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neonatal INTENSIVE CARE

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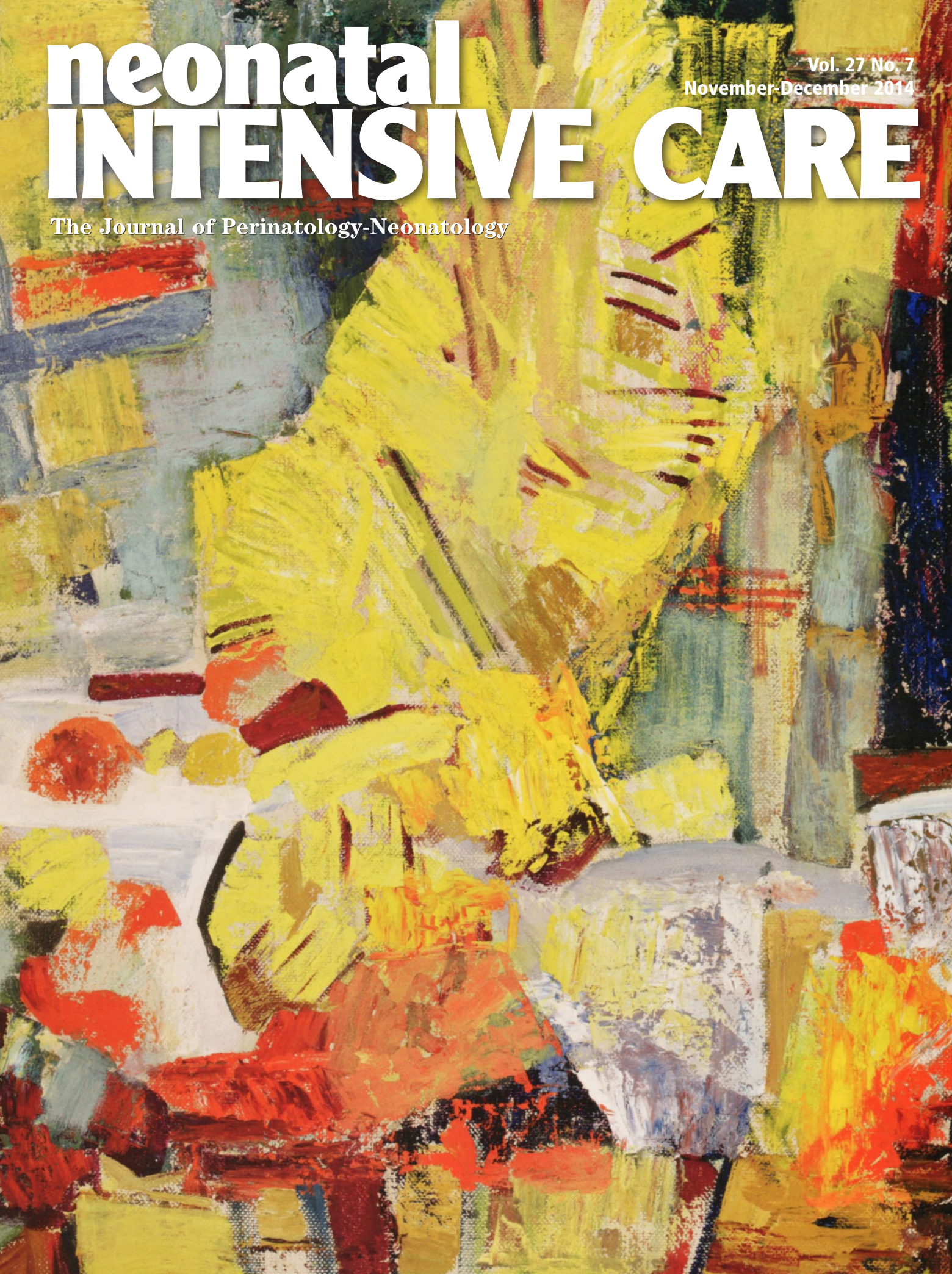

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References: 1. US Dairy Export Council. *Reference Manual for US Whey and Lactose Products*. June 2004. **2.** HPLC analysis. Data on file. Nestlé. 2002. **3.** Florendo KN et al. Growth in preterm infants fed either a partially hydrolyzed whey or an intact casein/whey preterm infant formula. *J Perinatol*. 2009;29:106-111. **4.** Cooke R et al. High protein pre-term infant formula: effect on nutrient balance, metabolic status and growth. *Pediatr Res*. 2006;59:265-270. **5.** American Academy of Pediatrics. Committee on Nutrition. *Pediatric Nutrition Handbook*. 6th ed. 2009.

