sensorimotor movement that will aid them after birth, according to researchers at the University of Tokyo.

What to know: The hundreds of neurons that control each muscle are synchronized in a fetus to create strong contractions that stimulate its activity. Random movements of babies in the womb help their development and boost the growth of their sensorimotor system, which supports everything from language development, cognitive growth, and hand-eye coordination to problem solving skills and social interaction. Infants develop their own sensorimotor system based on explorational behavior or curiosity, so they are not just repeating the same action but a variety of actions implying a linkage between early spontaneous movements and spontaneous neuronal activity.

Newborns and infants can acquire coordination skills through spontaneous whole-body movements without an explicit purpose or task, showing more common patterns and sequential movements, with an increase in coordinated whole-body and anticipatory movements as they grow older.

Understanding how the sensorimotor system develops starting in vitro could lead to understanding and treating a wide range of neurodegenerative disorders such as multiple sclerosis, spinal cord injuries, motor neuron disease, and even cerebral palsy.

NICU Use Up, Birth Weights Down in Babies of Mothers With HCV

Infants born to women infected with the hepatitis C virus (HCV) faced twice the risk of stays in the neonatal ICU (NICU) and 2.7 times the risk of low birth weight, a new analysis finds, even when researchers adjusted their data to control for injectable drug use and maternal medical comorbidity. Clinicians should be "aware that the infants of pregnant people with HCV may have a high rate of need for higher-level pediatric care," said Brenna L. Hughes, MD, MSc, chief of maternal fetal medicine at Duke University Medical Center, Durham, NC. She spoke in an interview about the findings, which were presented at the meeting sponsored by the Society for Maternal-Fetal Medicine. As Dr Hughes noted, "HCV remains a serious problem in pregnancy because it often goes undiagnosed and/or untreated prior to pregnancy. It can be passed to infants, and this can cause significant health-related outcomes for children as they age." For the multicenter US study, researchers identified 249 pregnant mothers with HCV from a 2012-2018 cohort and matched them by gestational age to controls (n = 486). The average age was 28; 71.1% of the cases were non-Hispanic White versus 41.6% of the controls; 8.4% of cases were non-Hispanic Black versus 32.1% of controls ($P < .001$ for race/ethnicity analysis); and 73% of cases were smokers versus 18% of controls ($P < .001$). More than 19% of cases reported injectable drug use during pregnancy versus 0.2% of controls ($P < .001$). The researchers adjusted their findings for maternal age, body mass index, injectable drug use, and maternal comorbidity. An earlier analysis of the study data found that 6% of pregnant women with HCV passed it on to their infants, especially those with high levels of virus in their systems. For the new study, researchers focused on various outcomes to test the assumption that "adverse pregnancy outcomes associated with HCV are related to prematurity or to ongoing use of injection drugs," Hughes said. There was no increase in rates of preterm birth or adverse maternal outcomes in the HCV cases. However, infants born to women with HCV were more likely than the controls to require a stay in the NICU (45% vs. 19%; adjusted relative risk, 1.99; 95% confidence interval, 1.54-2.58). They were also more likely to have lower birth weights (small for gestational age < 5th percentile) (10.6% vs. 3.1%; ARR, 2.72; 95% CI, 1.38-5.34). No difference in outcomes was seen when HCV

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Cover: Wassily Kandinsky, Color Study, Squares with Concentric Circles, 1913.

cases with viremia (33%) were excluded. "The most surprising finding was that the need for higher-level pediatric care was so high even though there wasn't an increased risk of prematurity," Hughes said.

Maternal COVID-19 Vaccine Curbs Infant Infection From Delta Variant

Maternal vaccination with two doses of the mRNA COVID-19 vaccine was 95% effective against infant infection from the delta variant, and 45% effective against infant infection from the omicron variant, a new study shows. Previous research has confirmed that COVID-19 neutralizing antibodies following maternal vaccination or maternal COVID-19 infection are present in umbilical cord blood, breast milk, and infant serum specimens, wrote Sarah CJ Jorgensen, MD, of the University of Toronto, and colleagues in their article published in The BMJ. In the study, the researchers identified maternal and newborn pairs using administrative databases from Canada. The study population included 8,809 infants aged younger than 6 months who were born between May 7, 2021, and March 31, 2022, and who underwent testing for COVID-19 between May 7, 2021, and September 5, 2022. Maternal vaccination with the primary COVID-19 mRNA monovalent vaccine series was defined as two vaccine doses administered up to 14 days before delivery, with at least one of the doses after the conception date. Maternal vaccination with the primary series plus one booster was defined as three doses administered up to 14 days before delivery, with at least one of these doses after the conception date. The primary outcome was the presence of delta or omicron COVID-19 infection or hospital admission of the infants. The study population included 99 COVID-19 cases with the delta variant (with 4,365 controls) and 1,501 cases with the omicron variant (with 4,847 controls). Overall, the vaccine effectiveness of maternal doses was 95% against delta infection and 45% against omicron. The effectiveness against hospital admission in cases of delta and omicron variants were 97% and 53%, respectively.

Moms' and babies' medical data predicts prematurity complications, Stanford Medicine-led study shows

By sifting through electronic health records of moms and babies using a machine-learning algorithm, scientists can predict how at-risk newborns will fare in their first two months of life. The new method allows physicians to classify, at or before birth, which infants are likely to develop complications of prematurity. A study describing the method, developed at the Stanford School of Medicine, was published online in *Science Translational Medicine*. "This is a new way of thinking about preterm birth, placing the focus on individual health factors of the newborns rather than looking only at how early they are born," said senior study author Nima Aghaeepour, PhD, an associate professor of anesthesiology, perioperative and pain medicine and of pediatrics. The study's lead authors are postdoctoral scholar Davide De Francesco, PhD, and Jonathan Reiss, MD, an instructor in pediatrics. Traditionally defined as birth occurring at least three weeks early, premature birth is linked to complications in babies' lungs, brains, vision, hearing and digestive system. Although earlier births generally carry higher risks, the timing of birth predicts only approximately how a specific infant will fare. Some infants who are born quite early develop no complications, while others born at the same stage of pregnancy become very ill or die. "Preterm birth is the single largest cause of death in children under age 5 worldwide, and we haven't had good solutions," Aghaeepour said. "By focusing our research on predicting the health of these babies, we can optimize their care." Many complications of prematurity take days or weeks after birth to emerge, causing substantial damage to newborns' health in the meantime. Knowing which infants are at risk could enable preventive measures. "We look mainly at the baby to make treatment decisions in neonatology, but we are finding that we can get valuable information from the maternal health record, really homing in on how individual babies' trajectories have been shaped by exposure to their specific maternal environment," said study coauthor David Stevenson, MD, a neonatologist at Lucile Packard Children's Hospital Stanford, professor of pediatrics and director of the March of Dimes Prematurity Research Center at the Stanford School of Medicine. "This is a move toward precision medicine for babies," he added.

Maternal COVID-19 Vaccine Curbs Infant Infection From Delta Variant

Maternal vaccination with two doses of the mRNA COVID-19 vaccine was 95% effective against infant infection from the delta variant, and 45% effective against infant infection from the omicron variant, a new study shows. Previous research has confirmed that COVID-19 neutralizing antibodies following maternal vaccination or maternal COVID-19 infection are present in umbilical cord blood, breast milk, and infant serum specimens, wrote Sarah C.J. Jorgensen, MD, of the University of Toronto, and colleagues in their article published in The BMJ. In the study, the researchers identified maternal and newborn pairs using administrative databases from Canada. The study population included 8,809 infants aged younger than 6 months who were born between May 7, 2021, and March 31, 2022, and who underwent testing for COVID-19 between May 7, 2021, and September 5, 2022. Maternal vaccination with the primary COVID-19 mRNA monovalent vaccine series was defined as two vaccine doses administered up to 14 days before delivery, with at least one of the doses after the conception date. Maternal vaccination with the primary series plus one booster was defined as three doses administered up to 14 days before delivery, with at least one of these doses after the conception date. The primary outcome was the presence of delta or omicron COVID-19 infection or hospital admission of the infants. The study population included 99 COVID-19 cases with the delta variant (with 4,365 controls) and 1,501 cases with the omicron variant (with 4,847 controls). Overall, the vaccine effectiveness of maternal doses was 95% against delta infection and 45% against omicron. The effectiveness against hospital admission in cases of delta and omicron variants were 97% and 53%, respectively. The effectiveness of three doses was 73% against omicron infant infection and 80% against omicron-related infant hospitalization. Data were not available for the effectiveness of three doses against the delta variant. The effectiveness of two doses of vaccine against infant omicron infection was highest when mothers received the second dose during the third trimester of pregnancy, compared with during the first trimester or second trimester (53% vs. 47% and 53% vs. 37%, respectively). Vaccine effectiveness with two doses against infant infection from omicron was highest in the first 8 weeks of life (57%), then decreased to 40% among infants after 16 weeks of age.

Hope for Catching Infants with CP Early

A new prognostic tool may help identify infants with cerebral palsy (CP) earlier, allowing them to receive therapies to improve later outcomes. Researchers from Canada used 12 clinical variables to predict the condition. The tool accurately predicted 75% of CP cases. The study was published in *JAMA Pediatrics*.

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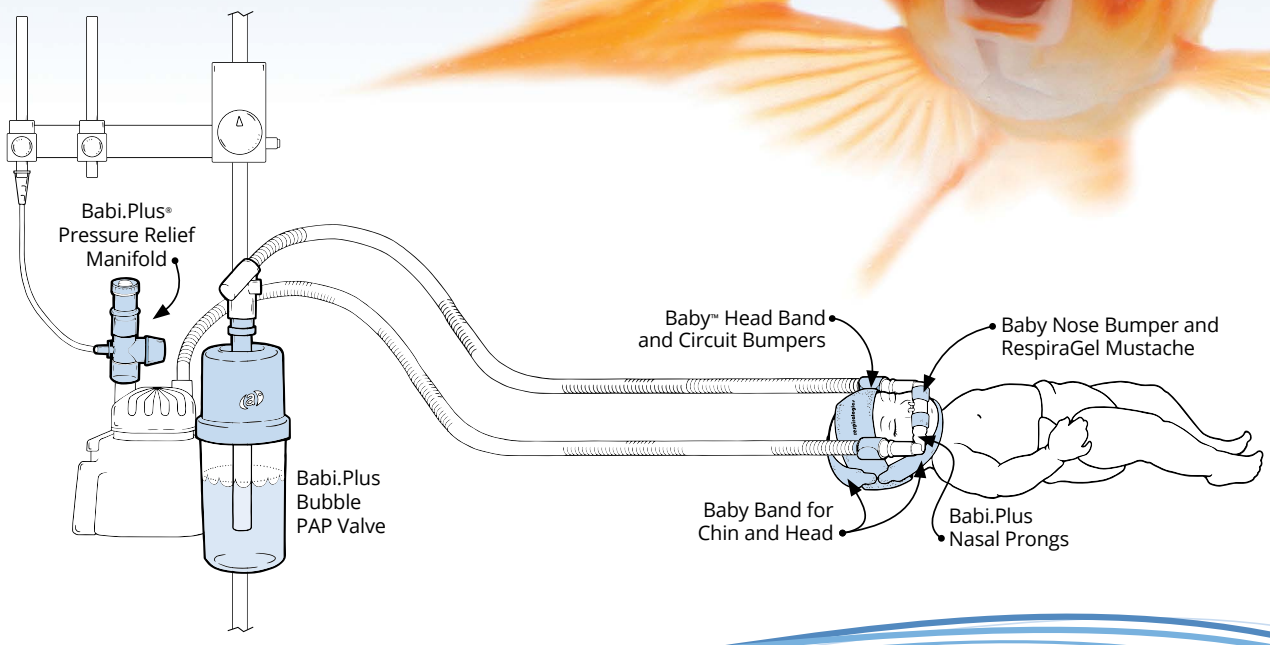

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The prevalence of CP in the US is two to three children per 1000, a rate that has been relatively unchanged for decades. Although recent innovations in diagnosis using motor scores and MRI scans have aided in diagnosis, these techniques have historically been reserved only for infants who were cared for in neonatal intensive care units, were born prematurely, or who had other neurologic risk factors, such as birth defects. The tool identified 2.4 times more children with CP than would have been detected using current diagnostic methods, according to the researchers. "We developed the prediction tool to try to make these findings accessible to any healthcare provider, which will hopefully help break down the long-held perception that CP is usually related to prematurity or a difficult delivery," said Mary Dunbar, MD, an author of the study. "We know that about half of children with CP aren't premature and didn't have a particularly difficult birth."

The bedside tool weighs factors such as the use by mothers of illicit drugs and tobacco; the presence of diabetes and preeclampsia during pregnancy; whether the infant is male; birth weight; and the number of miscarriages the mother had prior to the birth. The tool also factors in results from a test that measures how well the infant is adjusting to life outside the womb. Dunbar and her colleagues compared 1265 infants with CP from the Canadian Cerebral Palsy Registry from 2003 to 2019 to a control group of 1985 children without CP from the Alberta Pregnancy Outcomes and Nutrition longitudinal study. The study authors hope that the prognostic tool can be integrated into existing newborn screenings and completed by nurses or physicians as part of routine care. "Its cost is low especially in comparison to MRI and specialized neurological assessments," said Sarah Taylor, MD, section chief of neonatal-perinatal medicine at Yale New Haven Children's Hospital in Connecticut. Health systems and doctors may be more apt to adopt the tool, since it does not require specialized equipment or training, Taylor added.

State Quality Initiative Can Reduce Postpartum Hemorrhage and Maternal Morbidity

A statewide quality initiative can improve severe maternal morbidity (SMM) and reduce the incidence of maternal morbidity and mortality from postpartum hemorrhage (PPH), a modeling analysis found. Such measures could potentially provide savings to birthing hospitals, according to the California cost-effectiveness study, published in Obstetrics & Gynecology. A team led by Eric C. Wiesehan, MHA, MBA, a PhD candidate in health policy at Stanford (Calif.) University, examined the effects of the safety initiative of the California Maternal Quality Care Collaborative (CMQCC) in a theoretical cohort of 480,000 births across a mix of hospital settings and sizes. The CMQCC developed a PPH toolkit and quality-improvement protocol to increase recognition, measurement, and timely response to PPH. Drawing retrospectively on a large 2017 California implementation study, the simulation estimated that collaborative implementation of the CMQCC added 182 quality-adjusted life-years (0.000379 per birth) by averting 913 cases of SMM, 28 emergency hysterectomies, and one maternal mortality. Additionally, it saved $9 million ($17.78 per birth) owing to avoided SMM costs. According to the Centers for Disease Control and Prevention, pregnancy-related maternal deaths in the United States have increased from 7.2 per 100,000 live births to 16.9 per 100,000 live births over the past 20 years, making it the only country in the Organization for Economic Cooperation and Development with rising rates of maternal mortality. PPH accounts for 11% of maternal deaths. As to the study's broader applicability, Dr Wiesehan said in an interview, "findings of effectiveness in terms of reducing PPH-related SMM are well known outside of California. In terms of costs, however, it is more of an unknown how much is generalizable. It would go a long way if another state quality care collaborative implementing such a project recorded costs prospectively. Prospective costing, particularly microcosting, would be optimal to precisely place where the most, or least, value of this quality improvement project is achieved."

Infantile Hemangioma: Analysis Underscores Importance of Early Propranolol Treatment

Among patients with infantile hemangioma (IH), initiation of oral propranolol 3 mg/kg/day prior to 10 weeks of age was associated with a significantly better rate of treatment success, results from a post-hoc analysis of phase 2 and 3 clinical trial data showed. "It is widely accepted that oral propranolol should be started early to improve the success rate, but proposed thresholds have lacked supportive data," researchers led by Christine Léauté-Labrèze, MD, of the department of dermatology at Pellegrin Children's Hospital, Bordeaux, France, wrote in the study, which was published online in Pediatric Dermatology. In the pivotal phase 2/3 trial of propranolol of 460 infants, published in 2015, the mean initiation of treatment was 104 days, they added, but "in real-life studies, most infants are referred later than this." In addition, a European expert consensus panel set the ideal age for a patient to be seen by a specialist at between 3 and 5 weeks of age, while an American Academy of Pediatrics Clinical Practice Guideline set the ideal age at 1 month. To determine factors associated with a higher success rate with oral propranolol treatment, such as age at treatment initiation, the researchers analyzed data from the pivotal phase 2-3 clinical

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trial of oral propranolol in IH. They used Generalized Additive Model (GAM) charts with Generalized Linear Models (GLM), then a rule discovery algorithm, to identify subgroups presenting a high probability of occurrence of the predefined outcome: success at 6 months of treatment (defined as complete or nearly complete resolution of the target hemangioma). Study coauthors were Ilona J. Frieden, MD, of the department of dermatology at the University of California, San Francisco, and director of the UCSF Birthmarks & Vascular Anomalies Center; and Alain Delarue, MD, of medical affairs at Pierre Fabre Dermatologie, Lavaur, France, which markets the pediatric formulation of propranolol approved by the Food and Drug Administration in 2014 for treating IH. They found that patients who started oral propranolol 3 mg/kg/day before the age of 10 weeks had a success rate of 86%, while those who started treatment after 10 weeks of age had a success rate of 60%. "Our clinical experience suggested that starting early propranolol gave better results on infantile hemangiomas; however, we were surprised" by the significance of the difference, the three study authors stated in an e-mail reply to this news organization.

EU Commission Approves Delay in Medical Devices Law to Avert Shortages

The European Commission said it approved delaying the deadline for companies to comply with a new law regulating medical devices in order to prevent shortages of lifesaving equipment. The proposal now must be adopted by the European Parliament and Council through an accelerated process, the EU executive said in a statement. EU Health Commissioner Stella Kyriakides submitted the proposal, saying challenges in implementing the law were threatening supplies of critical devices, such as catheters used for surgeries on newborns with heart conditions. The law requires all devices to be re-certified by May 2024, but the new proposed deadlines push that back to the end of 2027 and the end of 2028, depending on the risk classification of the device. The Medical Devices Regulation came into effect in 2021 and was introduced after the 2010 scandal of exploding breast implants manufactured by a French company that exploited loopholes to sell faulty products at profit.

Nitric Oxide from Room Air – The Development of the LungFit PH 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 Dr Fred Montgomery, Inventor of LungFit and who has spent over 30 years developing and patenting nitric oxide delivery system technology that resulted in the commercialization of nitric oxide therapy.

Can you provide us with the history of your work in nitric oxide and designing technology to deliver nitric oxide to patients?

For the past three decades, I have devoted my career to developing nitric oxide delivery systems and making inhaled nitric oxide (iNO) more accessible for clinicians and patients. My involvement in nitric oxide began in 1993 at Ohmeda, the medical systems division of British Oxygen Company, an industrial gases company. We were one of the few businesses that had pharmaceutical and device development programs, as well as high quality, medical grade gas. We started iNO device development with a very basic delivery device that was being used in clinical trials. My development partner and co-inventor, Duncan Bathe, was on site in the UK when they put the first patient on iNO. The baby responded well and went home a few days later — it really was an exciting time for our team. After the clinical trial device, we started work on a commercial delivery system (INOvent) that could be taken to the bedside of a patient needing iNO treatment and be connected to any ICU ventilator. In January 2000, the FDA cleared the INOvent delivery system, making it the very first commercial system to be approved by the FDA.

Commercialization, along with the R&D program, shifted to INO Therapeutics, then Ikaria, followed by Mallinckrodt. We continued to develop delivery technology, focusing on ease of use, automation, and patient safety. In 2006 we received FDA clearance for the next-generation device, the INOmax DS. Additional next-gen system advancements quickly followed with the INOmax DSIR. These advancements and FDA clearances included adding a transport system for air and ground, increasing the geographic area and reach of iNO to treat and transport critically ill patients.

Our device development team also created the INOblender for bagging patients and the INOpulse for use in ambulatory patients with PPH. Duncan and I are on these patents as well and are proud of the work our team accomplished to innovate iNO delivery and make it easier for clinicians to access and treat with iNO.

However, the main challenge that we could not address at the time was how the iNO was supplied. The 45 lb pressurized iNO cylinders required for administration are big and bulky to transport. A sophisticated network and infrastructure are also required to manufacture and distribute the gas supply and then return the empty iNO cylinders. If you think about it, iNO cylinders might be the most inefficient drug packaging out there, with only 2 grams of actual iNO in a 45 lb package with 99.92% of the content being nitrogen. There was some promising research that provided evidence that we might be able to address this challenge, so Duncan and I decided to pursue development of an iNO system that took cylinders out of the equation.

What gave you the idea to create nitric oxide from air using electricity?

It's been known that lightning produces vast quantities of nitric oxide, and that it can also be produced in the lab by arc discharges. There was almost 20 years of published scientific research that demonstrated nitric oxide could be produced from electricity and room air. Various patents were filed, but the research did not progress to a level where you could produce the iNO in a controlled and continuous manner.

In 2011 Duncan and I left Ikaria to see if we could replicate what others had published and take it a step further to develop an alternative to iNO cylinders using electricity. The challenge was that if we wanted more iNO we had to use large spark discharges, and the bigger the spark, the more intense the current and the shorter time the electrodes lasted. The arcs only lasted a few micro-seconds and they could not generate the amounts of continuous nitric oxide we needed. While we were focusing on trying to control the quick, big spark, Duncan had an "aha" moment and asked a couple of crucial questions:

- What if we could inject current into the plasma and extend the time of the spark to produce more iNO?
- How do we control the current to a low level and maintain the spark for longer at a lower energy level?

Dr Fred Montgomery has spent over 30 years developing and patenting nitric oxide delivery system technology that resulted in the commercialization of nitric oxide therapy. He led the development of the INOvent delivery system for Datex-Ohmeda, which in 2000 was the first FDA 510(k) cleared nitric oxide delivery system that introduced inhaled nitric oxide therapy to the commercial market. He then established the Medical Device Group at INO Therapeutics, which developed the INOmax DS and INOmax DSIR products, the leading nitric oxide delivery systems in the United States to this day. Dr. Montgomery left Ikaria (formerly INO Therapeutics) in 2011 and cofounded NitricGen Inc. Since 2017 he has worked at Beyond Air, commercializing its LungFit PH family of products and further innovating delivery of iNO. He received his Doctor of Philosophy degree from the University of Salford, England and his Master of Business Administration from the University of Bradford, England.

Dr Fred Montgomery with the FDA-approved LungFit PH, the first and only 3-in-1 integrated system that generates and delivers inhaled nitric oxide from room air.

We brainstormed a few approaches and finally reached the stage where we could control the current at 70 mA in the plasma and extend the duration of the plasma from 10 µs to 20 ms, a range of 2,000:1. This change provided the flexibility to generate very small to very large quantities of NO from room air, a range of 0 ppm to 1500 ppm. We then developed a filter to remove the resulting impurities, O_3 and NO_2. In October 2011 we filed a patent.

In 2017 Duncan and I joined Beyond Air to commercialize this new technology, creating the LungFit family of products. At Beyond Air we have made significant investments in the technology with a robust product development team and research pipeline. Our current research focuses on a range of applications and therapeutic areas using high-concentration iNO (80 to 250 ppm) for antimicrobial treatment: Viral community-acquired pneumonia (VCAP), including COVID-19, bronchiolitis, and nontuberculous mycobacteria (NTM) lung infection, with additional research planned for the use of iNO to treat severe exacerbations due to lung infections in COPD patients.

What is the Ionizer™?

The Ionizer technology is the core of our LungFit platform and family of products. It is what generates the iNO from room air over a wide range of concentrations and at low electrical power. The LungFit product family includes the FDA (PMA) approved LungFit PH, which generates low concentration iNO (0-80 ppm) for use in the NICU to treat persistent pulmonary hypertension in newborns; as well as our LungFit PRO and LungFit GO, which generate high concentration iNO and are being studied for antimicrobial treatments in the hospital and home settings.

How does it work?

The Ionizer is a small chamber with two electrodes within each LungFit system that draws in room air and uses the power equivalent to a 60-watt light bulb to ionize the nitrogen and oxygen molecules. The molecules recombine as nitric oxide, and low levels of NO_2 are created as a by-product. The NO_2 Smart Filter then removes the NO_2 from the internal circuit. It's the Ionizer that gives us the flexibility to generate unlimited, on-demand iNO from room air.

We've come a long way since we first started testing this concept in 2011. With this simple, user-friendly technology, we can open access to iNO treatment at the global level in countries and on continents that don't have the infrastructure to support the use of cylinder-based iNO delivery systems. This is really exciting for me—removing barriers to care and empowering clinicians with this life-saving therapy.

Can you describe the LungFit Technology and how it fits into NICU operations?

We received US Food and Drug Administration approval for the LungFit PH on June 28, 2022. The LungFit PH generates iNO, a selective pulmonary vasodilator, and delivers it into the inspiratory limb of the patient breathing circuit of an ICU ventilator in a way that provides a constant concentration of nitric oxide, as set by the user. In the US, iNO is indicated to improve oxygenation in neonates with evidence of pulmonary hypertension. Outside of the US, including Europe, iNO has this same indication, and is also indicated for patients of all ages who are undergoing or have undergone heart surgery and develop pulmonary hypertension. We are currently working with the US FDA to expand our label to include this indication.

The LungFit PH is the first and only 3-in-1 integrated system for iNO generation, delivery, and monitoring. The system is fast, precise, and simple—power on and in seconds you can generate unlimited, on-demand iNO from room air, regardless of dose or flow. Because we generate iNO from air we are finally able to give clinicians in the NICU access to iNO at the bedside without pressurized 45-lb cylinders used by incumbent technology.

Given our involvement in the R&D that went into developing the first iNO system approved in the US and then subsequent systems, we had years of clinical, real-world feedback from users that helped inform the development of other key elements of the LungFit PH. It was also imperative to simplify the pre-use check and provide clinicians with immediate, on-demand access to iNO to treat their critically ill patients.

We kept the elements from our previous developments that worked well, such as the simple user interface, gas monitoring, and connections to the ventilator breathing circuit. At the same time, we eliminated the things that caused problems for users that came with using bulky 45 lb high-pressure cylinders. Examples are: no need to transfer heavy cylinders around in the NICU, no regulator connections to high pressure cylinders, no leak checking the connections, and no purging the gas lines of NO_2 before it is used on the patient. This all streamlines the workflow and reduces the setup time required to start iNO therapy.

How is the LungFit PH different than alternative nitric oxide delivery systems used in the NICU?

The incumbent iNO delivery systems in the NICU uses 45-pound

cylinders that deliver 800 ppm iNO and are pressurized to 2200 psi. The traditional cylinder-based iNO delivery system have worked well since their conception in 1999, but have various burdensome hurdles, specifically the iNO cylinders. These cylinders take up a large amount of storage space, must be returned to the manufacturer once used, require physical monitoring and cylinder emptying, pressure-testing, manual purging, and there is the potential for iNO leaks and wasted iNO before patient use.

The LungFit PH system generates iNO from room air, and consistently delivers and monitors iNO all in one compact system in under one minute. This system does not require reservoirs of iNO and automatically purges the delivery line with room air, eliminating the risk of unintended NO_2 bolus delivery to the patient. Additionally, the LungFit PH system includes a 2.5 oz NO_2 Smart Filter that removes NO_2 from the internal circuit, only takes a few seconds to replace, and can be stored at the point of care. The filters last 12 hours regardless of iNO dose or flow, creating predictability for the clinicians.

Nitric oxide has been researched and proposed as a therapeutic option to treat various cardiopulmonary conditions. What therapeutic areas are you currently focusing on as part of the research and development pipeline at Beyond Air?

Thanks to the Ionizer technology at the center of the LungFit family of products, Beyond Air is designing systems for a variety of clinical settings with the potential to treat across a broad spectrum of therapeutic areas. FDA approval currently includes PPHN, but we are not stopping there. We are researching treatments for viral and bacterial infections and NTM lung infections. Pending FDA review, we plan to conduct a pilot study to evaluate high concentration iNO to treat severe COPD exacerbations due to lung infections in hospitalized patients.

Beyond Air has conducted multiple pilot studies with the LungFit PRO system at 150 ppm of iNO to treat viral community-acquired pneumonia (VCAP), including COVID-19, resulting in promising safety and efficacy data. We are currently in discussion with the FDA on a US trial design for VCAP, including COVID-19.

This past fall, *The Annals of the American Thoracic Society* published a detailed review of our third pilot study of iNO in bronchiolitis patients. The study concluded that efficacy outcomes suggest intermittent administration of 150 ppm of iNO may be favorable, compared to the lower concentration, in shortening the time to improvement in clinically significant endpoints for hospitalized infants with moderate to severe bronchiolitis. The publication offers an overview of the study design and previously announced results, as well as the rationale for conducting a pivotal study.

We also released favorable safety, tolerability, and efficacy results from the at-home pilot study in patients with NTM lung infections treated with high concentration iNO using the portable LungFit GO System.

Beyond Air is the first bio-pharma company to prioritize investment in the research and development necessary to harness the power of nitric oxide. From hospital to home, our goal is to deliver global access to iNO and empower clinicians with more treatment options across a range of therapeutic areas.

Unplanned Extubations in the Neonatal Intensive Care Unit

Brian Walsh, PhD, RRT, RRT-NPS, RRT-ACCS, RPFT, FAARC

Well, thanks for that warm introduction and then happy Respiratory Care Week to all you guys out there. Appreciate everything that you do for our patients.

One of the things that we're going to talk about today is unplanned extubations, in the NICU. It's something that, I think, many of us have worked really hard to reduce, but it still often occurs, quite often.

When it comes to the introduction, we'll talk about the definition of unplanned extubations and we'll step through an overview of unplanned extubations in the NICU, clinical outcomes, financial implications, evidence-based approaches to try to reduce unplanned extubation and then it will end with technology solutions.

Let's first talk about what the definition is of an unplanned extubation.

You may have heard the term "accidental", which we shouldn't try to stay away from that term in this space, because it really is an intentional dislodgement of the endotracheal tube or unplanned, from that aspect of it.

Sometimes people say: "Hey, well the baby decided to remove the tube themselves", they know better, things like that. Really take the emphasis around this extraordinary event. We need to shy away from that and really use the UE or unplanned extubations because we don't want to take the emphasis off of this event. That obviously may occur when the child pulls at it, or bats at the tube or pulls the ventilator circuit. It could be done by hands-on care when we're actually carrying, weighing those types of things and then also family centered care that we often encourage as we go along.

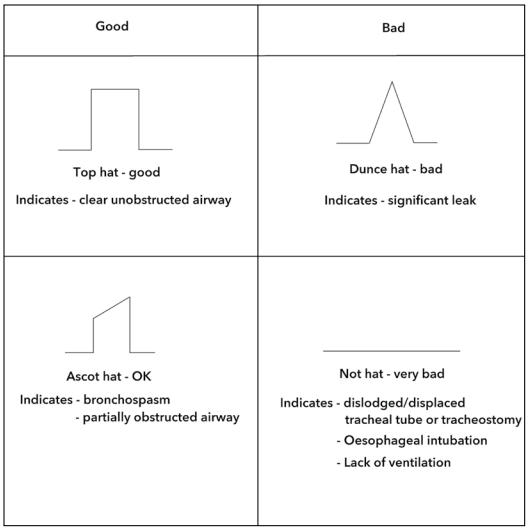
Let's talk about the incidences of unplanned extubation in the NICU. Reports vary between .54 to 16.1.

Obviously if you worked at that 16.1 place, that's pretty big range. It's per 100 endotracheal tube days, so, don't be confused with the associated pneumonia, which is per thousand days. Think about that as we go along. Often the benchmark is to try to get it to 0, but less than one is often preferred. The incidences are that between 14 and 41% of infants experience a UE during their hospitalization.

When it comes to quickly assessing the patient for an unplanned extubation, I often like to use the DOPE algorithm. And I'm not calling someone a DOPE, I'm just saying: "Hey, this is something that we need to think about." We're thinking about Displacement. And so, confirming that, whether it's Obstructed, Pneumothorax or Equipment failure.

So, let's step through this as we go along.

First is Displacement. One of the tools that we often use is look and see, like an inspection. Is there chest rise and fall, is there favor, and then the endotracheal tube will caution you. Chest rises and falls, particularly in the micro-preemies, it is really difficult to understand, especially when they're spontaneously breathing all around, because we tend to encourage that, obviously. Certainly, you can listen to breath sounds. And it may be louder on the right than the left showing you that there's maybe a right mainstem intubation. But really the hallmark is endtidal CO2, whether you use colorimetric CO2 or capnography, where you're actually looking at a waveform. I have some examples over here of waveforms that are actually detecting rather good versus bad.



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The one that we are really focused right now is obviously very bad, there is no rise of End Tidal CO2, which means that the patient is probably extubated.

When it comes to Obstruction, we'll quickly go through these. This is not really the focus of our talk today, but certainly in- line suctioning has become the standard. It allows us to quickly look and grab those secretions if we need to.

Obviously, we want to use our brain and wonder if they had a history of secretions after suctioning, do they improve their saturation, chest rise and fall, or EtCO2.

When it comes to Pneumothorax, this is an area in which I'm really proud of us. I think we've done a magnificent way of reducing the incidences of pneumothorax and to the point where it's actually become pretty rare. I often will suspect the airway before a pneumothorax just because of our improvements in our strategies of ventilating these kids.

When I first started in the early 90s, this was actually fairly common because we used pressure control ventilation and we did not monitor routinely tidal volumes. That's something that we have to think about. But you need to roll it out, that's part of the DOPE algorithm.

Inspecting that chest rise and fall, if you see asymmetrical chest rises and falls, that's obviously a very big sign that there's something going on there, whether it's an obstruction or a pneumothorax.

Certainly, auscultation is important, and then still the gold standard is transillumination, although I think ultrasound and those types of things are really starting to become standard of care at the bedside because ultrasound has become so much smaller, so much easier to use, and we're starting to train lots of people to actually be able to use that. But, until then, transillumination has been helpful in that.

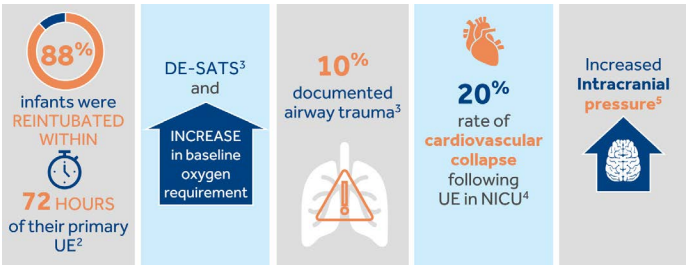
But what you need to do is to make the space dark, and sometimes that's a little bit difficult to do in a very busy ICU. Obviously, when it comes to Equipment Failure and troubleshooting the ventilator if the ventilators is alarming.

We train our nursing colleagues to take over and bag, until we can actually troubleshoot or get to the bed space to troubleshoot it if you're there already.

As a Respiratory Therapist, you can troubleshoot the ventilator and manually ventilate as appropriate, to see if you can actually improve the desaturation or whatever caused the event to begin with, from that aspect.

Let's talk about clinical outcomes. I think that is something that's really important as we go along. We'll think of short term as well as long term impacts to health.

Short term sequelae are that 88% of infants get reintubated within 72 hours. You may think that's high but pay attention to the 72 hours. In a study, they looked at that at a longer period of time, a lot of people do 24 hours, therefore the instances dropped quite a bit because sometimes these babies can do fairly well for the first day or two, but when you go out to three days then obviously that increases and so, so don't downplay it. It's an important event that actually occurs in the short term.



Of course, the desaturations increased baseline oxygen requirement 10% documented airway trauma from having to re-intubate 20%, and this is the ones that, at least in my experience, cost me the most heartburn, and often say one unplanned extubation is too many, because you can actually have patients who actually have cardiovascular collapse and it happens as high as 20% of the time.

And then, last but not least, it is particularly important in our micro- preemies the increased intracranial pressure either from the high CO2 that goes up because they can't ventilate themselves, or from the intubation itself causing high intracranial pressures. These guys have very delicate general metrics and they can actually have a bleed as a result of that as well. We certainly don't want to contribute to that if we can avoid it.

Some long-term ones are associated with bronchospasm, aspiration pneumonia, and particularly these children that we are feeding quite a bit, so they can actually have a pretty whopping pneumonia that occurs in these situations.

And even aspiration of just gastric content in the beginning can cause these bronchospasms and be really devastating to their lung function.

For the short-term as well as the long-term hypotension and arrhythmias can occur typically from hypoxia and then of course cardiopulmonary arrest and even death, which can occur in the wrong patient and certainly they are at increased risk of unplanned extubations or repeat of that as well.

There are a couple others where repeated unplanned extubations are associated with longer term. Tracheostomy, subglottic stenosis, and other injuries that can occur from the multiple repeated events of putting endotracheal tubes in there. And we already talked about the intraventricular hemorrhage.

When it comes to financial implications, this is something that was a little bit eye opening to me when we were diving into the literature about this topic, because a lot of times some people have the opinion that, you know, it's a risky business, right? We're saving kids' lives and things like that. Unplanned extubations are bound to happen and often many of the kids don't get re-intubated. So, these are attitudes that I've heard as time has gone on about unplanned extubations. Some of them, I think, are unfounded a lot of times, but we went through short term and the long-term implications, but now let's talk about the financial implications as well.

Some of the long-term financial implications are that they often, when they have an unplanned extubation and get re-intubated,

they have approximately a week longer duration of mechanical ventilation. This leads to about 10 days length of increased length of stay in the hospital and is associated with about 50,000 dollars of increased hospital cost. These are pretty recent data, only a couple years old.

Obviously, inflation is hitting all of us, right? And so, I have a feeling this may go up from there. When it comes to evidence-based approaches of how to look at reducing unplanned extubations, there's been a lot of work in this area and a lot of these folks should be applauded for their hard work. And of course, like we've done with ventilator associated pneumonia, extubation bundles have been really helpful in reducing unplanned extubations. We'll stop a little bit deeper into that.

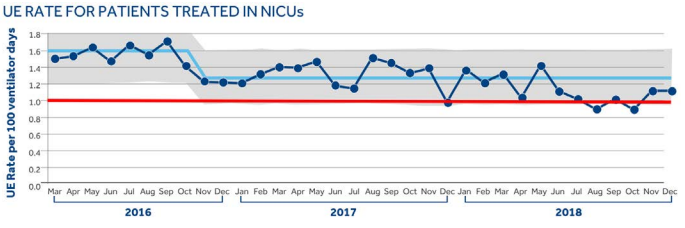
When it comes to unplanned extubation bundles, a lot of times what people are talking about is standardization. That's really important, in other words, the standardization of how we actually secure endotracheal tubes, how we manipulate those endotracheal tubes, even do chest X-rays. You want to make sure that we're all either assessing the landmarks from the gum or the nare, and not using things that move, like the lips or can swell because it can actually be hard to tell if it actually is in the same place or not. We all have to use the same landmark.

Same thing with standardized securing method, whether that's a NeoBar like we'll talk about it in a few minutes, or just taping, types of taping techniques that we're all doing it the same.

Protocols for invasive procedures, particularly when we're covering the head, kangaroo care, where we are giving over to the parents to hold and secure the ventilator circuits, routine positioning of babies when we turn them to help with their head molding and such, and then even switching beds or weighing a lot of times can be a high risk situation that we definitely want to do.

Multidisciplinary is also something that we need to continue to support and make sure that we have education across the dimensions, as well as doing root-cause analysis, or apparent-cause analysis following an unplanned extubation, and again, not contributing to a high-risk service that we provide and that we're always going to have some things. We really need to shoot for zero unplanned extubations if at all possible and always continue to strive until we get there.

When it comes to bundles, in this study, you can see here in their chart over a basically a three-year period of time, they've actually been able to reduce their unplanned extubation rates from roughly 1.55 to 1.2. Again, sadly not below that 1 threshold that we are still striving for.



When we assess the patients, we want to eliminate unplanned extubations and that harm that's associated with it, and we should always strive for that zero that I was mentioning before. I know it seems impossible, especially for you guys that take care of these guys day in and day out, but I promise you if we don't continue to strive for it, we will miss it every time, right?

Other things that have been on the focus now is looking at the endotracheal tube positioning and decreasing unplanned extubation by using that method.

Obviously, high endotracheal tubes are a problem. When it comes to these really small neonates, half a centimeter can mean the difference between intubation and extubation. So high ETT tubes are associated with that unplanned extubation. We want to optimize it to make sure that it's at that T1 level on chest X-ray.

Additionally, when we're advancing the endotracheal tubes, a lot of times there is a low risk, a lower risk of unplanned extubation during that. So, we should always be cautious when we're doing that, and I'll often stick my finger further back to keep it from kinking or pressing into the back and not actually advancing during that process.

Let's review some technologies that have come along the way. One of the things that we want to think about is that, when we have this current challenge of unplanned extubation, neonates have an increased risk factor for unplanned extubation. Some people contribute that to

sedation, or lack thereof sometimes, that we use in our neonates and their ability to move and pull their head away.

We already mentioned the high prevalence rate that 14 to 41% of infants will experience an unplanned extubation during their NICU hospitalization.

Obviously, if they do have an unplanned extubation, a lot of times they'll increase their cost and worsen their clinical outcomes. And because of those clinical outcomes, we have a longer length of stay.

They're obviously on the ventilator longer and that can actually contribute and makes this cycle continue on and repeat over and over again. So, we need to something to try to interrupt that cycle.

Some current limitations of our current practice are that unplanned extubation rarely can get below that one goal that we're often shooting for to review. People have obviously assessed the location of the endotracheal tubes by chest X-rays, which is technically the gold standard, but we often don't do them frequently or daily because we want to reduce the exposure to radiation. And it is a snapshot in time, it doesn't mean that it can't move from there a lot of times. So, when we're doing the chest X-rays and their routine assessments of the endotracheal tube and standardizing the markings, a lot of times that will help you understand where the tube is. But when it actually does occur, the DOPE mnemonic can address these decompensations. A lot of times.