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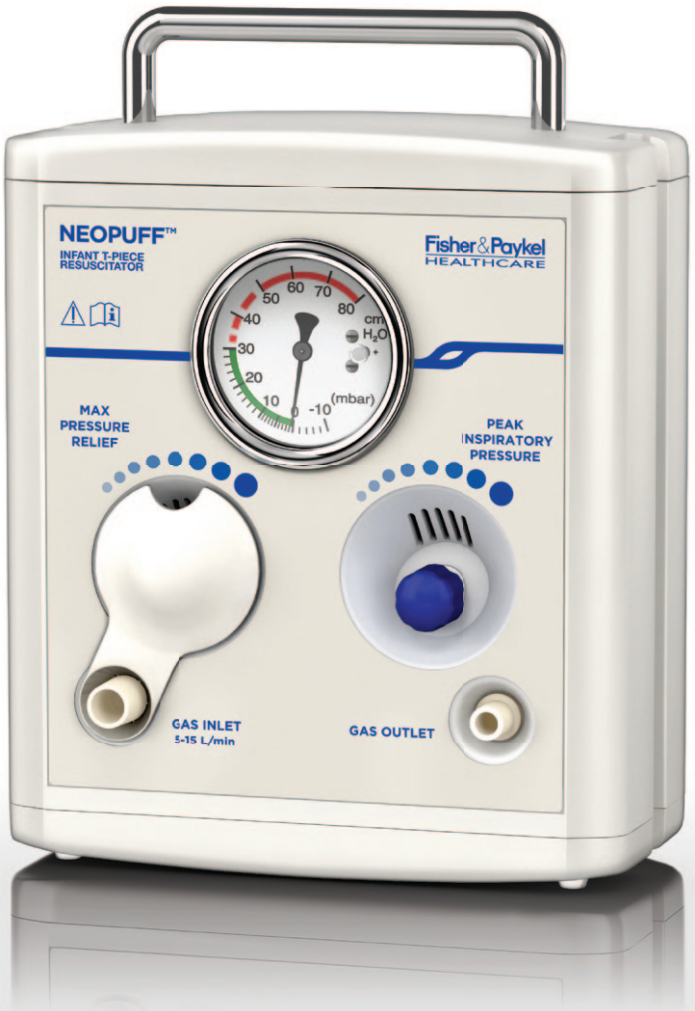

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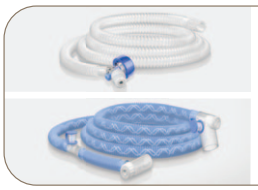

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Transport Ventilation for Neonates

Hamilton Medical has introduced a new item for the "most fragile patients" with the release of the HAMILTON-T1 with neonatal option — a high-end transport ventilator. Although not yet available in the US, during transport the HAMILTON-T1 delivers the same performance as a fully featured NICU ventilator at the bedside. Hamilton Medical says it supports tidal volumes of just 2 ml, and allows for effective, safe, and lung-protective ventilation for even the smallest preemies. The neonatal flow sensor accurately measures pressure, volume, and flow proximal to the patient. This guarantees the required sensitivity and response time, and prevents dead space ventilation. Therefore, the patient is better synchronized and the work of breathing (WOB) is reduced. The new neonatal expiratory valve can balance even the smallest differences in pressure and offers the neonate the possibility to breathe spontaneously in each phase of a controlled breathing cycle. In addition to all modern neonatal ventilation modes, the HAMILTON-T1 offers a new generation of nCPAP. In the new nCPAP-PC (pressure control) mode, you only define the desired CPAP target value for your patient and the ventilator automatically and continuously adapts the required flow to the patient's condition and possible leaks. Thanks to the demand flow technology, your patient will receive only as much flow as is necessary to obtain the set CPAP target. This reduces WOB, reduces the need for user interventions and ensures optimal leak compensation. You will also require less oxygen for transport and noise caused by the ventilator decreases distinctively. For mobility, the built-in high-performance turbine makes it independent of compressed air, gas cylinders or compressors. This saves weight and space and even noninvasively ventilated neonates can be transported over long distances. The combination of a built-in and an optional hot-swappable battery provides a battery operation of more than 9 hours. This can be extended indefinitely with additional hot-swappable batteries.