One of the things that I was thinking of when I was developing this talk is that we used to often encourage restraint therapists in particular if you could not quickly go through this DOPE algorithm and assess to pull the tube because we were actually encouraged by the neonatologist that the likelihood of it actually occurring was pretty hot and therefore it would be easier to bag

valve mask them and avoid the hypoxia related to a delay in diagnosis. So, it was almost preferred in those situations.

We've been able to reduce our unplanned extubations, pretty well, but sometimes the thought still goes through my mind that if I can't quickly do this, I'd rather just pull the tube and bag valve mask and call for help versus watching a baby who's really hypoxic and really struggling to breathe. And then, me trying to go through this algorithm in a quick fashion, because it happens so frequently; people have started to employ that approach. I'm not saying it's right or wrong, it's just one of those things that, in my practice, I've seen us do quite a bit, and I really would like to change that.

So, one of the technological solutions that I'm excited to chat with you guys about is SonarMed. What is this device? It's a pretty cool device in that it uses acoustic waves to interpret the changes in the distance, as well as the diameter of the endotracheal tubes. This can actually be a real time warning system and it can help us determining whether the endotracheal tube is in or out or up or down in these situations.

One of the really innovative ways that it does this is it has a really nice display that allows us to give real time feedback of the positioning of the endotracheal tube, and you can have an audible alarm when it changes from that baseline. It also can give you real time feedback on ET tube obstructions.

So, whether it's suction and secretions of the endotracheal tube, especially the really small ones when they get warm and they become pliable and they get like fish mouth as I call it.

And, depending on the positioning of the endotracheal tube, it can actually kink and so this would help identify those types of obstructions, as well as secretions.

You can use it to determine the quality of your suctioning, whether you got all the secretions out or removed those potential mucus plugs in those situations, as well as the airway circumference.

Another thing that I was thinking of is that some of you guys that have done this as long as I have, you've seen us keep babies intubated for prolonged periods of time and sometimes the numbers wear off of the tubes. So, we end up taking a permanent marker and lining up to where it used to be. Often, that has become a problem, as well, because these endotracheal tubes sometimes will stay in for weeks, if not months, in these infants, because we do a really good job of monitoring that. But sometimes we lose that location aspect in which this particular SonarMed tool would be able to help us with that aspect of it.

I want to share with you a really cool experience that Children's Hospital Illinois shared last year. They had no standardized collection of unplanned extubations. They discovered that they had a high rate of unplanned extubation compared to their other centers. They utilized the NeoBar in the majority of their cases, but they had no routine nasal intubation, and so their major interventions trial was that they used the SonarMed system and they did root cause analysis for all unplanned extubation.

They educated the RT's on proper use of the NeoBar and sizing and taping. They audited that bundle of measurements. They did ET tube annotation by radiology on the chest X-ray to verify the endotracheal tube positioning to be at that T1 space. They did mass education on the unit to proper positioning for chest X-ray. They encourage two-person care for all intubated subjects and then their baseline rate was 2.1 of 100 ventilator days prior to the interventions. They started off two times what we would like to see.

The "So what?" here is that they've chronically had a low lung disease rate compared to other centers. And unplanned extubations were associated with physiologic changes, and hypoxemia and hypercarbia, and increased arterial pressure, and increase in cranial pressure, which often leads to this less than controlled in an endotracheal tube intubation.

So, if you intubate at night in those types of situations, we know that repeat intubation is not great and especially when they're done emergently. They have a risk of airway injury, ventilator induced lung injury, associated pneumonia from potential aspiration pneumonia, and then other non-pulmonary complications such as HIVH.

Unplanned extubations obviously are a core measure by the US News and World Report, and that's an indication of quality, right?

If you're really particular about your endotracheal tubes and you can keep that number lower, then it is assumed that you provide a higher quality of care. So, the team knew that they could perform better.

The SonarMed system is the only intervention that we've been able to actually do recently. We've tried bundles and things like that, and they were able to drop their unplanned extubation from that baseline of 2.1 to 1.4, but they really still cannot go below that 1 that has been the goal of care.

They also noted that unplanned extubation were actually occurring during kangaroo time or skin to skin. We encourage two people to help with those situations, but sometimes there's just not a lot of space. Not all the staff were completely bought in on the two-person care that a lot of people have gone to with the standard of care and they presumed that the ET tube tended to move. Often, we've seen it, I've seen it. It's like I've almost magically re-intubated someone because I've seen the tube come way out and then I just pushed it back in as a natural reaction and somehow it stayed in. I have no idea sometimes how that actually occurred. The ways of measuring that shift when you're positioning, especially a child that's on the oscillator, it can be really difficult.

Then, the preference of the mother or the father or the caregiver to do skin to skin and positioning that they'd like to hold has been difficult to understand how to hold, how to actually tape or secure the ventilator circuit in the endotracheal tube.

Some of the unplanned extubations also occur outside of care. So, normal respirations, typical movements, they even have hiccups, they can swallow. I've even seen them turn the tube out that caused that endotracheal tube that seems to be at the right spot to be higher or lower in the trachea.

We obviously know the head position is important as well. Whether it's up or down can change the position of that endotracheal tube.

And so, they wanted a better way to monitor, particularly in real time, those situations of kangaroo care and such and so. They were actually one of the NICUs that were able to come lower than that 1 down to .51 per 100 ventilator days for the last six months. They certainly should be applauded for being able to do that.

They observed a more focused approach to invasive mechanical ventilator patients. They're doing the bundles, they're doing all the right things, but now they have that added tool of the SonarMed to be able to make us aware of where the tube is at any moment in time with that migration.

Here are all the things that they started to do. The two-person standard of care, the education with chest X-ray and the positioning and labeling of that, they did NeoBar reeducation, they did debriefings after unplanned extubation and they updated that process as they went along and then they implemented the SonarMed system, and they finally got below that 1 with the implementation of all these plus the SonarMed during this process. They continue to support, and they have an overwhelming belief of, that skin to skin or kangaroo care and bonding between that infant and that parent. It's part of their culture, and they do not want to limit that, especially for patients who are really sick, such as high frequency ventilation. That situation can be scary and intimidating for the parent because certainly no parent wants to cause the demise or a complication of their child.

They found it in those situations, the center has offered that reassurance that they needed to make sure that that endotracheal tube was in place allowed them to have a much more enjoyable time bonding with their infant and they tended to be more up to participate when it comes to monitoring the status of that airway.

So, eliminating unplanned extubation and harms associated with those events remains achievable. And now we have a new tool in the toolbox.

There's no such thing as the silver bullet, but now we have something that's really, really helpful.

I'll stop there and take any questions that may have come up.

The recording of this presentation can be found at https://www.medtronic.com/covidien/en-us/clinical-education/catalog/webinar-wednesdays.html on October 25, 2022.

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Engineering the extraordinary

Racial & Health Equity in the Form of a Free Wearable Breast Pump

Deb Discenza

Yes, really, a breast pump. So why would a free breast pump make a difference? Because it equalizes access in a way that is not already in place in the United States. That is where the **Alliance for Black NICU Families (https://BlackNICUFamilies.org)** comes in and proposes to make their first impactful change for Black NICU Families.

While insurance companies are supposed to increase access to a breast pump for all mothers, it is not easy and it is not equal whatsoever across payers. One insurance company may require a prescription, another may only cover a fraction of the pump's cost. Still another may not cover it at all stating that a customer can use their Health Savings Account (HSA). This maze of requirements makes it really easy for a parent to give up in times of distraction and stress. And the pumps themselves? They cover mostly the pumps that are not wearable and need to be plugged in to work. Many hospitals rent out pumps but the very same pumps are not only old and often extremely heavy (imagine a mother dragging that around after a c-section despite being told to avoid heavy lifting) thereby negating the supposed ease of providing milk.

For a family with a baby in the NICU, breastfeeding is optimal but absolutely challenging for even the most seasoned breastfeeding mother. Medical equipment, a child with a weak suck, developmental challenges and then of course medical issues create a tough situation for Mom and baby. So the Mom will pump breastmilk and deliver to the nurses in the NICU for feedings by NG or OG tubes. The goal is that at one point the Mother will be able to transition to breastfeeding. But again this can take time and so the pumping continues. That pump produces the liquid gold we NICU parents know all about but life has a way of creating obstacles.

For many Black NICU Families, those obstacles are numerous, everything from financial burdens to lack of safe and reliable transportation, balancing work and family obligations and just life in general. That mother may have to go back to work days or even a week after delivering the baby because of the need to feed the family and to pay for childcare

for older siblings is not free. And those same siblings require parenting thereby causing a challenge to balance that alongside pumping that breastmilk.

And for many, despite the increases in the federal law in the United States which notes, "Federal law requires employers to provide reasonable break time for an employee to express breast milk for her nursing child for one year after the child's birth each time such employee has need to express the milk (Section 7 of the FLSA). Employers are also required to provide a place, other than a bathroom, that is shielded from view and free from intrusion from coworkers and the public, which may be used by an employee to express breast milk." Reality check, a Black NICU Mom has a higher likelihood of working in a job that won't provide her with what she needs, period.

Enter the Elvie breast pump where Black NICU Moms have freedom to go about their lives using a wearable breast pump. Innovative and a game changer for Moms everywhere, the Alliance for Black NICU Families saw the opportunity to equalize access to these pumps by buying in bulk and giving them away free to Black NICU Moms through their NICUs, support groups etc. that sign up with our organization. We help the Mom, the baby, the family, the NICU and society all in one generous gesture. We give that Black NICU Mom freedom to handle her life as simply as possible by the use of a wearable

Deb Discenza is the mother to a 30-week preemie who is now 19 years old. She runs PreemieWorld.com, home of "The Preemie Parent's Survival Guide to the NICU" sold in bulk to NICUs and non-profit organizations nationwide. In 2020 she was a Co-Founder of the Alliance for Black NICU Families. Interested in the pump program? Please contact us at contact@blacknicufamilies.org.

pump, one that many would not be able to a and so we make it free and hassle-free.

This is the inaugural equalization program for the Alliance and we hope to provide many more in the coming years. Our organization depends on grants and donations to help further this program and create others. If you are interested in signing up for the Breast Pump program and/or assisting us with fundraising, please contact us at contact@BlackNICUFamilies.org.

Phototherapy in Newborns: Patient Protection and Best Practises

In this feature, Neonatal Intensive Care features informative webinars led by clinicians and healthcare providers about the actual application of specific products and therapies. This webinar features Vikash Dudhia, a NICU clinical expert and director of international business development at Maxtec, and Lexi Less, a Product Manager at Maxtec with a focus on neonatal and oxygen monitoring products.

This webinar is about the importance of eye protection during phototherapy treatment of newborns, highlighting how all forms of eye protection are not created equal and the important things to consider when choosing a phototherapy mask for your infant patients.

Lexi Less: Hi everyone. Thank you so much for taking the time to join us for our webinar today. Today, we're going to be talking about newborn phototherapy treatment and how to optimize patient protection and care during phototherapy. Any questions that you have about the presentation and the material we present today, we're going to get to during a Q&A at the end of the presentation. Without further ado, I am Lexi Less, and I am a product manager at Maxtec. Today I am presenting with Vik and I will let him introduce himself.

Vik Dudhia: Hi everyone, my name's Vik. I'm Vik Dudhia. I'm the director of international business Development here at Maxtec, and we're really pleased that you've joined us today.

Lexi Less: All right, so just to give you an idea of what we're going to be talking about today, we're going to be doing a quick overview on jaundice, which is the condition that phototherapy is used to treat. And then we're going to talk about phototherapy itself and the different forms of treatment and the risk. And then we're going to have different types of phototherapy eye protection, talk about different types of phototherapy eye protection with a focus on the EyeMax 2, and then we're going to end with a Q&A. And then throughout the presentation, we'll have just a couple questions for you to answer as well.

Vik Dudhia: Great. Thank you, Lexi. So what we're going to do is we'll start off with just a quick background on jaundice. And during this webinar, we're going to be specifically discussing jaundice in the newborn or the infant. Now, it affects three in five newborns, so it's relatively common, and it's described as yellowing of the skin. And that yellowing of the skin or the whites of the eyes, these are result of excess bilirubin in the system. The bilirubin, for those of you that may be not familiar, is actually formed as a result of the breakdown of the red blood cells.

Now, jaundice is classified in kind of two ways. We've got them listed here in front of you guys on the screen. The first one, which is the most common, is the physiological one. And as we said, it usually occurs around about day two and tends to resolve itself around about day 10 to 14. So within a couple of weeks, it resolves itself. It doesn't usually need any intervention, but sometimes it does need some extra support, and that comes in the form of light phototherapy. The second classification is common known as pathological. Now, that occurs when there might be an underlying cause to the jaundice. So it could be something like an infection, like a UTI, urinary tract infection, it could be like an endocrine disorder, it could be genetic, something like sickle cell anemia, for example. So really, what happens in the pathological classification is that the body isn't able to expel the bilirubin, which is why it's building up.

So what the clinician would have to do is trying to work out to determine what the root cause is, and then treat the underlying condition. From our experience, and I'm sure for you guys listening today, phototherapy is actually used for both physiological and pathological jaundice. So what's the treatment? So let's look at what the phototherapy is. So essentially, phototherapy is applying fluorescent light, which contains wavelengths of blue-green UV light to the baby's skin. And what that does is it causes a chemical reaction, and that's called photo-oxidation. And essentially what that does is kind of makes the bilirubin more water soluble, so that helps for it to be broken down and expelled from the body much, much easier.

In terms of the phototherapy treatment itself, there's two main types, and the first one is the conventional one, and that's the image that you see on the top right hand side there. And that's where you see the baby lying under the UV light, the phototherapy light with eye protection. The second one is probably not as common as the conventional one, but this is where the blue light is passed through a fiber optic cable, and that cable is woven into kind of a blanket, and the baby is kind of covered with that, and that's how the phototherapy is delivered. There are instances, and we've had discussions with clinicians and end users where both the conventional and the fiber optic therapies are used. But what's really important in both those instances is that the eye protection is used as well.

Lexi Less: Everyone, so you should see a question come up to the right of your screen. We're just curious, in your experience, what therapy do you see most often in hospitals? Do you see the conventional phototherapy being the sole use, or do you see a lot of hospitals supplementing with fiber optic phototherapy as well? Looks like there's a good mix. Awesome. All right. Thanks

If you would like to participate in this feature, as a company or healthcare provider, please contact Steve Goldstein at s.gold4@verizon.net

for participating. So it looks like a little bit more conventional, but there's a lot of fiber optic also.

Vik Dudhia: Oh, that's really good to see. Yeah, it kind of confirms what we've had, right, Lexi?

Lexi Less: Yeah. Yeah, we tend to see conventional being the sole phototherapy treatment.

Vik Dudhia: Great. So let's move on to the risks. And remember we said that jaundice is relatively common, and we also discussed the two main types of phototherapy treatment. Now, if jaundice is left untreated, bilirubin, which is passing through the blood system, can pass across into the brain through the blood brain barrier, as it's known, and then start depositing in parts of the brain. Now, that buildup of bilirubin can cause neurotoxicity. It's often referred to as bilirubin encephalopathy, that's hard to say, or BE. And that's a rare neurological disorder associated with high bilirubin levels in the body. Now, this was first detected in 1904, and it was called kernicterus. I think I got that right. And this is described as a brain dysfunction. And again, it is related to high bilirubin levels. Unfortunately, what this can lead to is brain dysfunction, brain damage, and hearing loss. So if left untreated, the repercussions can be quite serious on the patient.

Now, of course, with any therapy, there is a level of risk. But specifically when we are looking at light phototherapy, the risk is associated with the eyes and specifically to retinal damage. So if the appropriate eye protection is not used during phototherapy, there can be damage to the retinas, which eventually could lead to blindness. So that's something that we really want to avoid, and that's something that is avoidable because it's a common condition and can be very easily treated. So how do you choose what is the right mask? And there are lots of options on the market, and we appreciate that you always have a choice. But those options do have common features. And the common features are the sizes, the material, and the way to actually secure the mask. So usually, most of the options available in the market will have a number of size, maybe three, and that kind of covers from premature babies all the way up to full term.

And there are variety of materials as well. We have seen some where the eye protection almost fixes directly, using an adhesive material, directly onto the face, and that's great because it's allowing the rest of the skin to be exposed to the therapy, which is essentially what you want. But there may be, and this is a consideration where you're selecting your mask, there may be some irritation to the skin because of the adhesive that's used. And remember also, that this patient group does have very fragile and sensitive skin. So that's something to consider.

Other kinds of designs feature a mask that completely wraps around the head. And again, that's great in terms of securing the mask. But I guess on the flip side, sometimes the material is quite thick. So that doesn't allow full skin exposure to the light therapy, which is what you're trying to achieve. And also, it may kind of interfere the treatment in terms of how the clinician navigates around the patient. For example, if the patient's having CPAP, it could make it a little bit more difficult to access parts of the body.

Lexi Less: All right, so now we're going to talk about the Maxtec EyeMax 2. So one of the most common concerns that we hear from caregivers about different phototherapy masks that they've used is that they really struggle to find one that stays on the baby's head securely and doesn't move around. And infants, they move a lot more than you would expect, and it's important to have an eye mask on them that fits them really securely so that their eyes stay shielded from those harmful rays. So we're offering the EyeMax in three different sizes, and they're each adjustable, with not just one single adjustment point, but there's actually two Velcro points that you can adjust the fit to really make sure it fits perfectly on the patient. And we've heard by a lot of people that have used the EyeMax that the EyeMax stays put better than any other mask that they've used. So that's been really good feedback for us.

Vik Dudhia: Great. Thanks, Lexi. So I'm going to finish off. So the other two points to raise about the EyeMax is that it's gentle on the skin, right? The material that we use doesn't contain any adhesives because we know that the patient's skin is so fragile, so we don't want to introduce anything that may potentially irritate the skin. There is a little bit of flexibility. And people often say, "Well, what does that look like?" And I guess the closest we can get to that is the material is similar to the material used on diapers. It's gentle on the skin. And then the other kind of point, feature, I guess we should call it, is it's lightweight. So it's light and it's thin, so that allows the phototherapy treatment to pass through without any interruption. But also, on the inside, there is some special material that protects the eyes, and therefore the retinas during the treatment.

Lexi Less: Awesome. All right. So as a reminder, you are welcome to submit any questions that you have. We've gone over a good amount of information, so let's give you the opportunity to submit any questions you have in the chat box. So the first question is, does X-ray go through the mask? So yes, you can X-ray patients that have these masks on and the therapy continues to work.

Vik Dudhia: Yeah, that's a great question.

Lexi Less: Vik, here's one for you. How often do you change the mask?

Vik Dudhia: Yeah, again, another really good question, and we get asked this quite often. We would always refer to your local hospital protocols in terms of how often and the frequency of changing those masks.

Lexi Less: All right. And then here is one. How many are in a pack?

Vik Dudhia: There's 20 in a pack.

Lexi Less: Yes. And so each size is sold separately. And each size, you have 20 packs.

Vik Dudhia: You're right. I should have clarified that. So yeah, 20 in a pack of the same size.

Lexi Less: All right. How does the clinician tell when the patient doesn't need treatment anymore? So the bilirubin levels, they're usually tested every four to six hours or so after phototherapy treatment starts, and that can maintain checking if the treatment is working. And then once the infant's bilirubin levels are stable or they start to fall, they check them every six to 12 hours. And

then phototherapy stopped once the bilirubin falls to a safe level, which usually it takes a day or two.

Vik Dudhia: Good question.

Lexi Less: All right. Well, Vik, I think that's all we have today. So thank you everyone so much for joining. To inquire about EyeMax phototherapy mask product samples or to learn more, submit your request at maxtec.com/eyemax.

Vik Dudhia: Thank you so much.

Coping with Uncertainty in the Composition of Human Milk in the NICU

Andi Markell, RD, LD, MSL-BC, CSP and Emily Canata, MS, RDN, CLC

For preterm infants to grow and thrive, enteral nutrition (EN) is carefully calculated in the NICU to meet precise and evolving nutritional needs. A major problem with this approach, however, is that calculations are based on estimations and not the actual composition of human milk. Understanding the factors that contribute to variability in the composition of human milk helps in the development of strategies that ensure preterm infants receive the nutrition they need.

Current EN Feeding Recommendations

In 2022, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) updated its clinical EN recommendations for preterm infants.[1] In their current iteration, they are largely in line with the 2021 recommendations by Koletzko et al,[2] although there are minor differences. The ESPGHAN update features some important changes, including the elimination of different protein values for infants born < 1000 g or ≥ 1000 g, with birthweight no longer being a differentiating factor. It also includes an overall decrease in recommended protein intake down to 3.5-4 g/kg, although protein intake may still be increased to 4.5 g/kg/day in some cases. The ceiling for calorie intake was also increased, allowing intakes up to 160 kcal/kg/day.[1] Table 1 demonstrates the evolution over the past 20 years in the recommended protein and energy intakes for preterm infants.

Nuances of Human Milk Composition

Meeting recommended nutritional intake levels is difficult when the exact composition of the milk is unknown. Pragmatically speaking, most NICUs do not have a breastmilk analyzer, and the analysis of milk has not been standardized. In addition, there are many factors that influence the composition of human milk, including maternal nutrition, environment, maternal BMI, time of pumping, stage of lactation, and production volume.[9,10] Term babies adjust their intake based on breastmilk composition while premature infants are prescribed set volumes.

Even greater uncertainty is introduced when using donor milk. Most donor milk comes from mothers who have been lactating for up to a year postpartum. This milk will have quite different nutrient composition from that of mothers who have recently given birth to a preterm or term infant.[11] While milk banks may measure the nutrient content of donor milk, the same challenges exist with regard to a lack of a standardized milk analysis process.[12]

If that were not enough to introduce considerable uncertainty, the composition of human milk that actually reaches the infant can be altered by the method of administration. Human milk fat has been shown to adsorb onto the tubing, resulting in loss of fat, calcium, and phosphorus. This phenomenon increases with longer time of delivery. Thus, the fat and mineral content of milk received by NICU infants could vary depending on whether they receive continuous or bolus feeding and even the length of the feeding tubes.[13-15]

Throwing Off the Numbers

Striking the right balance between protein and energy is paramount to optimizing EN in NICU infants. In their chapter titled "Energy Requirements and Protein-Energy Metabolism and Balance in Preterm and Term Infants," published in *Neonatal Nutrition and Metabolism*, Kashyap and Schulze write that "protein requirements cannot be determined without considering concurrent energy intake and energy requirements cannot be determined without consideration of simultaneous protein intake."[16]

How can NICUs accurately calculate protein and energy intake given the known inconsistencies in the nutritional composition of human milk? Most NICUs simply use average numbers to calculate calories, typically estimating 20 kcal/oz, but this could be an over- or underestimation of actual calories present. As for protein, some may use preterm protein of 1.6 g/100 g values for a defined time frame, such as until 2 or 4 weeks of age. Others may use term milk values (0.9 g/100 g) only. In reality, we do not know when mother's own milk transitions from preterm to mature, and there is no guarantee that preterm milk is "high protein" or term milk is "low protein."

The 2022 ESPGHAN update recommends that "optimal Protein Energy Ratio (PER) for enteral intake in preterm infants is likely to be around 2.8 to 3.6g/100 kcal,…with PERs at the higher end of this range associated with improved weight gain and [free fat mass] accretion."[1] Nevertheless, PER should not be used alone. Total calorie and protein intakes within the recommended range are still the primary target.

PER can be thrown off dramatically when the milk being fortified is actually higher or lower in protein or calories than estimated. When both calorie and protein concentrations are balanced at

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Table 1. Evolution of Recommended Protein-to-Energy Ratio for Preterm Infants (1993-2022)

Nutrient	Tsang 1993[3] < 1000 g	Tsang 1993[3] 1000-1500 g	Tsang 2005[4] AAP v7[5] <1000 g	Tsang 2005[4] AAP v7[5] 1000-1500 g	Koletzko 2014[6] AAP v8[7]*	Koletzko 2021[2]	ESPGHAN 2010[8]	ESPGHAN 2022[1]
Energy (kcal)	110-120	110-120	130-150	110-130	110-130	110-130	110-135	115-140 (160)
Protein (g)	3.6-3.8	3-3.6	3.8-4.4	3.4-4.2	3.5-4.5	3.5-4.5	3.5-4.0 (1-1.8 kg) 4-4.5 (< 1 kg)	3.5-4.0 (4.5)

*The American Academy of Pediatrics (AAP) V8[7] takes its recommendations from Koletzko 2014.[6]

higher or lower than anticipated in the same direction (ie, both high or both low), PER remains within the estimated range. But if one is high while the other is low, or one is low while the other is high, thus unbalanced, this can have a clinically meaningful effect on the quality of growth.[17] Due to the unknowns of breastmilk composition, the use of PER in the clinical setting has limitations.

Towards a Tailored Fortification Approach

Targeted fortification is an emerging approach where human milk is analyzed, and the dose of fortification is added based on the nutrient content of human milk. This requires access to a reliable milk analyzer, which is not the reality in most NICUs. While this is not currently common practice, it shows promise toward tailoring nutrition therapy.

What NICUs have no lack of, however, is patients themselves. Careful observation of preterm infants, including scrupulous assessment of growth metrics, can be used to individualize fortification. The assessment of growth should be a thorough review of data points including weight, length, head circumference, proportionality of measurements, and change in z scores in relationship to diagnosis, medical therapies, laboratory test results, and actual intake. Simply put, preterm infants who are not receiving the nutrients they need will not grow optimally.

Role of an Exclusive Human Milk Diet (EHMD)

An EHMD, defined as feeding infants human milk fortified only with human milk-based fortifiers, has been shown to have multiple health benefits for infants born very low birthweight, including increased feeding tolerance and reduced risk of necrotizing enterocolitis.[18-23] It also works well with a tailored approach to fortification as Prolacta Bioscience offers four human milk-based fortifiers with differing nutritional compositions. All four of these options have an osmolality below 450 mOsm/kg, allowing clinicians to meet protein goals at any ordered volume. In addition, Prolact CR provides an opportunity to add calories without more protein. This can be helpful when recommended protein targets have been reached but growth goals are not being met, as well as when there are concerns of fat loss related to feeding methods.

Conclusion

Preterm infants have highly specific nutritional needs. It is important to meet these goals as well as consider the balance between protein and energy for optimal growth. This is a challenge based on the unknowns of human milk. In the absence of reliable numbers, clinicians should use anthropometrics and other nutrition-focused assessments to determine if nutritional needs are met. The EHMD, while reducing comorbidities, also offers the flexibility to meet infants' individual nutritional needs.

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Do Vitamins and Nutritional Supplements Affect Fetal Brain Development?

BM Petrikovsky, MD, PhD

In the Summer 2021 issue of the *Neonatal Intensive Care* Journal, we proposed a new protocol for vitamin administration during pregnancy and postpartum.[1] This new protocol in vitamin administration was based on the changing demands by the mother and her fetus throughout different gestational ages.[1]

According to current recommendations, prenatal vitamins should be taken daily in the same dose throughout the entire pregnancy irrespective of gestational age. The American College of Obstetricians and Gynecologists (ACOG) recommends vitamins with 600 mg of folic acid, 200 mg of DHA, 27 mg of iron, 1.00 mg of calcium, and 600 mg of vitamin D. Current brands of prenatal vitamins are nearly identical in their content. Some components of prenatal vitamins, such as folic acid, have proven benefits for fetal development, while others have not shown to be effective.[2] Folic acid supplements have been associated with a decrease in neural tube defects, and preliminary results suggest that vitamin B3 may improve early pregnancy outcomes and possibly prevent miscarriages.[3] However, these benefits are limited to the first trimester of pregnancy only.

A modified table of major vitamins and micronutrients used during pregnancy and lactation is provided below (used with permission):

Table 1

	Pregnant Women	Lactating Women
Folic acid	600 mcg	500 mcg
Vitamin B12	2.6 mcg	2.8 mcg
Vitamin D	5 mcg	5 mcg
Calcium	1000-1300 mg	1000-1300 mg
Iron	27 mg	9-10 mg
Iodine	220 mcg	270 mcg
Zinc	10-11 mcg	11-12 mg

Vitamins, minerals, and nutritional substances may also have side effects. For example, the combined use of vitamins C and E increases the risk of fetal loss or perinatal death. Vitamin A is a known teratogen; its intake is associated with an increased risk of congenital malformations.[5,6] In view of the above, we proposed a modified protocol for the administration of vitamins, microelements, and nutritional substances during pregnancy, with the type and dose changing with gestational age. For instance, there is no need for folic acid after the closure of the neural tube. Calcium is more needed in the second trimester as the fetal skeleton grows, and iron supplements are much needed in the second and third trimesters to prepare for blood loss in labor and delivery.

Trimester friendly vitamin administration will allow for maximum benefit with minimal side effects. For example, in a healthy pregnancy, patients in the first trimester without anemia often experience limited benefits from iron supplements and may also experience constipation.

Can vitamins and supplements enhance fetal brain function and improve intelligence in the future?

The term 'designer babies' refers to genetic manipulations of preimplantation embryos to influence the traits of the child. This editorial does not address genetic interactions aimed at securing phenotypic characteristics, such as height, eye color, skin color, etc. The genetic enhancement of embryos is currently a science fiction, but there are those who advocate for a ban on such practices before they become a reality. Omega-3 fatty acids are known to impact fetal brain development. This has led to recommended intakes of 250-375 mg of Docosahexaenoic acid daily (DHA) for pregnant and lactating women by the Dietary Guidelines.[7] DHA plays a key role in the maturation and functions of the fetal and infant brain and retina. A DHA deficiency tends to decrease learning skills in rat and monkey models.[8,9]

Based on evidence-based medicine, several leading health organizations such as the Academy of Pediatrics, ACOG, and the March of Dimes recommend the consumption of fish or omega-3 fatty acids during pregnancy resulting in ~200 mg DHA per day. Despite these recommendations, consumption of omega-3 fatty acids remains low across the US population. Ninety five percent of pregnant women do not meet the recommendations.[10] Polysaturatured fatty acids (PFA) of the omega-3 and omega-6 families are not synthesized by humans.[12] Therefore, the Canadian Health Authority recommended that pregnant women take 150 mg of fish oil weekly.[13,14] However, despite disagreements on benefits, the majority of authors failed to find any side effects associated with fatty acid supplementations in recommended doses.[11-15]

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From a neurodevelopmental perspective, the third trimester of pregnancy is crucial for the accumulation of fatty acids in the brain. These fatty acids play a vital role in cell signaling, gene expression regulation, and neurotransmission.[16,17] Based on the current scientific evidence, we include the following nutritional substances in a regimen of trimester-specific vitamin supplementation to promote fetal brain development:

Vitamin D: Research has shown that there is a positive correlation between psychomotor and mental development as measured by the Bayley Scales of Infant Development (BSID) and higher levels of vitamin D. Additionally, low maternal vitamin D levels have been linked to an increased risk of attention deficit hyperactivity disorder (ADHD) in offspring.[19]

Docosahexaenoic Acid (DHA): The fetal demand for fatty acids peaks in the third trimester, and maternal circulating levels of DHA decline across pregnancy.[20] There is evidence to suggest that DHA supplementation can have positive effects on infant problem-solving, preschool-age processing, elementary-age verbal abilities, and IQ scores.[21]

Choline: Research has shown that the supplementation of choline at double the recommended amount (930 mg/day) during the third trimester resulted in an improved speed of processing in infants, whereas supplementation with a lesser amount (750 mg/day) did not improve memory.[22]

Concluding Remarks

Currently, women are administered the same content and dose of prenatal vitamins and nutritional substances irrespective of trimester/ gestational age. However, maternal and fetal demands change as pregnancy progresses. Thus, much-needed folic acid in the pre-conceptional periods and early pregnancy has no known benefits in the 3rd trimester of pregnancy. Demands in fatty acids grow from minimal in the first trimester as the fetal brain is growing and developing. Therefore, the slogan "one size fits all", is no longer applicable. We propose a new term "Fitamins" (trademark pending) to better describe a new type of pill, which combines various doses of vitamins and nutritional substances to better respond to maternal and fetal needs as pregnancy progress.

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Saving Neonates with Extended Dwell Peripheral Intravenous (EPIV) Catheters

Machele Duvall, RN, BSN

Any nurse who has had the privilege of working with preterm infants in the neonatal intensive care unit (NICU) will readily tell you that one of their greatest concerns is addressing the vessel fragility of a patient requiring vascular access. Innovative approaches and devices are slow to enter this arena and are, understandably, viewed with a wary eye until evidence-based research is properly, and convincingly, provided for the clinical staff. One very effective approach incorporates the use of Extended Dwell Peripheral Intravenous (EPIV) catheters, which is the focus a new, carefully conducted study by Marchetti, et al., 2023 in the February 2023 edition of *Advances in Neonatal Care.* The project also provides invaluable decision-making information for the practitioners who dedicate themselves to this noble cause.

Neonatal vessels are extremely fragile and when medication or nutritional needs require vascular access, many nurses have difficult choices to make and the wrong choice may seriously compromise the morbidity, or the mortality, of the neonate. Therefore, choosing the proper clinical reason, the proper device, and the proper insertion technique, are all critical elements.

The study clearly indicates that previously the use of peripheral intravenous (PIV) catheters and peripherally inserted central catheters (PICCs) were the standard in the NICU. PIVs are easily placed bedside into the scalp or an extremity by a registered nurse (RN) but cannot be used for medications or nutritional fluids that are irritants to the vessels and they have a very short dwell time of less than 2 days. On the other hand, PICCs may be used for any type of medication or nutritional fluid, require extremely sterile conditions for placement by highly skilled personnel, an x-ray to confirm placement, and are extremely susceptible to central line infections.

Then a new product, the EPIV, pioneered by Neo Medical, Inc. as part of the NeoMagic line of products, arrived on the scene. The device allows placement at bedside into the peripheral venous circulation using the same technique used for PICC insertion (the Modified Seldinger Technique, or MST) without the concern for serious central line infections. The greatest benefit is that *the dwell time is up to 29 days.* The study suggests that EPIV use reduces the number of placement attempts, thereby reducing pain and the resulting neurodevelopmental issues, as well as reducing the risk of infection and complications.

The researchers, after careful and extensive literature review and consultation with many highly skilled professionals, developed a three-part implementation strategy for use of the EPIV. The first part, called the NICU Vascular Access Decision Tree, addressed infant weight and clinical reasons for treatment such as therapeutic hypothermia, hypoglycemia, antibiotic use, cardiac issues, and osmolarity of fluids administered. The second part addressed in-depth training criteria for a highly skilled Vascular Access Team (VAT). The final part incorporated the Assessment of Clinical Outcomes based on comprehensive statistical analysis during the three-year study.

Overall, their evidence-based research clearly suggests EPIV use in the NICU is truly an outstanding choice. Results reflected a significant reduction in occlusions, infiltrations, and infections. Additionally, the incidence of leakage was tremendously reduced with the proper use of securement devices such as adhesive glue.

These results, combined with the use of a high-quality EPIV offering a 29-day dwell time, will help NICU professionals to confidently move forward in their efforts to save our most vulnerable patient population.

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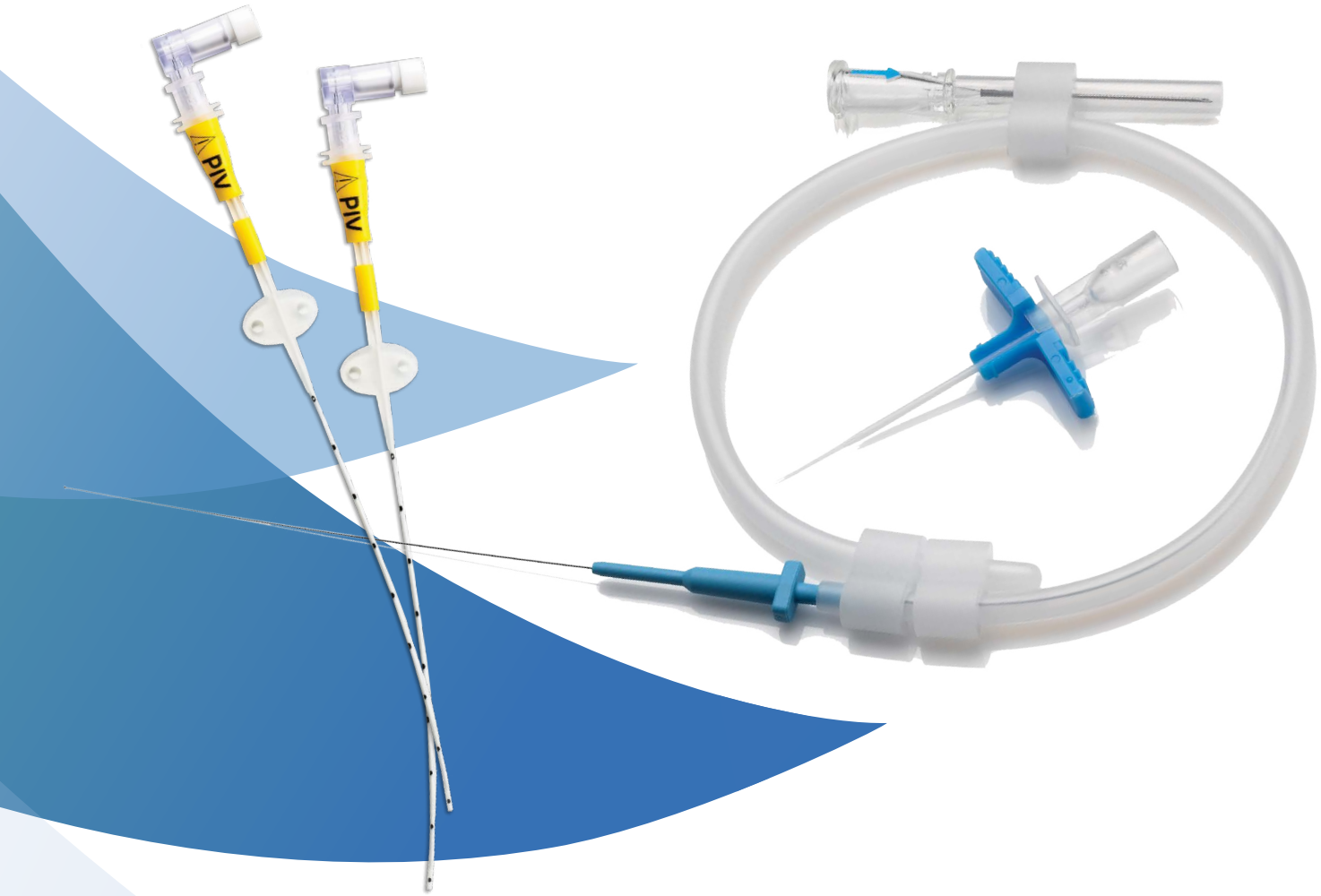

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Protocols to Ease Transport of Extremely Low Birth Weight Babies Improves Safety while Maintaining Low IVH Rates

This webinar features Ashley M Childress, MPA, BSN, RNC-NIC, Nurse Manager at Bryan Neonatal Intensive Care Unit in South Carolina, discussing protocols to ease transport of extremely low birth weight babies, including using an ATOM Dual Incubator while traveling to the NICU.

Please tell us about yourself.
My name is Ashley Childress. I am a second career nurse with a previous background in Non-Profit Management and Social Work. After graduating from nursing school, I began as an adult oncology nurse and transferred into neonatal intensive care about a year and a half later. After a few years at the bedside, I moved into an Assistant Nurse Manager role and recently became our unit's Nurse Manager about a year and a half ago. When not at work, I enjoy spending time with my husband and two children.

Can you tell us about your NICU?
Our organization's mission is "Inspire Health. Serve with Compassion. Be the Difference." Our unit is classified as a Level IIIC as defined by the American Academy of Pediatrics. Our unit has an internal transport and delivery team, a unit-based vascular access team, and a specialized Small Baby unit that recently began resuscitating infants at 22-weeks gestation.

Have you recently implemented any new protocols or procedures that you would like to share?
Over the course of the last several years we have addressed the significant issues extremely low birth weight (ELBW) often face by adopting the use of Prolacta and developing new protocols like our ROP protocol, early CPAP stabilization protocol, a new bathing protocol, and our IVH Bundle that includes admitting small babies directly into an ATOM Incubator before traveling to the NICU.

What clinical factors made you see a need for a change in said protocols or procedures?
We look at our outcomes annually and brainstorm as a team to determine how we can change our practices to better serve our patients. Our nurses work closely with physicians to develop and tweak the protocols as needed based on nursing workflow and constraints of our physical space, budget, etc.

What are the parameters for this new process?
Any baby born prior to 28-weeks gestation or weighing less 1000gm is defined as ELBW and admitted to our Small Baby Unit.

Was staff accepting of the new process and how did this hinder or assist in implementation?
Absolutely. We encourage our specialty teams to engage in process improvement projects within the unit. We see that their passion for the project and buy-in is far more effective than anything our leadership team could achieve with a management or physician-only driven process change.

How was your success measured?
We follow our VONN data trends, as well as unit-tracked measures (post-delivery temperatures, feeding intolerance, etc.) on a monthly, quarterly, and annual basis.

What were the benchmarks and who was the team that oversaw this change?
Typically, we have a nurse team paired with leadership and a physician to discuss an area of opportunity and work through the process of setting goals, developing strategies to address the issue, implementing the changes, and collecting data in relation to the changes that were made.

How long had you been utilizing the new protocol before you noticed that the process was improving patient outcomes?
In regard to taking the ATOM Incubator to deliveries, we immediately had anecdotal from nursing that transporting babies from the delivery room to the unit was smoother, took less time, and the babies settled much more quickly. We see continued success in maintaining a low IVH rate in our ELBW population represented in our VONN results.

Was new technology used to implement the new process?
The ATOM Dual Incubator.

What specifically about the new technology helps make success possible?
The extended battery life (lithium ion technology) of this incubator allows us to keep the bed prewarmed and ready for admission even when unplugged from a power source. It also continues to effectively warm the patient regardless of whether it is serving as a radiant warmer or isolette, which is good during prolonged deliveries. Finally, the bed drives smoothly and is not difficult to maneuver like some older transporters or incubators.