Life-saving Treatment Not Given to All Moms

A steroid injection that prevents disability and even death in premature babies is only being administered to half of the mothers who give birth prematurely in hospital. The World Health Organization conducted a study that has been published in The Lancet, from May 2010 to December 2011. It examined the use of antenatal corticosteroids, which cost less than one US dollar each, and reduce the risk of respiratory distress syndrome in premature babies. The use of tocolytic drugs to slow down labour and allow the antenatal corticosteroids to work was also studied. Using data from a WHO Multicountry Survey, 29 countries and over 300,000 births in 359 hospitals were studied. It showed that only 52 percent of women who gave birth at 26-34 weeks' gestation and were eligible for the injection actually received the treatment while in labour. The rate varied between countries as the majority studied were of low-and-middle income. More so, only 18 per cent of women who could receive both the antenatal corticosteroids and tocolytic drugs were actually given them.

Drug Treatment Reduced for Neonates

Use of a stringent protocol to treat neonatal narcotic abstinence syndrome (NAS) reduces the duration of opioid exposure as well as the length of hospital stay, according to a new study. The benefits of a stringent protocol are significant, regardless of the opioid used for treatment. NAS is increasing in prevalence in the US, and yet there is currently no consensus with regard to the best treatment drug or best taper strategy for NAS management. The study advances medical understanding of the "best practice" for NAS management. Eric S. Hall, PhD, from the Prenatal Institute at Cincinnati Children's Hospital in Ohio, and

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colleagues present the results of their cohort analysis in July 28 article in Pediatrics. The multicenter cohort includes charted data from 547 pharmacologically treated infants and is larger than any other previously published study or meta-analysis. "Our study identified key differences in NAS management strategies that translated into shorter opioid exposures and reduced length of hospital stay. Results indicate that the use of a stringent weaning protocol, rather than the particular opioid chosen for treatment, was the most important predictor of length of hospital stay and duration of opioid treatment," the authors write. "Consistent with previous literature describing improvements in pediatric outcomes through standardization of care, study results suggest that the greatest impact on outcomes is achieved through implementation and adherence to a formalized NAS treatment protocol with agreed-upon starting doses, explicit instruction about dose escalation, and strict weaning parameters," the authors explain. The study included only infants who required opioid therapy (417 managed with an established weaning protocol and 130 managed without an established weaning protocol). After the researchers accounted for hospital variation, infants who received protocol-based weans had a significantly shorter duration of opioid treatment (17.7 vs 32.1 days; $P < .0001$) and shorter hospital stay (22.7 vs 32.1 days; $P = .004$). Among those who received protocol-based weaning, the duration of opioid treatment and length of stay were no different in infants treated with morphine compared with those treated with methadone. When the authors analyzed the data from patients who were treated with phenobarbital, they found a longer duration of phenobarbital administration in patients treated with morphine compared with those treated with methadone ($P \leq .002$). The protocol-driven wean described in the current study has the advantage of reducing the length of drug treatment.