Was the manufacturer of this technology supportive in helping you achieve your goals and how?
Yes – ATOM helped us customize the incubator for our needs and did on-site training when we purchased them. While we didn't originally intend to take these beds to ELBW deliveries, we quickly saw the benefit of utilizing them in this way.

Would you say that this process improvement supported by new technology would be beneficial for other facilities as well?
If the physical space of a delivery room/OR can support admitting directly to an ATOM incubator, this process could certainly positively impact infants born in other facilities.

Outcomes from Novel Hearing Protective Device for Neonates Exposed to Noise during Critical Care Transportation

Daleen Penoyer, PhD, RN, CCRP, FCNS, FCCM, FAAN, Geraldine B Martinez, BSN, RNC-NIC, C-NPT and Linda Lowman, MEd

Introduction

In normal circumstances, while in utero, fetuses are protected from exposure to high-frequency sounds but experience low frequency sounds that are important for neurosensory development.[1-2] Prematurely born neonates have immature neurosensory development. Excessive auditory stimulation caused by exposure to noise outside the womb may have negative consequences for premature infants, including increased stress, unstable physiologic responses, and may influence auditory and neurological development.[3-4]

The National Vital Statistics report in 2021 indicated that nearly 400,000 newborns in the United States were born prematurely prior to the expected due date—before the 37th week of gestation.[5] Most of those premature neonates are admitted to Neonatal Intensive Care Units (NICUs), with 20% requiring emergency transport via ground or air ambulance to receive specialty care. External noises created by emergency transport vehicles, such as helicopters, airplanes, or ground ambulances are generally loud and noxious and could elicit stress responses in these neonates.[6-7] A study by Karlsson, et al, linked air and ground transport to increases in heart rate that may be attributed to an increase in stress. Their recommendation was to reduce exposure to noise during transport of neonates.[8]

As part of a program to reduce noise exposure during neonatal transport, sounds levels inside transport incubators within emergency transport vehicles were evaluated at various points of travel on simulated transports by the study organization personnel along with the transport team from the NICU. A sound level meter was placed inside the vehicle and a separate sound level meter was also placed inside the transport incubator. Measurements of sound level (decibels) were obtained in the ground ambulance during idle, cruise and end idle time periods, and in the helicopter, from start-up, cruise, and final approach. These periods were deemed those with the highest degree of noise/sound. Figure 1 summarizes typical sound level meter readings recorded during the beginning, middle and end of these of these experiments.

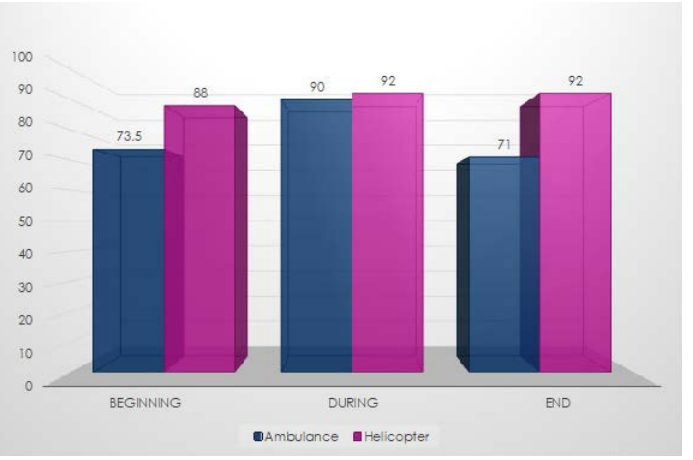

Figure 1. Typical sound level meter readings (dBA) measured in rotor wing and ground ambulance transports.

Some commercially available ear protection devices are appropriately sized for small infants or children, but they do not properly fit smaller, premature neonates. While noise levels in transport vehicles are known, the frequency composition of that noise is not generally known and is an important factor for developing proper hearing protection devices. Additionally, the actual degree of protection provided by commercially available ear protector devices in actual transportation environments has not been published. The study organization partnered with NEATCap Medical, LLC to measure both the sound levels and frequency distribution of noise experienced within transport vehicles. These measurements were a precursor to a real-world test to evaluate a novel ear covering hearing protection device designed for premature neonates exposed to those specific transport noises.

A simulated premature baby manikin fitted with one microphone at each ear location was used to record the sound level and sound frequency distribution within the transport incubator. Continuous recordings were made before, during and after completion of transport runs in both helicopter and ground ambulances. One ear was covered with a hearing protector and the other ear was uncovered, allowing for an assessment of the effectiveness of sound blocking of actual transport sounds. Figure 2 shows the manikin inside the transport incubator.

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Ms Martinez is a neonatal advanced clinical nurse in the Neonatal Intensive Care Unit Level IV at the Orlando Health Winnie Palmer Hospital for Women and Babies in Orlando, Florida and was the lead transport nurse for the neonatal team at the time of this study.
Ms Lowman is a developmental specialist in the Neonatal Intensive Care Unit at the Orlando Health Winnie Palmer Hospital for Women and Babies in Orlando, Florida.

Figure 2. Simulated patient setting for recording sounds within the incubator during transport. Manikin in photo is wearing DREAMIES T-M device.

Data obtained with the manikin verified that both air and ground transport sound environments are dominated by very low frequency sounds less than 300 Hz. Such low frequency sounds are often difficult to block.

A hearing protection device (NEATCAP DREAMIES) designed to block high-frequency alarm sounds during routine care in the NICU was studied with 50 babies (clinicaltrials.gov #NCT02744066)[9] at another hospital site with no serious adverse events after 3 days of use with perceived observations of increased patient sleep.[10] The company developed a new product with modified ear cups (DREAMIES T-M) to mitigate transport noise. With the manikin wearing these ear cups, an actual sound reduction of 18-20 dB was measured during helicopter transport at sound levels up to 90 dBA. A sound reduction of 17-18 dB was measured during ground ambulance transport at sound levels up to approximately 80 dBA. Prior to this study, this device had not been studied with actual patients during transport.

Purpose of the Study

The purpose of this study was to evaluate the performance of and outcomes of using DREAMIES T-M in a real-world setting during ground and air transport of actual neonatal patients to the organization's neonatal intensive care unit. The study was a prospective, descriptive, exploratory, and observational pilot design to evaluate the performance of the device and infant stress markers during transportation to the NICU. Inclusion criteria for the study were neonates transported ≤ 96 hours after birth without skin or head trauma during delivery with parental consent.

Methods

After obtaining parental informed consent, transport team members measured the infants' occipital frontal head circumference to determine the appropriate device size, then applied the device over the neonate's ears per the instructions for use. During transport, the transport team monitored and recorded movements and stress signals of the infant, skin condition before and after application and use of the device, maintenance of the device position, and ease-of-use by transporters.

Vital signs and NPASS (Neonatal Pain, Agitation and Sedation Scale) scores were recorded as indicators of neonatal distress. During length of time of the transport, vital signs including heart rate, respiratory rate, oxygen saturation and NPASS scores were measured every 5-30 minutes depending on the stability of the infant. NPASS is a validated tool for neonatal stress with agitation/stress scores of 0, 1 or 2 assigned in five categories (Crying/Irritability, Behavior State, Facial Expression, Extremities Tone, Vital Signs) resulting in a minimum score of 0 (appropriate, normal, relaxed) and a maximum score of 10 (significant pain/agitation).[11]

Results

The study sample included 49 infants; 57% were male. Mean gestational age was 37 ±3.43 weeks (range 23.7-42 weeks) and mean weight was 2910 ±880 grams (range 570-4810 grams). Sizes used with the device were extra small (n=2), small (n=6), medium (n=22), and large (n=18). Data for the size of one infant was missing. Skin condition was assessed before and after application and 41/49 (84%) had no issues, 8 had some redness that quickly resolved after removal (16.3%).

Device use

Transporters reported that the application process for the device was very easy (96%, n=47) or easy (4%, n=2). The device stayed in place during transport 98% of the time and one device ear cup fell off one ear, requiring adjustment. The same number of responses (98%) indicated that the device did not interfere with care, except for the one device that fell off the ear, requiring the device to be adjusted back into place.

The length of time that infants wore the device during transport ranged from 29 to 155 minutes. Thus, comparison of all vital signs (VS) was challenging. Since the number of VS taken were dictated by the length of the transport ride, the first and last VS taken were recorded and evaluated for differences to determine stress signals from VS over the transport period. Table 1 summarizes these results. There were no statistical differences between these values.

Likewise, in measuring the NPASS scores, a similar approach was taken to measure the start (First) and ending (Last) values for differences. One infant had no NPASS scores documented. To note, no infants were sedated during transport, thus sedation scores were not included. There were no statistical differences between the first and last NPASS scores. NPASS results are presented in Figure 3.

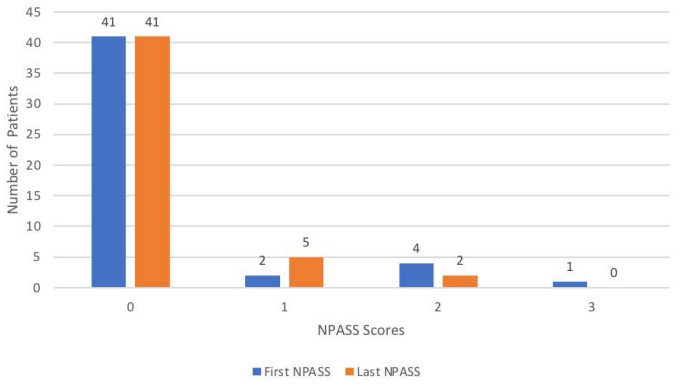

Figure 3. First and Last NPASS scores and comparison

We found that the first and last, and first and mean values of all measures were highly correlated and statistically significant.

Table 1. Vital Signs measurements and comparison.

Measurement	FIRST Measure	LAST Measure	MEAN of all Measures	Significance First vs Last P < .05
Heart Rate/min	135.4 ± 17.69	141.12 ± 19	137.8 ± 13.6	P = .054 NS
Resp. Rate/min n=48	54.73 ± 14.08	50.4 ± 14.62	51.41 ± 11.03	P = .111 NS
SpO2 in %	95.2 ± 6.33	96.27 ± 3.56	95.4 ± 6.34	P = .109 NS

There was no correlation between demographics of weight, gender, or gestational age on any of the vital sign measures.

In conclusion, very few issues were found by transporters using the novel ear protection devices. The devices were generally easy to apply, remained in place, and did not interfere with care. Vital signs and measures of neonatal stress using the NPASS scores were stable across the transport period while neonates wore the device and neonates were generally calm without visible signs of stress.

Manikin measurements revealed that overall sound levels inside an actual transport incubator were reduced by about 20 dB using the novel hearing protection device during simulated ground and air transport runs. This means the noise exposure was reduced from very loud, stress-inducing levels to a volume typical of normal human conversation.

Recommendations for practice are to consider some form of ear protection in premature neonates, particularly during transport in vehicles known to have excessive noise and vibration. Further studies are needed in this area to determine long-term impact of noise-reduction strategies in neonates over time.

Funding

This study was conducted without funding for research activities, however NEATCap Medical, LLC provided the DREAMIES T-M devices for the study.

Institutional Review Board Review

The study was approved by the Orlando Health Institutional Review Board with expedited review. Informed consent was required by parents of neonates involved in the study.

Conflict of Interest

None of the investigators in the study declare any conflict of interest with regards to the study. No payments were made to any investigators for the study.

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Impacts of a Tracheostomy Tube and Speaking Valve within the Pediatric Population

Kristin A King, PhD, CCC-SLP

Critical development of anatomy, physiology, swallowing, mobility, and other skills begins in utero and continues from birth through childhood. Immediately after birth, speech, language, and cognition are added to the many areas of development that a child is quickly undergoing. Ruano et al. (2021) reported on the follow-up care of an infant who underwent surgery in utero for two life-threatening conditions. They reported on use of the Passy-Muir® Valve (PMV®) after birth for stimulation of the upper airway and to assist with vocalizations and swallowing.[1] Even as an infant, their patient was able to wear the PMV all-day. It is well documented that primary speech and language development occurs from birth to age three, and during this same timeframe, infants and toddlers are making vast changes in gross and fine motor development.[1] These skills continue to develop throughout childhood but at a slower pace than initially seen in infancy and early childhood (birth to age three years).[2] When this process is complicated by medical conditions requiring a tracheostomy, the manner in which the systems interact for development are compromised even further.

Pediatric tracheostomy placement is occurring with greater incidence due to the advancements in medical interventions and the increased survival rate of infants who are premature and those with congenital abnormalities. Long term tracheostomy placement has been associated with delayed acquisition of language and social development.[3] Additionally, long term tracheostomy may impact parent-child bonding and the ability of the child's family to know their wants and needs due to the communication impairment.[4]

Impact of a Tracheostomy Tube: Pressure

A tracheostomy in a developing infant or toddler can impact speech and language development, fine and gross motor development, oral-motor sensory awareness, and feeding and swallowing. When a tracheostomy is placed, the patient transitions from having a typical respiratory system with positive end-expiratory pressure (pressure remaining in the lungs after expiration) to an open system, with a loss of pressures. Pullens & Streppel (2021) reviewed the importance of restoring normal airway physiology to improve swallowing when a tracheostomy is present in a child. The authors report that they found that 70.5% of the children whom they studied with a tracheostomy demonstrated swallowing problems and 94.7% had aspiration, with 50% being silent.[5] Some issues that they share as typical findings for tracheostomy-related swallowing problems are sensitivity problems, lack of force during swallowing, difficulty with laryngeal elevation, and decreased relaxation of the upper esophageal sphincter.[5] They strongly advised to use a speaking valve with these patients and to assess swallowing with the valve both on and off to determine differences.

The human body functions with a pressurized system and this system impacts coughing, speech, swallowing, trunk support, and postural stability, among others. Gipsman et al. (2022) discussed the benefits of using speaking valve to mitigate the risk of developing respiratory infections. Due to the restored pressure and cough effectiveness, the authors found that secretion management was improved in children.[6] In infants and toddlers, restored pressure is particularly important as it also impacts gross motor development for trunk control during sitting, crawling, standing, and walking which have a direct correlation with self-feeding and advancement of oral intake.[7]

Assessment and Treatment Plan

With the wide range of developmental skills which may be impacted by a tracheostomy, it is essential for clinicians to have an assessment plan that incorporates the use of a Valve and then a treatment intervention plan that includes both Valve use and activities for speech and language development, gross motor development, and feeding skills. General activities that may be used with children who have a tracheostomy to assist with improving upper airway use, sensory awareness, and speech and language development include enriching the speech and language environment (e.g., reading aloud, parallel talk during play or medical care, play therapy), training use of the upper airway through interactive tasks (e.g., bubble blowing, whistles, blowing through a straw), and providing infants and children with access to a more typical aerodigestive system through use of a Passy-Muir Valve.

A study which addressed the physiologic effects of a tracheostomy on feeding and swallowing also included some considerations for gross motor function and development. The authors hypothesized that the children would exhibit delays

With 25 years of experience in medical, academic, and industry settings, Dr King brings a unique perspective of medical speech pathology. Her research, publications, and teachings focus on traumatic brain injury, swallowing disorders, and critical care (tracheostomy and mechanical ventilation) for both pediatric and adult patient populations. She has been an invited speaker both domestically and internationally and has published in peer-reviewed journals. Currently, Dr King is the Vice President of Clinical Education and Research for Passy-Muir, Inc.

Enhancing language development through Valve use and language play.

secondary to the tracheostomy.[8] The authors found that growth delays, G-tube (gastrostomy tube) feedings, and feeding skill delays were attributed to the tracheostomy. They found that ventilator-dependence, cuffed tracheostomy, and in-line speaking valve use were infrequently associated with the feeding and swallowing evaluation.[8]

Research also has shown that feeding and swallowing improve feeding and swallowing. Therefore, increasing function through use of the Valve and providing oral stimulation and access to oral intake provides an opportunity to improve oral nutrition. The impact of a tracheostomy and links between gross motor and language development have been discussed in the literature. It is important to provide a closed system which assists with restoring more normal thoracic and abdominal pressures impacting gross motor movements and even swallowing. While a tracheostomy may cause an open system in an otherwise closed environment, restoring more typical function through use of the Valve not only closes the system, restoring pressures, but ends the vicious cycle that begins with a tracheostomy.

Decannulation

Kolb et al. (2021) completed a retrospective chart review that analyzed 18-years of data. From this review, they found that for successful decannulation in the pediatric population, the weaning process should include speaking valve use and capping.[9] They also reported that the longer a patient had a tracheostomy, then the more difficult the decannulation, with only 25% decannulated if trached longer than 25 months, and almost no decannulations occurred if trached longer than 75 months.[9] Another study also was conducted to look at decannulation in pediatrics. The authors explained that transtracheal pressure is a measurement that allows assessment of the upper airway and discussed the need to downsize the tracheostomy while simultaneously alternating use of the speaking valve.[10] They discussed using the speaking valve for strengthening of the supraglottic musculature as a rehabilitation strategy. They authors found that use of the speaking valve is a tool to assist with progressing toward decannulation.[10]

Overall, research has shown that tracheostomies are performed in young children, including infants, and that benefits exist for early intervention to address use of a speaking valve to close the system and restore upper airway airflow and pressures. It also has shown that without use of a speaking valve, delays may occur in areas related to gross motor development, feeding and swallowing, voicing, and parent-child bonding. Research supports that early intervention and treatment to improve function and healthcare interventions are critical.

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A Newborn with Cardiac Murmur and Tachycardia

Faiza Javed, MD and Shabih Manzar, MD, FAAP

Objectives

1. For providers involved in caring for newborn infants it is very important to diagnose heart diseases in a timely manner and start appropriate interventions.
2. Interpretation of the neonatal electrocardiogram requires skill and training. Knowing the types of arrhythmias is important.

A study case is presented as a question to achieve these objectives. The answer with explanation is provided at the end.

Case Study

A newborn infant was admitted to the nursery at 2 am following a cesarean section. The mother was a 24-year-old primigravida. The pregnancy was uneventful. The infant was noted to have tachypnea and was placed on pulse oximetry. The nurse reported a heart rate of 178 beats per minute with an oxygen saturation (SpO_2) of 92% in room air. The point-of-care glucose was 20 mg/dL. A glucose gel treatment was provided, and the infant was fed 20 mL of commercial formula. The repeat glucose was 25 mg/dL. The infant was then transferred to neonatal intensive care for intravenous glucose administration. The infant was noted to have dusky episodes while feeding with SpO_2 of 88-94% in room air. A loud cardiac murmur was heard on examination. The on-call senior house officer was called at the bedside, and she ordered a chest X-ray and electrocardiogram (Figures 1 A and B). Blood pressures in all four extremities were similar. Based on the patient's history and physical examination, chest X-ray, and ECG, which is the most likely diagnosis?

A. Transposition of great arteries
B. Coarctation of the aorta
C. Sinus tachycardia
D. Atrial flutter

The answer is D: atrial flutter. Atrial flutter (AF) is one of the common tachycardia rhythms noted on the electrocardiogram (ECG) during the neonatal period. The presence of an umbilical venous catheter in the right atrium (Figure 1A) could also be a triggering factor for AF. The saw-tooth appearance on the ECG suggests AF (Figure 1B).[1] In some instances of AF, a simultaneous M-mode tracing of the atria and ventricle showing a 2:1 atrial to ventricular contraction pattern could be helpful.[2] The cardiac murmur noted in the infant was due to an A-V canal defect. The treatment of AF depends upon the severity. It includes adenosine bolus, synchronized cardioversion, antiarrhythmic medications, and ablation therapy. The case presented responded to cardioversion and was started on antiarrhythmic medications.

In the neonatal period, transposition of the great arteries (TGA) is the most common cause of cyanotic heart disease. TGA is characterized by ventriculoarterial discordance and the classic "egg on a string" appearance on chest X-ray.[3] The infant had no cyanosis, and cardiomegaly was not typical of TGA. In an emergency, a continuous infusion of prostaglandin E1 is the mainstay of treatment, followed by balloon septostomy and later arterial switch procedure.[4]

Coarctation of the aorta (CoA) occurs in 0.3 per 1000 live. Newborn (NB) infants with severe CoA may be completely asymptomatic at birth because the ductus arteriosus (DA)

Summary Table

Condition	Characteristics
Atrial flutter (AF)	The electrocardiogram showed the saw-teeth appearance with indistinguishable P and T waves. The cardiomegaly seen on the chest X-ray was due to the fact that the infant had an AV-canal defect, which is also the cause of the re-entry pathway causing AF.
Transposition of great arteries (TGA)	Cardiomegaly can occur in TGA, but it gives an impression of an egg-on-a-string appearance. Also, TGA is associated with cyanosis and desaturation, while the infant's SpO_2 was 92% in room air.
Coarctation of aorta (CoA)	Once ductus arteriosus constricts, the newborn with coarctation could present with cardiogenic shock. The BPs were normal in all extremities, ruling out CoA.
Sinus tachycardia (ST)	ST in possible but ECG showing saw teeth waves in not typically seen in ST.

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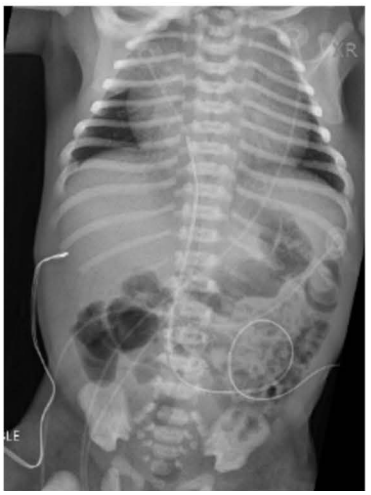

Figure 1A. Chest X-ray

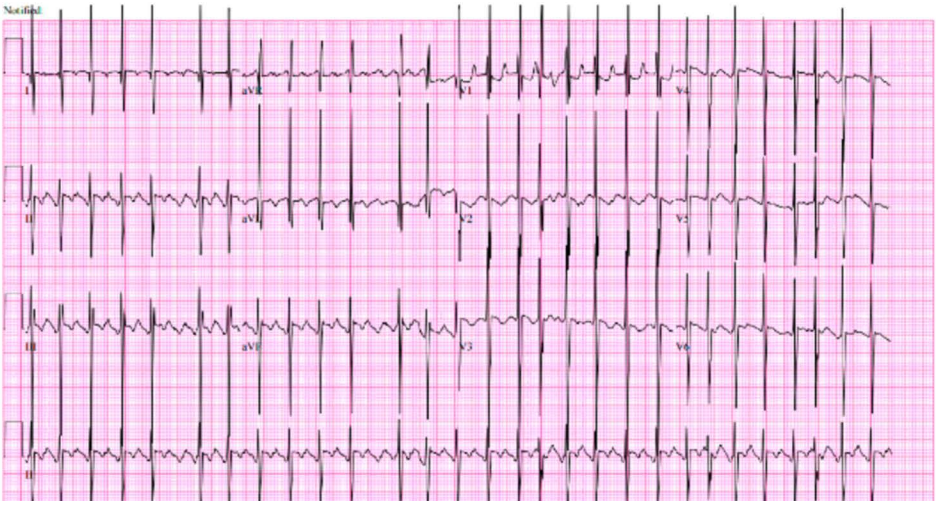

Figure 1B. Electrocardiogram

provides blood flow to the descending aorta. Radio femoral delay may not be evident with open ductus in NB, but BPs would be affected due to reduced blood flow in the lower extremities. In the infant, BPs were similar in all extremities ruling out CoA. With the closure of the DA, the NB infant could present with cardiogenic shock.[5]

The heart rate of 178 could be sinus tachycardia (ST), but the saw teeth appearance on ECG waves is atypical of ST. For a heart rate of > 220 beats/min, a diagnosis of supraventricular tachycardia (SVT) could be entertained. SVT is usually caused by an accessory pathway resulting in reentrant tachycardia. The HR was 178 beats/min in the case. The treatment consists of adenosine and cardioversion in refractory cases.[6]

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Vascular and Pulmonary Effects of Ibuprofen on Neonatal Lung Development

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Abstract

Background: Ibuprofen is a nonsteroidal anti-inflammatory drug that is commonly used to stimulate closure of a patent ductus arteriosus (PDA) in very premature infants and may lead to aberrant neonatal lung development and bronchopulmonary dysplasia (BPD).

Methods: We investigated the effect of ibuprofen on angiogenesis in human umbilical cord vein endothelial cells (HUVECs) and the therapeutic potential of daily treatment with 50 mg/kg of ibuprofen injected subcutaneously in neonatal Wistar rat pups with severe hyperoxia-induced experimental BPD. Parameters investigated included growth, survival, lung histopathology and mRNA expression.

Results: Ibuprofen inhibited angiogenesis in HUVECs, as shown by reduced tube formation, migration and cell proliferation via inhibition of the cell cycle S-phase and promotion of apoptosis. Treatment of newborn rat pups with ibuprofen reduced pulmonary vessel density in the developing lung, but also attenuated experimental BPD by reduc- ing lung inflammation, alveolar enlargement, alveolar septum thickness and small arteriolar wall thickening.

Conclusions: In conclusion, ibuprofen has dual effects on lung development: adverse effects on angiogenesis and beneficial effects on alveolarization and inflammation. Therefore, extrapolation of the beneficial effects of ibuprofen to premature infants with BPD should be done with extreme caution.

Background

Major advances in neonatal intensive care have not reduced the incidence of bronchopulmonary dysplasia (BPD) or neonatal chronic lung disease (CLD) in premature infants, because increased neonatal survival has shifted the affected population to premature infants born at less than 28 weeks of gestation.[1,2] The incidence of BPD is stable at 35-40% of extremely premature infants.[2,3] Treatment of respiratory failure due to lung immaturity and surfactant deficiency in these extremely premature infants with invasive respiratory support and supplemental oxygen may injure the developing lung permanently.[4] BPD is characterized by a reduced alveolar surface and impaired lung function due to enlarged alveoli caused by oxidative stress-induced lung damage and arrested alveolar development.[1] Prenatal insults, perinatal inflammation, oxidative stress and pulmonary arterial hypertension (PAH) complicate BPD pathogenesis and contribute to adult lung disease, like COPD, at relatively young ages.[2,3,5,6] Effective pharmacological treatment for BPD is lacking and badly needed.

The neonatal rat is a suitable animal model for studying BPD pathogenesis and novel treatment options.[7-10] These rodents are born during the saccular stage of lung development, mimicking the lung development stage of infants at high risk for BPD, and develop chronic lung inflammation, followed by persistent alveolar simplification, lung fibrosis, PAH and right ventricular hypertrophy (RVH) after exposure to hyperoxia.[1,11]

Ibuprofen is a potent nonsteroidal anti-inflammatory drug (NSAID) that is extensively used for the treatment of colorectal cancer, lung inflammation in cystic fibrosis, and closure of a patent ductus arteriosus (PDA) in premature neonates.[12-15] However, information about its effect on aberrant lung development after premature birth and the pathogenesis of BPD is incomplete and controversial, ranging from concerns about adverse effects, no impact, to beneficial effects on BPD in premature infants.[15-20] Our previous clinical study and a meta-analysis have indicated an increased risk for BPD in ibuprofen-treated infants.[16,20] Other experimental studies suggested an anti-angiogenic effect of ibuprofen in ocular angiogenesis in neonatal rats[21] and embryonic development in zebrafish.[22] Considering the essential role of angiogenesis in the pathogenesis of BPD[2] and the fact that each year millions of premature infants receive ibuprofen for PDA closure,[17] of which some are exposed to repeated or prolonged courses of ibuprofen treatment,[23] there is an urgent need to unravel the potential role of ibuprofen in normal lung development and BPD pathogenesis after premature birth.

To advance our knowledge on the effect of ibuprofen treatment on perinatal lung development and BPD, we studied the effect of ibuprofen on endothelial cell function in cultured human umbilical vein endothelial cells (HUVECs), and the effect on alveolar and vascular development and lung inflammation in

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neonatal rats kept under conditions of normoxia or hyperoxia to induce experimental BPD.[24]

Materials and methods

In vitro studies

Human umbilical vein endothelial cells (HUVECs)

HUVECs were isolated from the umbilical cord, as previously reported.[25] Briefly, an umbilical cord of 10-20 cm in length was collected after obtaining consent from the parents and processed in a biohazard cabinet.

The umbilical vein was cannulated and rinsed using sterile 0.09% saline to remove blood. One millilitre of 0.2% collagenase (C0103, Sigma-Aldrich, St. Louis, MO, USA) was injected into the umbilical vein and incubated for 10 min at 37°C. The umbilical cord was gently squeezed to facilitate the detachment of endothelial cells. Hereafter, the umbilical vein was rinsed with endothelial cell medium (ECM, 1001, ScienCell, Carlsbad, CA, USA), containing 10% fetal bovine serum (FBS, SV30208, HyClone, Marlborough, MA, USA) and Pen/Strep (#15140-122, Gibco, Fremont, CA, USA) to harvest the cells. HUVECs were collected by centrifugation at 750×g for 10 min, resuspended in medium and cultured at 37°C in 95% air/5% CO_2 humidified with water. Cells within passage 3-7 were used for the experiments.

Tube formation assay

96-well plates were pre-cooled and coated with 50 µL of Matrigel basement membrane matrix (#354234, Corning, NY, USA) per well. The plates were incubated at 37°C for 1 h to allow the basement to polymerize. After the gel became solid, 1×10^4 HUVECs were seeded with 100 µL of complete ECM containing DMSO (0.1%, v/v) or 100 µM, 500 µM or 1000 µM of ibuprofen. Plates were incubated at 37°C in 95% air/5% CO_2 for 6 h. Tube formation of HUVECs under different conditions was photographed with a camera mounted on a microscope (IX73, Olympus, Tokyo, Japan) and analyzed with Image J software (NIH, USA). Five technical repeats were performed.

Wound healing assay

HUVECs were seeded into a 12-well-plate at 2×10^5 cells/well in ECM supplemented with 10% FBS and 1% Pen/Strep. The cells reached a confluency of 80-90% after 24 h of incubation. Then, the culture medium was removed, monolayers were scratched using a 200 µL pipette tip to make a straight wound. The wound was rinsed twice with phosphate-buffered saline (PBS) and incubated with ECM free of FBS and treated with DMSO (0.1%, v/v), 100 µM, 500 µM and 1000 µM of ibuprofen dissolved in DMSO for 24 h at 37°C in 95% air/5% CO_2. The healing process was monitored under a microscope. The wounds were photographed at 0, 12 and 24 h after the scratch and analyzed with Image J software. At least 5 pictures of each well in three technical repeats were analyzed.

Immunofluorescence

HUVECs treated with different concentrations of ibuprofen were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. The cells were then incubated overnight at 4°C with Ki67 antibody (ab16667, Abcam, Fremont, CA, USA; diluted 1: 250), DNA damage marker, 8-hydroxy-2′-deoxyguanosine (8-OHdG, 12501, QED Bioscience, San Diego, CA, USA; diluted 1:1000), or 1% BSA (A1933, Sigma-Aldrich, St. Louis, MO, USA) as a control, followed by incubation with Alexa Fluor 488/555-conjugated donkey anti-rabbit/mice antibody (A-21206 and A21422, Invitrogen, Waltham, MA, USA, diluted in 1:1000) for 2 h in the dark. The cells were covered with Prolong Gold antifade reagent with DAPI (8961, Cell Signaling Technology, Danvers, MA, USA) and incubated in the dark for 24 h and then sealed with nail polish. The cells were studied with an Olympus IX73 microscope (Tokyo, Japan), equipped with a camera. Pictures were taken with a consistent exposure time, and analyzed with Image J software. At least 6 pictures of each well in three (Ki67) or five (8-OHdG) technical repeats were analyzed.

Paraffin-embedded rat lung tissue sections were incubated with an antibody against vWF (A0082, Dako Cytomation, Glostrup, Denmark; diluted 1:2000) overnight at 4°C or with FITC conjugated TUNEL (C1088, Beyotime Biotechnology, Shanghai, China) for 1 h at 37°C, using 1% BSA as a control, followed by incubation with Alexa Fluor 555-conjugated goat anti-rabbit antibody (A32732, Invitrogen, Waltham, MA, USA, diluted in 1:1000) for 2 h in the dark to visualize vWF. Sections were covered with Prolong Gold antifade reagent supplemented with DAPI (8961, Cell Signaling Technology, Danvers, MA, USA).

RNA-seq

Because all parameters studied on HUVEC proliferation, wound closure, tube formation and migration were significantly inhibited after treatment with 500 µM of ibuprofen and to exclude (1) a potential suboptimal (100 µM) and (2) a potential toxic (1000 µM) effect of ibuprofen on HUVEC we performed the RNA Seq experiments with 500 µM of ibuprofen. RNA-seq was performed on a BGIseq500 platform (BGI-Shenzhen, China). Briefly, isolated RNA from HUVECs treated with 0 or 500 µM ibuprofen was quantified using a NanoDrop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA), and purified with Oligo(dT)-attached magnetic beads. After synthesis of the first-strand cDNA, A-tailing mix and RNA index adapters were added by incubating to end repair. The amplified cDNA fragments were purified by Ampure XP Beads and validated on the Agilent Technologies 2100 bioanalyzer for quality control. The double-stranded PCR products from the previous step were heated, denatured and circularized by the splint oligo sequence to get the final library. The single-strand circle DNA (ssCir DNA) was formatted as the final library. The final library was amplified with phi29 to make DNA nanoballs (DNB) which

Table 1 Sequences of oligonucleotides for forward and reverse primers for real-time RT-PCR

Gene product	Forward primer	Reverse primer
IL6	5′-ATATGTTCTCAGGGAGATCTTGGAA-3′	5′-TGCATCATCGCTGTTCATACAA-3′
CINC1	5′-GCACCCAAACCGAAGTCATA-3′	5′-GGGGACACCCTTTAGCATCT-3′
MCP1	5′-ATGCAGTTAATGCCCCAGTCA-3′	5′-TTCTCCAGCCGACTCATTGG-3′
TF	5′-CCCAGAAAGCATCACCAAGTG-3′	5′-TGCTCCACAATGATGAGTGTT-3′
β-Actin	5′-TTCAACACCCCAGCCATGT-3′	5′-AGTGGTACGACCAGAGGCATACA-3′

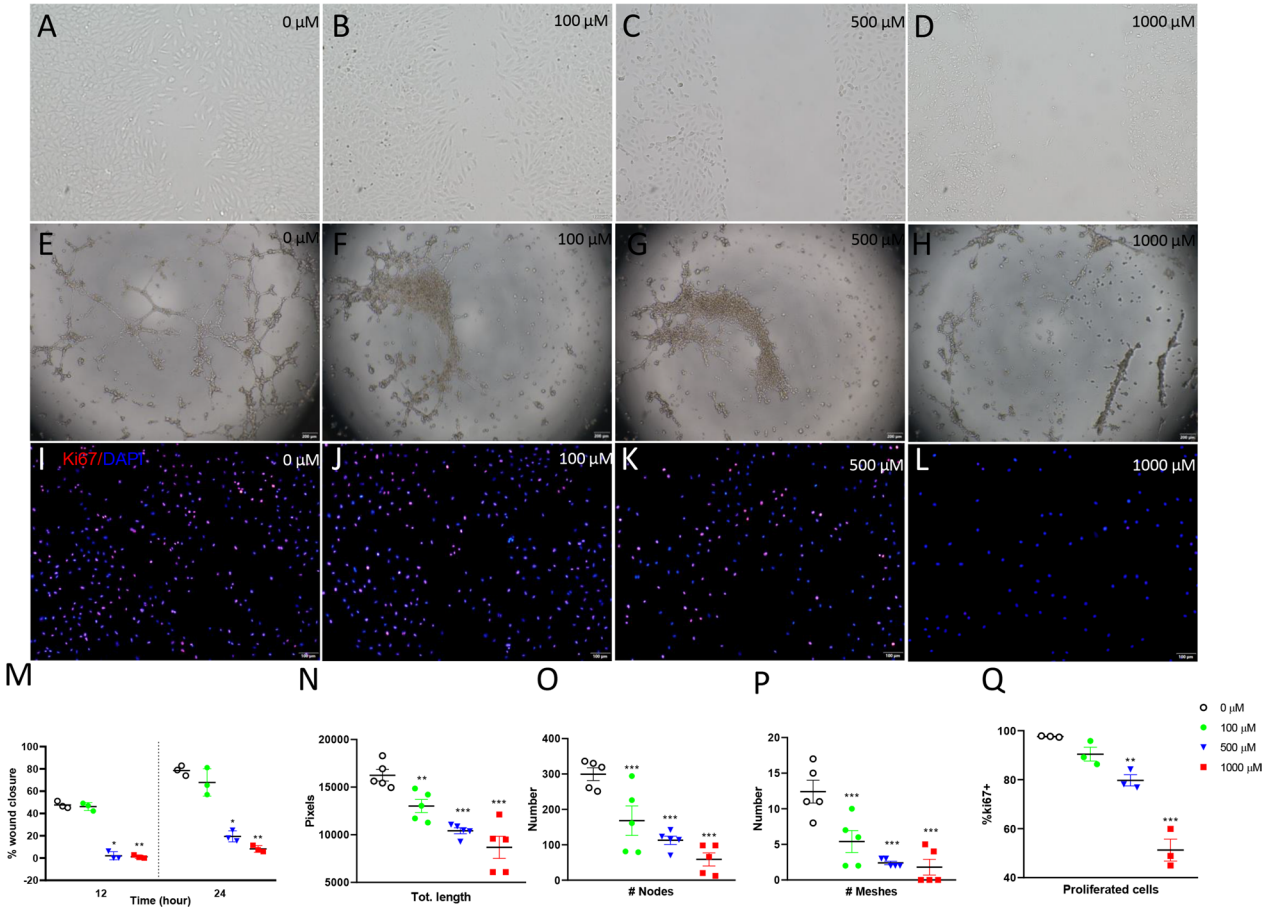

Fig. 1 Representative photos of migration (**A**–**D**), tube formation (**E**–**H**) and proliferation (**I**–**L**) of HUVECs treated with DMSO (0.1%, v/v, **A**, **E**, **I**, open circles), 100 μM ibuprofen (**B**, **F**, **J**, green circles), 500 μM ibuprofen (**C**, **J**, **K**, blue triangles) and 1000 μM ibuprofen (**D**, **H**, **L**, red squares). Wound closure rate at 12 and 24 h after treatment (**M**, N = 3), total length, number of nodes and meshes (**N**–**P**, N = 5) and Ki67-positive cell ratio (**Q**, N = 3) were used to quantify the migration, tube formation and proliferation ability of HUVECs. Values are expressed as mean ± SD. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus DMSO controls

had more than 300 copies of one molecular. DNBs were loaded into the patterned nanoarray and single end 50 bases reads were generated on the BGIseq500 platform. The data was first filtered with SOAPnuke (v1.5.2, https://github. com/BGI-flexlab/SOAPnuke) to remove reads with sequencing adapter, high low-quality base ratio (> 20%), and unknown base ratio higher than 5%. Clean reads were mapped and the expression level was calculated by RSEM (V1.2.12, https://github.com/deweylab/RSEM). Differential expression analysis was performed using DESeq2 (v1.4.5). GO (http://www.geneontology.org/) and KEGG (https://www.kegg.jp/) enrichment analysis was performed to summarize the pathways involved. The significance was corrected by Q value with a rigorous threshold (Q value ≤ 0.05) by Bonferroni. The analysis was performed on the Dr. Tom platform generated by BGI (https://biosys.bgi.com/).

Cell cycle analysis and apoptosis by flow cytometry

For cell cycle analysis, HUVECs were fixed overnight with 70% ethanol at 4°C after treatment with DMSO (0.1%, v/v) or ibuprofen for 48 h. Hereafter, HUVECs were incubated with Triton X100 (T8787, Sigma-Aldrich, St. Louis, MO, USA; 0.1%) and RNAse A (R-4875, Sigma-Aldrich, St. Louis, MO, USA; 100 μg/mL) and stained for 30 min with propidium iodide (PI, P4170, Sigma-Aldrich, St. Louis, MO, USA; 40 μg/mL). The fluorescence was excited at 488 nm and measured with a 585 nm filter. Data were analyzed with Modfit software (Verity Software House, Topsham, Maine, USA). For apoptosis analysis, HUVECs were treated with DMSO (0.1%, v/v) and ibuprofen for 72 h and stained with a FITC Annexin V Apoptosis Detection Kit I (556547, BD Life Sciences, Franklin Lakes, NJ, USA) according to the manufacturer's instruction.

Animal studies

All animal procedures in this study were approved by the Institutional Animal Care and Use Committee of the Shenzhen Institutes of Advanced Technology of the Chinese Academy of Sciences. Newborn pups were randomized into 4 groups (N = 6-7 for each group): two experimental BPD groups raised in 100% oxygen and 2 control groups raised in room air, assuming a similar sex ratio among the groups. Experimental BPD was induced by exposure to hyperoxia as previously reported.[24] Briefly, newborn pups were raised and fed by foster dams in a Plexiglas chamber filled with 100% oxygen for 10 days. Foster dams had access to water and food ad libitum and were rotated daily to prevent hyperoxia-induced lung damage and, importantly, balance maternal care given to pups in control and experimental groups.