Neonatal Jaundice and Brain Damage

Early detection of jaundice and immediate treatment can ensure neonatal damage to a baby's brain won't happen. The condition is the result of a yellow substance, bilirubin, which breaks down in the liver and is usually removed from the body via stools. While the fetus grows in the womb, it is fed by the mother's placenta, which is also responsible for removing this bilirubin. Bilirubin is created when the body replenishes the old red blood cells. After birth, the newborn's liver must independently start casting this substance out, a process which can take some time to develop adequately. While there is a high level of bilirubin in the baby's blood, it causes the skin and whites of the baby's eyes to appear yellow. Breastfeeding jaundice is another type of neonatal jaundice commonly seen in the first week of life among breastfed babies. It occurs when the baby does not nurse well, or the mother's milk supply is low. If there are any signs which appear after the newborn goes home, the bilirubin levels should be measured right away. Besides a simple blood test, hospitals today use probes that estimate the bilirubin level by merely touching the baby's skin. A baby with neonatal jaundice must be kept well-hydrated, either with breast milk or formula food. A regular feeding schedule encourages bowel movements, which will help remove bilirubin through the stool. Some newborns diagnosed with jaundice may need to be treated in the hospital for one to two days. Sometimes, the infant will be placed under special blue lights for a treatment called phototherapy. The light helps break down bilirubin in the baby's skin. It is a rare occurrence that a baby has severe neonatal jaundice. This may occur if there is a phenomenal advancement in the number of red blood cells the body needs to replace. This is a chronic symptom in small-for-gestational-age babies, and sometimes in twins. It may also occur if there is a mismatch in the blood type between the mother and the baby. Very high levels of bilirubin can be toxic to the brain and can lead to complications associated with neonatal jaundice. When the bilirubin gets into the central nervous system, it can lead to a condition called kernicterus. Kernicterus may have begun to develop if the infant begins to exhibit extreme lethargy, changes in muscle tone, and a high-pitched cry. Information in this article was compiled by Nilofar Neemuchwala.

Obese Women Need Medical Help Too

Personal biases and concerns about professional liability are leading some obstetricians to avoid obese patients, putting babies and the moms at greater risk of complications. Dr Sigal Klipstein, chairwoman of the committee on ethics of the American College of Obstetricians and Gynecologists, says it is time for doctors to push aside prejudice and fear. They must take more positive steps to treat obese women who are pregnant or want to become pregnant. Obesity affects 36 percent of women of childbearing age and is linked to a host of difficulties during pregnancy, labor and delivery, including gestational diabetes, hypertension and pre-eclampsia to miscarriage, premature birth, emergency cesarean delivery and stillbirth. The infants of obese women are more likely to have congenital defects, and they are at greater risk of dying at or soon after birth. Babies who survive are more likely to develop hypertension and obesity as adults. A published analysis of 38 studies found that even modest increases in a woman's pre-pregnancy weight raised the risks of fetal death, stillbirth and infant death. Dr Klipstein and her colleagues recently issued a report on ethical issues in caring

for obese women. Obesity is commonly viewed as a personal failing that can be prevented or reversed through motivation and willpower. But the facts suggest otherwise. Although some people manage to shed as much as 100 pounds and keep them off without surgery, many obese patients say they've tried everything, and nothing has worked. "Most obese women are not intentionally overeating or eating the wrong foods," Dr Klipstein said. "Obstetricians should address the problem, not abandon patients because they think they're doing something wrong." The committee report emphasizes that "obese patients should not be viewed differently from other patient populations that require additional care or who have increased risks of adverse medical outcomes." Obese patients should be cared for "in a nonjudgmental manner," it says, adding that it is unethical for doctors to refuse care within the scope of their expertise "solely because the patient is obese." Obstetricians should discuss the medical risks associated with obesity with their patients and "avoid blaming the patient for her increased weight," the committee says. Any doctor who feels unable to provide effective care for an obese patient should seek a consultation or refer the woman to another doctor. Obesity rates are highest among women "of lower socioeconomic status," the report notes, and many obese women lack "access to healthy food choices and opportunities for regular exercise that would help them maintain a normal weight."

High-Volume Units Best

A new study says that treating preemies in high-volume neonatal hospital units increases survival rates. The British study included babies born before 33 weeks of pregnancy who were admitted for extra care. Full term is considered 39 to 40 weeks. The analysis confirms results of a 2010 US study led by Dr Judith Chung. For the new study, the researchers analyzed data from 20,554 very premature infants delivered at 165 hospitals with neonatal units across the UK. About 4.5 percent of them died in the hospital. Infants were 32 percent less likely to die if they were admitted to high-volume neonatal units compared to low-volume units, the researchers found. The earliest preemies, those born before 27 weeks of pregnancy, benefited the most from high-volume units. Those babies had half the odds of dying when they were treated in neonatal units that handle a high number of premature births, compared to low-volume units, the study published in BMJ Open found.