Pups received daily subcutaneous injections of 50 mg/kg of ibuprofen (I4883, Sigma-Aldrich, St. Louis, MO, USA), dissolved in 100 μL arginine buffer (10 mg/mL, SinePharm, Shanghai, China) or arginine buffer only as treatment control. Pups were anesthetized on day 10 by intraperitoneal injection of pentobarbital (40 mg/kg). Three independent experiments were

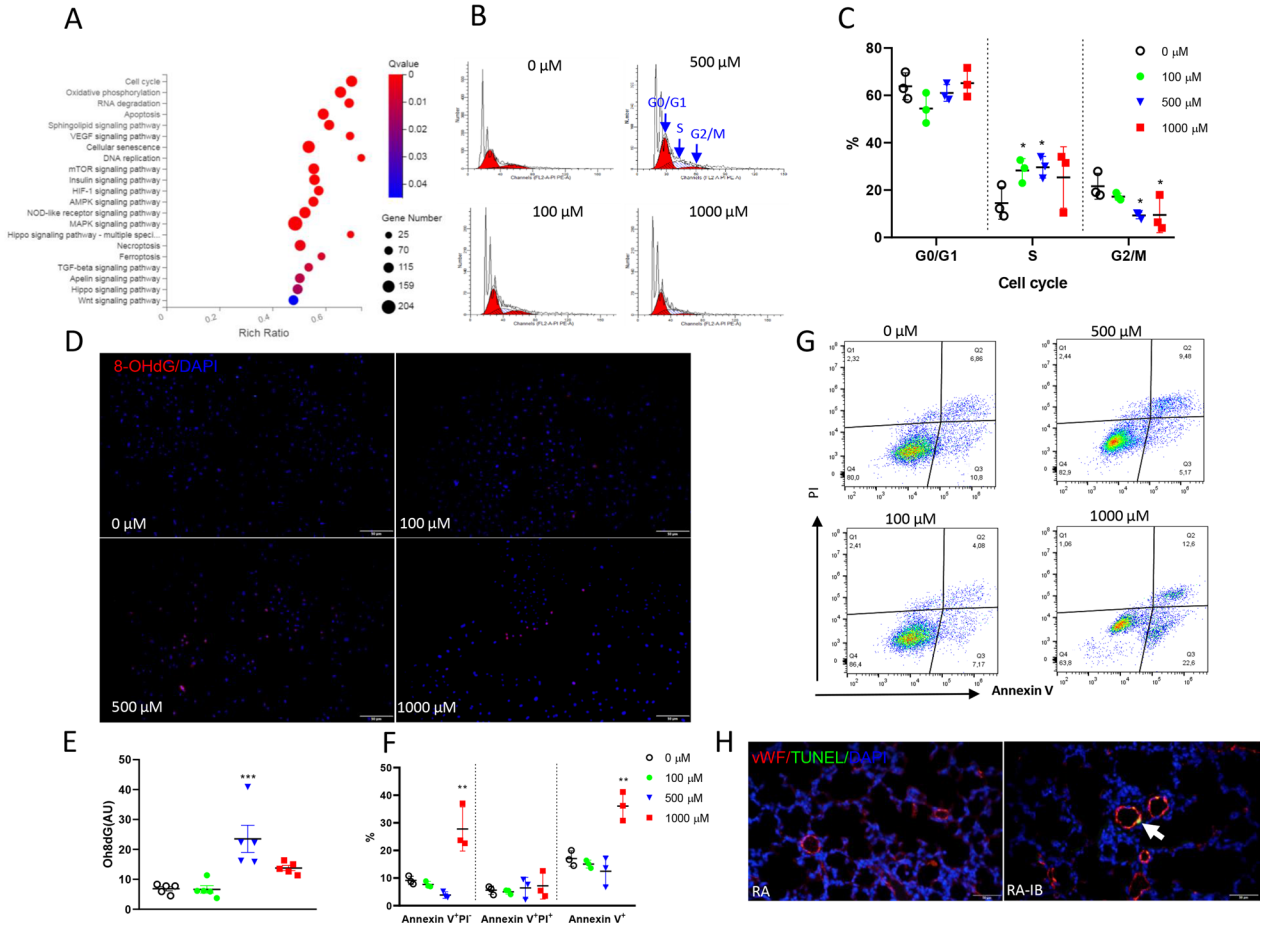

Fig. 2 Bubble plot of pathways significantly affected by 500 µM of ibuprofen (**A**, N = 4–5). Representative photos of cell cycle analysis of HUVECs treated with DMSO (0.1%, v/v), 100, 500 or 1000 µM of ibuprofen (**B**) and proportion of cells at different cell cycle stages, G0/G1, S and G2/M (**C**, N = 3). Representative photos and fluorescence intensity quantification of the DNA damage marker, 8-hydroxy-2′-deoxyguanosine (8-OHdG) staining in HUVECs treated with DMSO or ibuprofen (**D** and **E**, N = 5). Representative photos and quantification of apoptosis analysis by propidium iodide (PI) and Annexin V staining in HUVECs treated with DMSO or ibuprofen (**G** and **F**, N = 3). Representative photos of TUNEL staining of neonatal rat lung tissue exposed to ibuprofen. Rat pups were kept in room air (RA) and were daily injected subcutaneously with arginine buffer (RA) or ibuprofen (50 mg/kg/day; RA-IB) until 10 days of age (**H**). DMSO, open circles; 100 µM ibuprofen, green circles; 500 µM ibuprofen, blue triangles; and 1000 µM ibuprofen, red squares. Values are expressed as mean ± SD (**C** and **F**) or mean ± SEM (**E**). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus DMSO controls

performed, two for histological data and one for RT-PCR data. Lung tissue from pups raised in different litters was either fixed in situ with formalin or frozen in liquid nitrogen and stored at –80°C for RT-PCR as previously reported.[24]

Histology and lung morphometry

Formalin-fixed, paraffin-embedded, 4 µm-thick lung sections were stained with hematoxylin and eosin. In addition, lungs were stained with specific antibodies against von Willebrand factor (vWF, A0082, Dako Cytomation, Glostrup, Denmark; diluted 1:5000), CD31 (ab182981, Abcam, Fremont, CA, USA; diluted 1:2000), CD68 (monocytes and macrophages, ab31630, Abcam, Fremont, CA, USA; diluted 1:500), myeloperoxidase (MPO, ab208670, Abcam, Fremont, CA, USA; diluted 1:1000), α smooth muscle actin (ASMA, A2547, Sigma-Aldrich, St. Louis, MO, USA; diluted 1:10,000), or 1% BSA (A1933, Sigma-Aldrich, St. Louis, MO, USA) as a control, followed by staining with HRP conjugated anti-mice/rabbit antibodies accordingly (ab6721 or ab6728, Abcam, Fremont, CA, USA; diluted 1:1000). Antibody staining was visualized using the chromogenic substrate NovaRed as recommended by the manufacturer (SK-4800, Vector, Burlingame, CA, USA). Sections were counterstained briefly with hematoxylin using standard methods.[24] Mean linear intercept (MLI) was determined on HE stained lung sections, as previously reported.[8,24] Briefly, 10 non-overlapping photos of lung tissues were made with an Olympus CX43 microscope (Tokyo, Japan) at a 200× magnification. Structures, including big vessels and airways, were excluded. The photos were analyzed using a coherent system of 21 lines and 42 points embedded in the CellSens software (Olympus, Tokyo, Japan).

Vessel density was assessed in lung sections stained for vWF or CD31 at a 200× magnification by counting the number of vessels per field. At least 10 representative fields per experimental animal were investigated. The density of ED-1 positive monocytes and macrophages or MPO-positive neutrophilic granulocytes was determined in the alveolar compartment by counting the number of cells per field. In each experimental animal 20 fields in one section were studied at a 400× magnification. Pulmonary alveolar septal thickness was assessed in HE-stained lung sections at a 400× magnification by averaging 100 measurements per 10 representative fields. Medial wall thickness was calculated from the formula "percent wall thickness = $\frac{2\times wall\cdot thickness}{external\cdot diameter} \times 100$".[26] Structures, including big vessels and airways, were excluded. Two independent researchers blinded to the experimental groups performed the analysis.

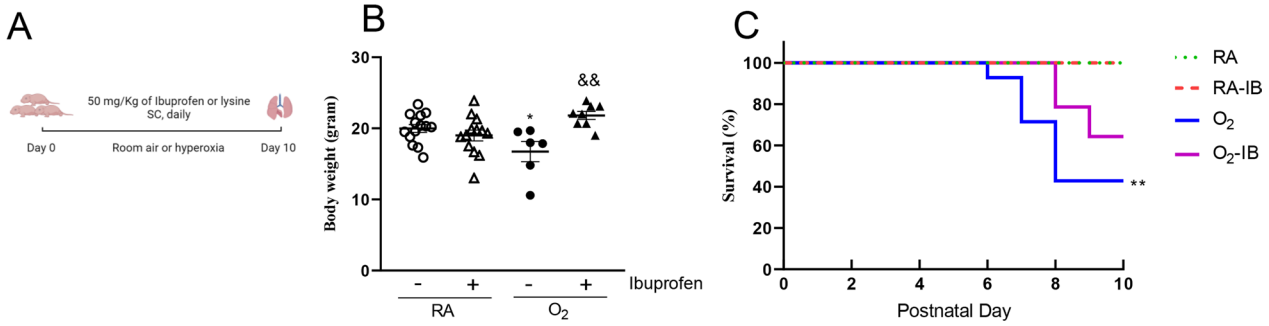

Fig. 3 Experimental scheme (**A**), growth (**B**) on day 10 (N = 14 for the RA control group and N = 13 for the RA-ibuprofen group, N = 6 for the oxygen control group, and N = 8 for the oxygen-ibuprofen group) and Kaplan–Meier survival curve (**C**) (calculated from 14 pups for each group). Room air (RA) pups (open symbols) and age-matched O_2-exposed (O_2) pups (solid symbols) were injected daily with arginine buffer (circles) or ibuprofen (50 mg/kg/day; triangles) until 10 days of age. Data are expressed as mean ± SEM. *$p < 0.05$ and **$p < 0.001$ versus RA controls. $^{\&\&}p < 0.05$ versus age-matched arginine buffer-treated O_2-exposed controls

Real-time RT-PCR

RNA was isolated from 30 mg lung tissue homogenates or 5×10^5 HUVECs using TRIzol (#15596026, Invitrogen, Waltham, MA, USA) according to the supplier's manual. cDNA was synthesized with the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific, Waltham, MA, USA). Real-time quantitative PCR was performed on an Applied Biosystems 7300 Plus system (Applied Biosystems, Foster City, CA, USA). β-actin was used as a housekeeping gene reference. Relative mRNA expression was normalized to room air controls. Primers are listed in Table 1.

Statistics

Parameters were displayed as mean ± standard error of the mean (SEM), unless otherwise stated. Differences between experimental groups were analyzed by one-way ANOVA, followed by Sidak multiple comparisons test. For comparison of survival curves, Kaplan–Meier analysis followed by a log rank test was performed. Statistical analysis was performed using a GraphPad Prism version 8 software package (San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

Data availability

RNA sequencing data analyzed in the article (Fig. 7A) have been deposited into the CNGB Sequence Archive (CNSA)[27] of China National GeneBank DataBase (CNGBdb)[28] with accession number CNP 0002466.

Results

Effects of ibuprofen treatment on endothelial cell proliferation, migration and tube formation ability

Since experimental evidence pointed to an inhibitory effect of ibuprofen on angiogenesis, we investigated the role of ibuprofen in endothelial function of human umbilical vein endothelial cells (HUVEC) in vitro by studying migration, neovascularization and proliferation. After 12 and 24 h of treatment with 500 or 1000 µM of ibuprofen, wound healing was attenuated by 4.0-fold (24 h; 500 µM; $p < 0.05$, Fig. 1C, M) or 9.6-fold (24 h; 1000 µM; $p < 0.01$, Fig. 1D, M). After 6 h of treatment with ibuprofen, tube formation ability was attenuated (Fig. 1E–H), as shown by reduced total length, number of nodes and number of meshes in HUVECs treated with 100, 500 or 1000 µM of ibuprofen ($p < 0.01$ and $p < 0.001$, Fig. 1N–P). After 24 h of treatment with 0, 100, 500 or 1000 µM of ibuprofen, cell proliferation was reduced by 1.2-fold (500 µM, $p < 0.01$, Fig. 1K, Q) and 1.9-fold (1000 µM, $p < 0.001$, Fig. 1L, Q), respectively.

Ibuprofen arrested cell cycle at S stage in HUVEC

To further explore the effects of ibuprofen on HUVEC, RNA-seq was performed in control and 500 µM ibuprofen treated HUVECs. Several pathways in HUVECs were affected by ibuprofen, including VEGF signaling, apoptosis, oxidative phosphorylation and cell cycle regulation (Fig. 2A). Because the cell cycle was the most affected pathway, we studied this pathway in more detail in flow cytometry experiments and demonstrated that significantly more ibuprofen-treated HUVECs were in S phase and less cells in G2/M phase compared to controls, indicating that the cell cycle in HUVECs was arrested in S phase (Fig. 2B, C), when DNA was synthesized. Since DNA might be damaged by ibuprofen during the synthesis in S stage by oxidative stress,[29] we studied the expression of the DNA damage marker, 8-hydroxy-2′-deoxyguanosine (8-OHdG). Increased expression of 8-OHdG indicated that ibuprofen induces oxidative stress in HUVECs, damages the DNA (Fig. 2D, E) and subsequently causes apoptosis (Fig. 2F, G). This was confirmed in neonatal rats treated with ibuprofen in which we observed more vWF-positive endothelial cells in lung tissue sections that were stained for TUNEL, a marker for the final phase of apoptosis (Fig. 2H).

Effects of ibuprofen on growth and survival in neonatal rat pups

Because vascular development and angiogenesis play a critical role in alveolarization during lung development and BPD pathogenesis these in vitro data in HUVECs prompted us to investigate the effect of ibuprofen on normal postnatal lung development and BPD pathogenesis in neonatal rats kept in normoxia (room air; RA) or in hyperoxia (100% O_2), respectively. The experimental scheme is displayed in Fig. 3A. On neonatal day 10, body weight was comparable in arginine- and ibuprofen-treated rat pups kept in RA (19–20 g; Fig. 3B). Pups exposed to hyperoxia for 10 days showed a significant decrease in body weight (17 g), which was prevented by ibuprofen treatment (21 g). Exposure to hyperoxia resulted in a 43% survival on day 10 in arginine-treated rat pups (Fig. 3C). Treatment of experimental BPD with 50 mg/kg/day of ibuprofen resulted in a tendency towards less mortality (survival: 64%; Fig. 3C), which started 2 days later (Fig. 3C) compared to hyperoxia-exposed controls. All RA-exposed pups showed no morbidity or mortality during the experimental period of 10 days.

Effects of ibuprofen on postnatal pulmonary vascular development

Administration of ibuprofen to RA-exposed controls reduced

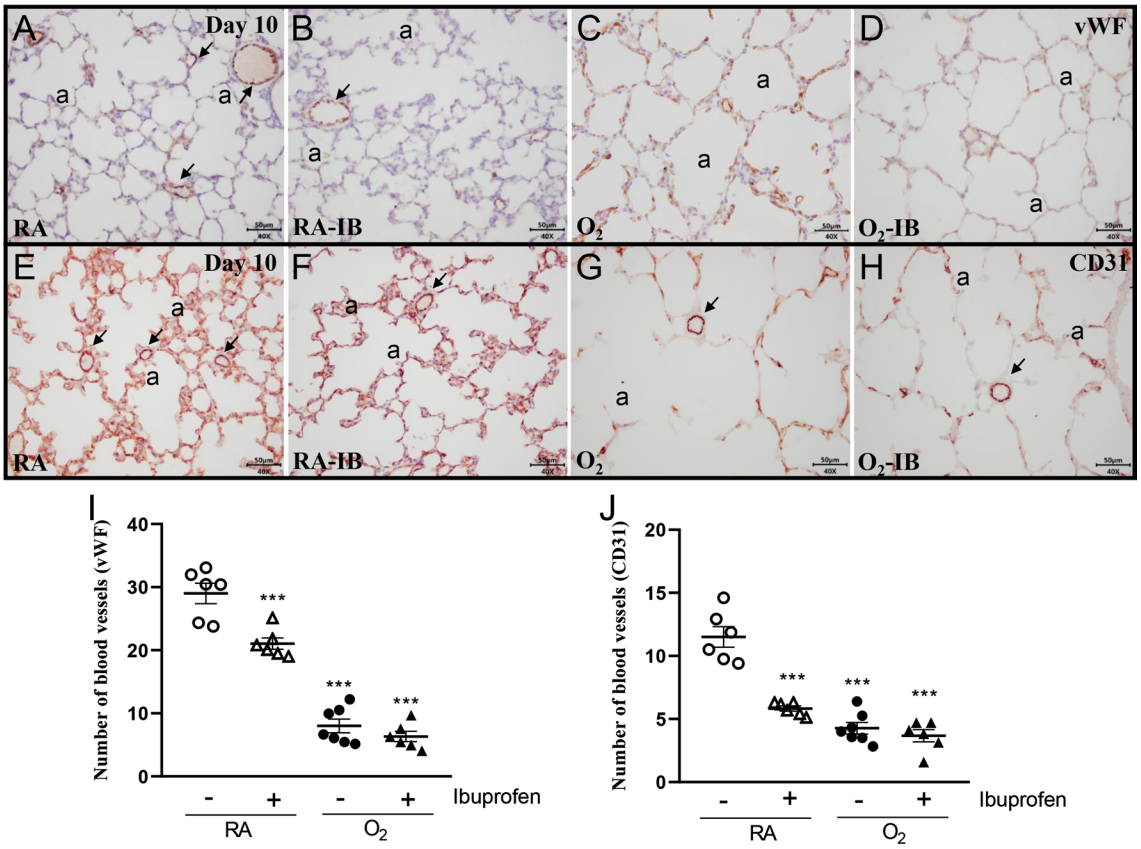

Fig. 4 Representative lung sections stained for von Willebrand Factor (vWF; **A**–**D**) or CD31 (**E**–**H**) of rat pups kept in room air (RA; open symbols; **A**, **B**, **E** and **F**) or 100% O_2 (solid symbols; **C**, **D**, **G** and **H**). Pups were injected daily with arginine buffer (circles; **A**, **C**, **E** and **G**) or ibuprofen (50 mg/kg/day; triangles; **B**, **D**, **F** and **H**) until 10 days of age. Arrows indicate blood vessels. The number of pulmonary vessels stained with vWF (**I**) or CD31 (**J**) was determined on paraffin sections in rat pups on day 10. Values are expressed as mean ± SEM (N = 6–7). ***$p < 0.001$ versus RA controls

blood vessel density: 1.6-fold for vWF staining (Fig. 4B, I) and 2.0-fold for CD31 staining (Fig. 4F, J), ($p < 0.001$). The anti-angiogenic effect of ibuprofen was less significant than exposure to hyperoxia, which dramatically reduced pulmonary vessel density (2.3-fold for vWF staining and 2.7-fold for CD31 staining, $p < 0.001$; Fig. 4C, G, I and J) compared to RA controls. In hyperoxia-exposed pups treated with ibuprofen, blood vessel density was not significantly different from hyperoxia-exposed treatment controls (Fig. 4D, H, I and J).

Effects of ibuprofen on pulmonary inflammation and lung airway development

Since ibuprofen is a potent anti-inflammation drug, we studied the effect of ibuprofen on pulmonary inflammation induced by hyperoxia. Oxygen exposure for 10 days resulted in an influx of macrophages (11.8-fold, $p < 0.01$ Fig. 5C, I) and neutrophils (29.2-fold, $p < 0.001$, Fig. 5G, J). Administration of 50 mg/kg/day of ibuprofen to hyperoxia-exposed pups significantly reduced the influx of macrophages (2.2-fold, $p < 0.05$; Fig. 5D, I) and neutrophils (2.7-fold, $p < 0.001$; Fig. 5H, J). Because inflammation and vascular development are key mediators in BPD, we investigated the effect of ibuprofen on lung airway development. Hyperoxia led to a heterogeneous distribution of enlarged airspaces (1.6-fold, $p < 0.001$; Fig. 6C, I), surrounded by septa with increased thickness (2.6-fold, $p < 0.001$; Fig. 6C, J), and increased pulmonary arterial wall thickness (2.2-fold, $p < 0.001$; Fig. 6G, K). Compared to hyperoxia exposed controls, ibuprofen reduced alveolar size (1.3-fold, $p < 0.001$; Fig. 6D, I), alveolar septal thickness (1.5-fold, $p < 0.001$; Fig. 6D, J), and pulmonary arterial wall thickness (1.8-fold, $p < 0.001$; Fig. 6H, K). Administration of ibuprofen to RA-exposed controls did not affect the parameters investigated.

Effects of ibuprofen treatment on mRNA expression in lung tissue of genes involved in inflammation and coagulation

To explore the anti-inflammatory effect of ibuprofen in experimental BPD, presented in Fig. 7, we studied mRNA expression of key genes involved in inflammation and coagulation. Exposure of neonatal rat pups to hyperoxia for 10 days increased mRNA expression in the lung of the inflammatory factors interleukin 6 (IL-6, 49.7-fold, $p < 0.001$; Fig. 7A), chemokine-induced neutrophilic chemoattractant-1 (CINC-1, 9.8-fold, $p < 0.001$; Fig. 7B), and monocyte chemoattractant protein 1 (MCP-1, 13.8-fold, $p < 0.001$; Fig. 7C), the procoagulant factor tissue factor (TF, 3.3-fold, $p < 0.001$; Fig. 7D), compared to RA controls. Administration of ibuprofen for 10 days to hyperoxia-exposed rat pups significantly reduced mRNA expression of IL-6, MCP-1 and TF ($p < 0.001$, $p < 0.01$ and $p = 0.05$, respectively, Fig. 7A, C and D) compared to hyperoxia controls.