Company Offers Human Milk-derived Formula

Prolacta Bioscience, Inc., the pioneer in human milk-based neonatal nutritional products, now offers the first and only ready-to-feed (RTF) human milk-derived premature infant formula, Prolact RTF. It is made from 100 percent human donor milk, as opposed to cow milk, and is used when mother's own milk is not available and fortification is needed. Prolact RTF provides optimal nutrition for premature infants in the neonatal intensive care unit (NICU). The benefits of Prolact RTF include less risk of mixing errors and more efficient preparation time. It is a cost-effective way to provide a 100 percent human milk diet when mother's own milk is not available. "Prolact RTF now provides healthcare providers with an alternative to the traditional cow milk-based

infant formula, when mother's own milk is not available," said Scott Elster, CEO of Prolacta Bioscience. "This product reduces the amount of time it takes to prepare feedings and significantly decreases the opportunity for mixing errors. It is a huge step forward for those babies without access to mom's own milk." Clinical studies have shown that extremely premature infants (500 grams – 1250 grams at birth) who are fed a 100% human milk diet instead of preterm formula containing cow-milk protein have a significantly lower incidence of surgery associated with the intestinal disease necrotizing enterocolitis (NEC) and require fewer days of intravenous feedings through total parenteral nutrition (TPN). One such clinical trial compared the duration of TPN, morbidities, and growth patterns in extremely premature infants fed exclusive diets of either cow milk-based preterm formula or donor human milk and human milk-based human milk fortifier (H2MF). The trial was a clear comparison of the effects of formula versus donor human milk. The trial's conclusion supported the use of a 100 percent human milk diet to nourish extremely preterm infants in the neonatal intensive care unit. The American Academy of Pediatrics (AAP) recommends the use of human milk for all preterm infants, be it mother's own milk or pasteurized donor human milk when mother's own milk is unavailable. Premature infants are given superior protection when their nourishment begins with human milk, and the odds of developing NEC are reduced by 77% when infants are fed a 100 percent human-milk diet, as opposed to one containing cow milk.

Parents Get Emergency Training

A new, first-of-its-kind program in Texas provided by the Simulation Center at Texas Children's Hospital will help reassure and prepare parents whose babies are being discharged from the hospital's neonatal intensive care unit (NICU) on ventilators with tracheostomies. Texas Children's Hospital cares for babies with complex medical issues such as chronic lung disease from extreme prematurity, which frequently requires mechanical ventilation for long periods of time. Despite needing mechanical ventilation, babies can have a tracheostomy placed, so that they can be sent home safely. The pilot program, currently in a study phase, was developed to allow parents to practice real-life airway emergency scenarios that can occur when a baby on a ventilator with a tracheostomy is released from the NICU to be cared for at home. The goal of the program is to educate and empower parents to respond skillfully and confidently in a variety of emergency scenarios and to reduce readmissions to the hospital and/or accidental death. Mortality after discharge from the hospital directly associated with airway emergencies ranges between 0.5 and 3 percent in this patient population. Most of these events result from one of two types of airway emergencies– accidental dislodgement or blockage of the tracheostomy tube. Using a highly technical and realistic mannequin that has been modified with a tracheostomy, 10 families have participated in the pilot program which involves practicing four airway emergency scenarios including water dumping from the humidified ventilator circuit into the tracheostomy tube, a tracheostomy tube obstruction, accidental dislodgement of the tracheostomy and what to do in the event of a power failure. The emergency scenarios parents practice in simulation training are based on actual experiences of former Texas Children's NICU parents after their child was discharged from the hospital. After each simulation scenario, parents receive video-review assisted feedback about their performance during one-on-one debriefing sessions where deviations from expected actions are discussed.

Hospital Banks on Donor Milk

The Children's Hospital of Philadelphia (CHOP) announced it will develop a non-profit milk bank to provide donor human milk for hospitalized infants at the Hospital's Main Campus, with the goal of opening in late summer of 2015. CHOP will develop the bank in cooperation with the Human Milk Banking Association of North America, a professional organization that sets the standards and guidelines for non-profit donor milk banking in North America. Once open, it will be one of the only non-profit milk banks located inside a freestanding children's hospital in the United States. At CHOP, more than four out of five infants discharged from the Hospital's intensive care units are receiving human milk. The Hospital has used donor human milk since 2006 for at-risk infants to supplement a mother's own milk supply if it is insufficient or if the mother is unable to provide milk for her infant. This milk is ordered from an HMBANA-certified milk bank, where it is processed and pasteurized in accordance with stringent safety guidelines, and then shipped to CHOP. There are 17 association milk banks throughout the US and Canada. At CHOP, many mothers choose to become human milk donors and CHOP facilitates the donation process in partnership with a HMBANA milk bank. In order to become a HMBANA donor, the mother must meet strict donor criteria to ensure that she is healthy and the milk is safe. Donors must complete a medical history and lifestyle questionnaire and obtain the approval of their healthcare provider prior to donating milk, as well as have a blood test to screen for diseases including HIV, hepatitis B and syphilis. HMBANA-approved donors are volunteers and are uncompensated. These same guidelines will be followed in the future; however, the process will be completed at CHOP and the donated milk will be pasteurized and processed for CHOP's inpatient infant population.

Placentas Get a Bad Rap

Scientists at the UCSF Medical Center in San Francisco are studying placentas in an effort to unlock more potential benefits. The placenta is a disk of tissue attached to the uterine lining on one side and to the umbilical cord on the other, which grows from the embryo's cells, not the mother's. It is sometimes called the afterbirth: It comes out after the baby is born, usually weighing about a pound, or a sixth of the baby's weight. It provides oxygen, nourishment and waste disposal, doing the job of the lungs, liver, kidneys and other organs until the fetal ones kick in. Dr Susan Fisher, a professor of obstetrics, gynecology and reproductive sciences, and other researchers have studied the placenta for decades, but she said: "Compared to what we should know, we know almost nothing. It's a place where I think we could make real medical breakthroughs that I think would be of enormous importance to women and children and families." The National Institute of Child Health and Human Development the placenta "the least understood human organ and arguably one of the more important, not only for the health of a woman and her fetus during pregnancy, but also for the lifelong health of both." In May, the institute gathered about 70 scientists at its first conference devoted to the placenta, in hopes of starting a human placenta project, with the goal of finding ways to detect abnormalities in the organ earlier, and treat or prevent them.

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Predictors for an Unsuccessful Intubation-Surfactant-Extubation Procedure: A Cohort Study

Brix N, Sellmer A, Jensen MS, Pedersen LV, Henriksen TB

Background

The Intubation-Surfactant-Extubation (INSURE) is a procedure that is increasingly being used to treat the respiratory distress syndrome in preterm infants. The objective of this study was to identify predictors for an unsuccessful INSURE procedure.

Methods

The neonates included were less than 32 weeks' gestation, treated with surfactant in the neonatal intensive care unit, and born 1998-2010. INSURE was defined as surfactant administration during intubation for less than 2 hours without the need for mechanical ventilation. INSURE success was defined as no re-intubation within 72 hours after INSURE, and INSURE failure was defined as re-intubation within 72 hours after INSURE. An unsuccessful INSURE procedure was either INSURE failure or mechanical ventilation for more than 24 hours immediately after surfactant administration. All predictors were defined a priori and were present before surfactant administration. Multivariate logistic regression was performed.

Results

In total, 322 neonates were included: 31% (n = 100) had INSURE success, 10% (n = 33) had INSURE failure, 49% (n = 158) needed mechanical ventilation for more than 24 hours, and the remaining 10% (n = 31) needed mechanical ventilation for less than 24 hours. Predictors for INSURE failure were low gestational age and hemoglobin below 8.5 mmol/l. Predictors for mechanical ventilation for more than 24 hours were low gestational age, Apgar at 5 minutes below 7, oxygen need above 50%, CO2 pressure above 7 kPa (~53 mmHg), pH below 7.3, lactate above 2.5 mmol/l, need for inotropes, and surfactant administration shortly after birth, whereas preeclampsia reduced the risk.

Conclusions

We identified specific predictors associated with an unsuccessful INSURE procedure. Keeping high-risk neonates with one or several predictors intubated and treated with mechanical ventilation after surfactant may prevent a re-intubation procedure.

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The Value of Human Colostrum in the Care of NICU Infants

Jean Rhodes PhD, CNM, IBCLC

Introduction

Human colostrum is a vital first food tailored to assist the newborn's transition to extrauterine life. During gestation, humans receive nutrients, immunological agents and growth factors from both the placenta and amniotic fluid. After birth, newborns must orally ingest what they need to survive. From colostrum's typically small volumes to its unique composition, colostrum nurtures and protects human infants until they are ready and able to consume larger volumes of mothers' milk. In this article, we will explore information specific to colostrum components, administration of mothers' own colostrum to her NICU infant and new evidence of potential colostral benefits.