Discussion

Ibuprofen compromised endothelial function in HUVECs by inducing oxidative stress-related DNA damage and arresting the cell cycle in the S-phase, which subsequently promoted endothelial apoptosis. The anti-angiogenic effect of ibuprofen in HUVECs, demonstrated by inhibition of cell proliferation, migration and tube formation ability, confirms previously published data.[30] Because ibuprofen is frequently used to treat a patent ductus arteriosus after premature birth, a patient

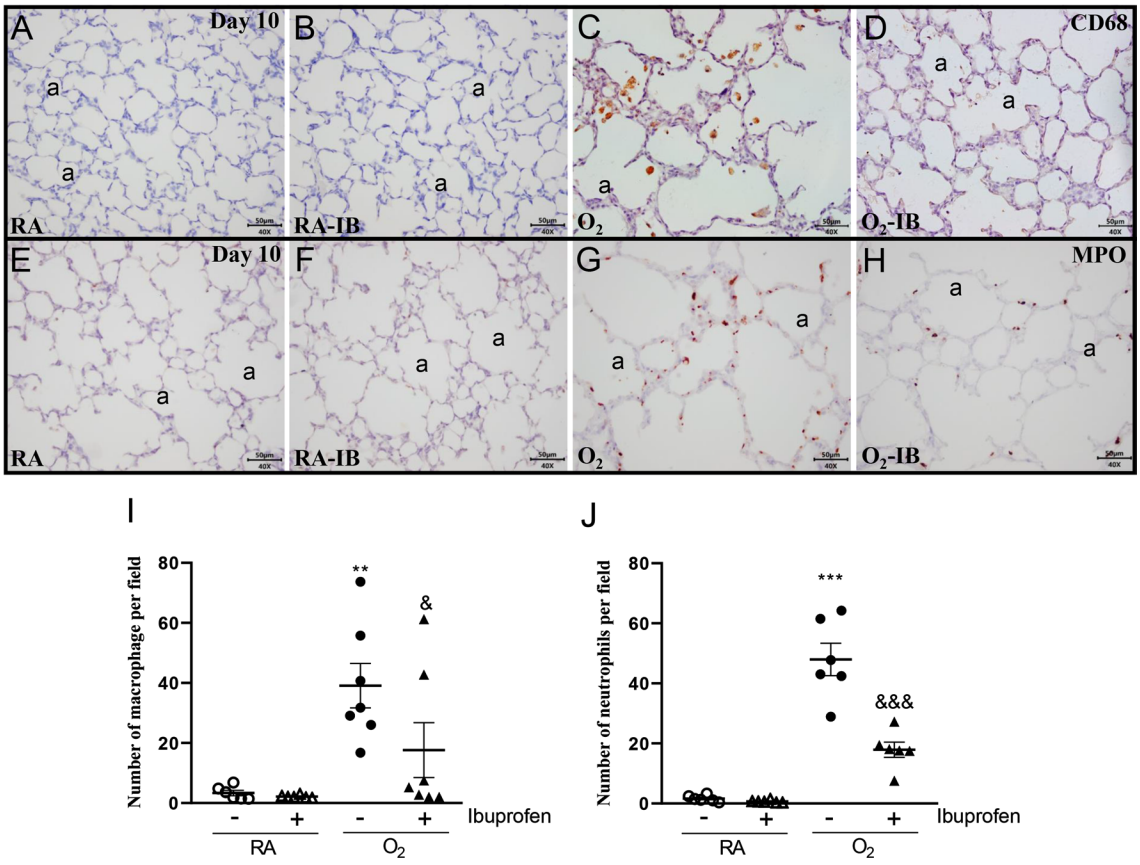

Fig. 5 Representative lung sections stained for the macrophage marker CD68 (**A**–**D**) or myeloperoxidase (MPO) as a marker for neutrophilic granulocytes (**E**–**H**) of rat pups kept in room air (RA; **A**, **B**, **E** and **F**) or 100% O_2 (**C**, **D**, **G** and **H**). Pups were injected daily with arginine buffer (**A**, **C**, **E** and **G**) or ibuprofen (50 mg/kg/day; **B**, **D**, **F** and **H**) until 10 days of age. a = alveolus. The influx of monocytes and macrophages (**I**) and neutrophilic granulocytes (**J**) was determined by morphometry on lung sections. RA pups (open symbols) and O_2 pups (solid symbols) were injected daily with arginine buffer (circles) or ibuprofen (50 mg/kg/day; triangles) until 10 days of age. Values are expressed as mean ± SEM (N = 6–7). $^{**}p < 0.01$ and $^{***}p < 0.001$ versus RA controls. $^{\&}p < 0.05$ and $^{\&\&\&}p < 0.001$ versus age-matched arginine buffer-treated O_2-exposed controls

population at risk of developing BPD, and angiogenesis plays a crucial role in normal and aberrant postnatal lung development, these findings prompted us to study the role of ibuprofen in normal neonatal lung development and in the pathogenesis of experimental BPD in rats.[18,31] The anti-angiogenic effect of ibuprofen in HUVECs was confirmed in vivo in rat pups in which ibuprofen treatment during normal neonatal lung development had adverse effects on pulmonary vascular development that resulted in a reduced vascular bed. However, beneficial effects were also demonstrated in rat pups with hyperoxia-induced experimental BPD in which treatment with ibuprofen attenuated disease progression and lung injury by reducing lung inflammation, preventing pulmonary vascular remodeling and preserving alveolar development.

Ibuprofen-induced inhibition of angiogenesis was demonstrated by a reduced vascular bed in newborn rat pups raised in normoxia showing a reduced number of blood vessels after ibuprofen treatment using two different endothelial markers: vWF and CD31. An ibuprofen-induced inhibition of vascular development was observed in rat ocular development,[21] cardiovascular development in zebrafish[22] and tumor growth and metastasis.[30,32] The potential mechanisms involved include inhibition of vascular growth factors, like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hypoxia-inducible factors (HIF),[33-35] inhibition of the mitogen-activated protein (MAP) kinase (ERK2) activity,[36] and direct inhibition of cell function.[32] Here, we found that multiple pathways were affected in ibuprofen-treated HUVECs, including the well-known angiogenesis related pathways: VEGF signaling, HIF signaling and Hippo signaling, as well as processes involved in cell cycle, apoptosis, senescence, necroptosis and ferroptosis, of which the cell cycle was the most significantly affected one. We further confirmed the arrest of the cell cycle at S stage in ibuprofen treated HUVECs. Although much is known about the regulation of the G1/S, G2/M, and metaphase/anaphase transitions by different cyclin-dependent kinases (cDKs) and their activating cyclin subunits, less is known about the control mechanism for the S/G2 transition. The expression of CDK1/2, CHK1 and cyclin A, which were suggested to be involved in S phase or S/G2 transition,[37] were significantly down regulated by ibuprofen in the RNA-seq data (data not shown). In addition, experimental evidence strongly suggests that DNA damage is a trigger for S/G2 arrestment.[38] Therefore, we examined the 8-OHdG level to indicate DNA injury and found that ibuprofen increased 8-OHdG expression, probably reflecting DNA damage in HUVECs, thereby leading to the arrest in the S stage and resulting in cell apoptosis shown in this study and by others.[29,34,39] Besides, we also found the apelin/APJ pathway as one of the most affected pathways by ibuprofen in our RNA seq data (Fig. 2A), in which apelin is a potent vasodilator and protects effectively against experimental BPD in rat pups.[9]

Inflammation plays a pivotal role in the pathogenesis of BPD and may contribute to severe lung tissue damage and fibrosis, and treatment with anti-inflammatory agents protects against

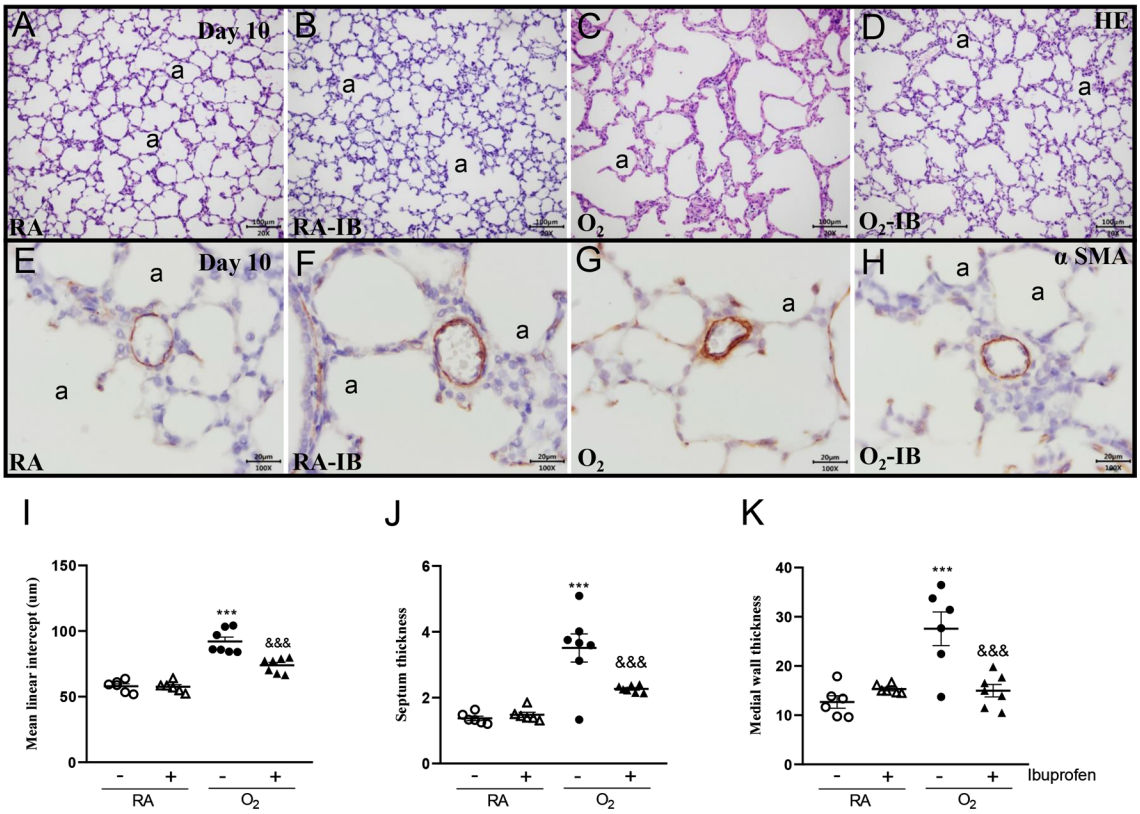

Fig. 6 Representative lung sections stained for HE (**A**–**D**), or α smooth muscle actin (αSMA; **E**–**H**) of rat pups kept in room air (RA; **A**, **B**, **E** and **F**) or 100% O_2 (**C**, **D**, **G** and **H**). Pups were injected daily with arginine buffer (**A**, **C**, **E** and **G**) or ibuprofen (50 mg/kg/day: **B**, **D**, **F** and **H**) until 10 days of age. a = alveolus. Lung morphometry, including the quantification of mean linear intercept (MLI, **I**), septal thickness (**J**), and arterial medial wall thickness (**K**), was determined on paraffin sections from rat pups on day 10. RA pups (open symbols) and O_2 pups (solid symbols) were injected daily with arginine buffer (circles) or ibuprofen (50 mg/kg/day; triangles) until 10 days of age. Values are expressed as mean ± SEM (N = 6–7). ***$p < 0.001$ versus RA controls. $^{\&\&}p < 0.01$ and $^{\&\&\&}p < 0.001$ versus age-matched arginine buffer-treated O_2-exposed controls

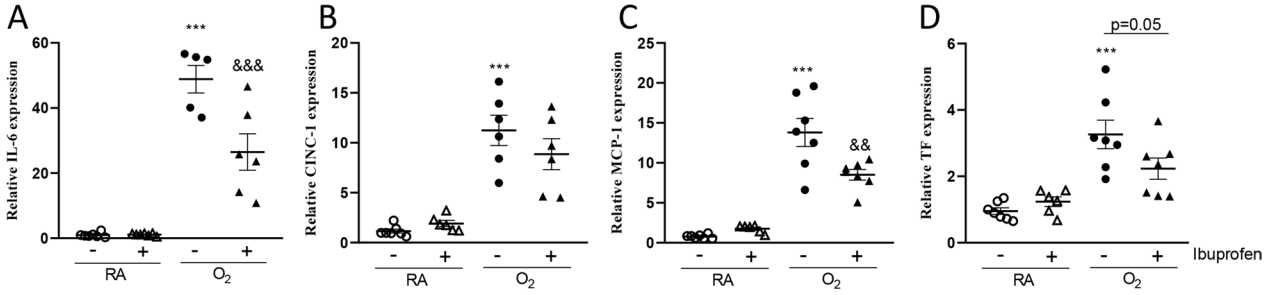

Fig. 7 Relative mRNA expression in lung homogenates of Interleukin 6 (IL6; **A**), chemokine-induced neutrophilic chemoattractant-1 (CINC-1; **B**), monocyte chemoattractant protein 1 (MCP1; **C**) and tissue factor (TF; **D**) on day 10 in rat pups. RA pups (open symbols) and O_2 pups (solid symbols) were injected daily with arginine buffer (circles) or ibuprofen (50 mg/kg/day; triangles) until 10 days of age. Values are expressed as mean ± SEM (N = 5–7). ***$p < 0.001$ versus RA controls. $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ versus age-matched arginine buffer-treated O_2-exposed controls

hyperoxia-induced experimental BPD.[11,40,41] Since ibuprofen is a potent nonsteroidal anti-inflammatory drug, we expected an anti-inflammation effect of ibuprofen in our experimental model. Indeed, ibuprofen protected against hyperoxia-induced lung injury in rat pups by reducing the influx of inflammatory cells, mRNA expression of pro-inflammatory genes, vascular remodeling and alveolar enlargement in the current study. The anti-inflammatory effect of ibuprofen in neonatal rats with experimental BPD is supported by observations in multiple in vivo models of lung disease in dogs, rabbits and sheep with sepsis, in mice with trauma and septic challenge, in rats with ventilator-induced or endotoxic lung injury, in sheep with thrombin-induced lung vascular leakage[42-44] and in cystic fibrosis patients with lung inflammation.[45] The mechanism of anti-inflammation by ibuprofen has been established by blocking COXs activity, thereby attenuating prostaglandin mediated inflammation.[46] Our data confirm previous studies demonstrating protection against hyperoxia-induced BPD in rodents treated with (selective) COX2 inhibitors, including aspirin and celecoxib and in genetically modified $COX2^{-/-}$ mice.[47]

The absence of alveolar enlargement in ibuprofen treated rat pups kept in normoxia and the beneficial effect of ibuprofen on aberrant alveolar development and vascular remodeling in experimental BPD was unexpected, because in BPD pathogenesis alveolar enlargement is believed to be driven

by aberrant vascular development.[1,31] We speculate that (1) despite its anti-angiogenic effect ibuprofen preserves vascular integrity, thereby preventing alveolar enlargement in rat pups kept in normoxia and (2) ibuprofen alleviates BPD pathology in rat pups kept in hyperoxia by reducing the inflammatory response and preserving vascular integrity thereby preventing aberrant alveolar development and vascular remodelling. Our findings are supported by experimental data by Kuniyoshi et al.,[48] who found reduced alveolarization in neonatal rats treated with indomethacin, but not in ibuprofen treated neonatal rats. Their histological data clearly demonstrate that, in contrast to indomethacin treatment, early and late treatment with ibuprofen prevents alveolar enlargement in neonatal rats with experimental BPD. However, this beneficial effect of ibuprofen on alveolar enlargement in experimental BPD was not claimed by Kuniyoshi et al.[48] A protective effect of ibuprofen in the lung was also demonstrated in premature baboons on lung development and in adult rats with ventilator-induced lung injury.[43,49]

Although clinical studies suggest that ibuprofen treatment for PDA closure in very premature infants might be a risk factor for PAH,[19] our data do not support this potential adverse effect. We demonstrated that treatment of neonatal rats with ibuprofen had no adverse effects on arterial vascular remodeling during normal postnatal development and even prevented vascular remodeling in pups with experimental BPD, which is a readout for PAH in this experimental BPD model.[11,50] The beneficial effects of ibuprofen on pulmonary vascular remodeling were unexpected, because reduced intracellular cAMP levels caused by prostaglandin inhibition in vascular smooth muscle cells are expected to exacerbate PAH.[51,52] This unexpected finding may be explained indirectly via a dampening of the inflammatory response by ibuprofen, thereby preserving endothelial cell integrity and function, and reducing smooth muscle cell proliferation and contraction.[53] Alternatively, the beneficial effect on vascular remodeling can also be mediated via ibuprofen's off-target effect of elevating intracellular cGMP levels via cGMP-selective phosphodiesterase (PDE) inhibition.[54,55] This explanation is supported by the beneficial effects of agents that increase intracellular cGMP levels, either by stimulating the NO-eNOS-cGMP pathway with inhaled NO, apelin or soluble guanylate cyclase modulators or inhibiting cGMP breakdown with the specific cGMP-selective PDE5 inhibitor sildenafil, in newborn rats with experimental BPD that our group and others described previously.[9,50,56-58] Interestingly, the beneficial effects of ibuprofen on experimental BPD may be explained by activation of the apelin/APJ pathway, which we have demonstrated to protect against experimental BPD in rats.[9]

In this study, we exposed pups to ibuprofen for the whole experimental period (10 days), which varies from clinical practice, where ibuprofen is usually given to preterm infants for 3 days to close a PDA. Short versus prolonged and early versus late exposure to ibuprofen may affect its anti-inflammatory and anti-angiogenic effects. The anti-inflammatory effect of ibuprofen might be absent if ibuprofen is given for a short period and inflammation has not yet been established. Similarly, the anti-angiogenic effect of ibuprofen might be absent if given at a later stage when vascular growth is less vulnerable. We have recently demonstrated that ibuprofen reduces vascular growth factors, such as PDGF-BB, VEGF-A and HIF-2α, in infants with PDA,[59] confirming that the anti-angiogenic effect of ibuprofen is already present in human infants exposed for 3 days. Although this adverse effect on vascular growth might be absent when ibuprofen is given at a later stage, it may compromise its positive effect on PDA closure. This is in line with a recent clinical trial showing that ibuprofen significantly increased the risk of BPD in infants with a PDA[60] in the absence of its anti-inflammatory and presence of its anti-angiogenic effect. In the experimental BPD pups both the anti-angiogenic and anti-inflammatory effects were present and this might explain the different findings between our and clinical studies.

We acknowledge several limitations in this work. We used HUVECs in the in vitro experiments to study the effects of ibuprofen on angiogenesis. Although HUVECs are primary endothelial cells isolated from the umbilical cord vein and widely used in endothelial function studies, there might be fundamental differences between pulmonary micro vessels and the umbilical cord vein. Furthermore, since the ductus arteriosus closes naturally within 3 days in newborn rodent pups, we could not investigate the influence of ibuprofen on ductus closure, and the associated effect on BPD conferred by our and other clinical studies. Furthermore, we did not determine the gender of the pups in our study, obliviating the possibility to establish a potential difference in ibuprofen effect between males and females.

Ibuprofen exhibits an anti-angiogenic effect in HUVECs and the developing lung, which is considered an adverse effect in lung development and the pathogenesis of BPD, and beneficial effects in experimental BPD by promoting alveolarization, reducing inflammation and preventing vascular remodeling. This suggests that the beneficial effects of ibuprofen outperform the adverse effects in hyperoxia-induced experimental BPD in rat pups. However, extrapolation of the beneficial effects of ibuprofen and other NSAIDs to premature infants with BPD should be done with extreme caution. Similarly, prolonged and repeated courses of ibuprofen treatment for PDA closure in premature infants should be carefully considered.

Abbreviations

ACTB	Beta-actin
ASMA	α Smooth muscle actin
BPD	Bronchopulmonary dysplasia
CINC1	Chemokine-induced neutrophilic chemoattractant-1
CLD	Chronic lung disease
ECM	Endothelial cell medium
HUVECs	Human umbilical vein endothelial cells
IL-6	Interleukin 6
MLI	Mean linear intercept
MCP1	Monocyte chemoattractant protein 1
MPO	Myeloperoxidase
NSAID	Nonsteroidal anti-inflammatory drug
8-OHdG	8-Hydroxy-2′-deoxyguanosine
PAH	Pulmonary arterial hypertension
PDA	Patent ductus arteriosus
TF	Tissue factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VEGF	Vascular endothelial growth factor
vWF	Von Willebrand factor

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Author contributions

CY, GW and XC conceptualized and designed the study, XC

and GW wrote the first draft of the manuscripts. DH, XW, JZ and ZH carried out the experiments. XH and YL performed the data analysis. GW, CY, FW and XC reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All animal procedures in this study were approved by the Institutional Animal Care and Use Committee of the Shenzhen Institutes of Advanced Technology of the Chinese Academy of Sciences.

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