Colostrum Components and Differences between Human Infants and Other Mammals

Unlike humans, many mammals—for example, horses, pigs, cows and goats—do not receive maternal antibodies in utero and for them, early ingestion of colostrum is absolutely essential for newborn survival; these animals attain gut closure within a day or so after birth, leaving very little time for intracellular absorption in the intestine of maternal colostral antibodies. If they do not receive colostrum before gut closure, they will die of infection from environmental pathogens.[1]

Unlike these mammals, humans receive IgG antibodies via the placenta during the third trimester, which provide systemic immunity against specific diseases for approximately 6 months.[1-3] After birth, newborns will receive additional but different immune globulins from mothers' colostrum and milk to prevent mucous membrane invasion of the gastrointestinal and respiratory tracts.[1,4,5] Secretory IgA is the most abundant immunoglobulin in colostrum. These mucosal-related antibodies bind to invading microbes, preventing them from attaching to and invading the mucosa.[6] They are also capable of neutralizing bacteria, viruses and bacterial toxins and reducing the risk of inflammation.[6]

In addition, colostrum provides infants with other antimicrobial components, as well as immunomodulary and anti-inflammatory agents, growth factors, antioxidants and substances that promote tolerance and/or priming of the immune system.[1,4,7-14]

Jean Rhodes is an independent consultant for Medela. She has 30 years of experience as a nurse, lactation consultant, nurse-midwife, educator and researcher. Rhodes was formerly with the Medical University of South Carolina. This article was provided by Medela.

For preterm infants, colostrum is critically important as these infants—due to their early births—miss some of the passive immunity conferred by placental IgG. Human colostrum contains high concentrations of many substances also found in mature milk: growth factors,[15,16] antioxidants[14,17,18] and anti-infective agents—sIgA, lysozyme, lactoferrin, macrophages, lymphocytes, and neutrophils.[6,19]

Potential Roles of Cytokines (and TRAIL) in Human Colostrum

In 2013 Davanzo and associates[20] identified for the first time a cytokine called TRAIL in human colostrum and mature milk. Cytokines are cell-signaling protein molecules secreted for the purpose of intercellular communication. In the context of human milk, cytokines are most recognized as having an immunomodulary role in cellular communication during microbial invasion and infection. Cytokines signal neutrophil movement towards pathogens and influence inflammatory responses to fight off harmful foreign body invasion. Cytokines can have opposing actions; they can trigger manifestations of disease-like inflammation and cell death or they can act to suppress inflammation and promote cellular proliferation.[21] A superfamily of cytokines called tumor necrosis factors (TNF) can induce death in tumor cells, controlling the growth of organs and tissues. TRAIL—or tumor necrosis factor–related apoptosis inducing ligand—is a tumor necrosis factor cytokine found in many human tissues, including serum, breast tissues, human colostrum and milk.

Davanzo et al determined TRAIL levels could be up to 400 times higher in colostrum than in human serum. TRAIL concentrations in human milk decline in the first 4-5 days after delivery but are still higher in mothers' milk than maternal serum. Researchers in earlier studies had determined that TRAIL, unlike other tumor necrosis factors, could induce apoptosis (death) of tumor cells in vitro and in vivo with minimal or no toxicity to normal cells and tissues. In a review of studies on TRAIL, Di Pietro and Zauli[21] reported effective but selective killing of tumor cells from lung, breast, kidney, colon, prostate, thyroid and skin cancers by TRAIL without harmful effects to healthy cells. Given its potential to kill malignant cells, TRAIL is currently under study as an anti-cancer therapy.[22]

Davanzo and associates identified TRAIL levels in human colostrum and milk that were within the range of concentrations that can kill cancer cells, concluding, the presence of TRAIL in colostrum and milk may be associated with the reduced risk

of certain cancers in children who were breastfed, specifically, lymphoblastic leukemia, Hodgkin's disease and neuroblastomas.

Colostrum Administration and Interaction in the Oropharynx

Despite small colostral volumes produced in the first few days, the amount is usually sufficient to coat infants' oropharyngeal-associated lymphoid tissue (OFALT) located in the tonsils and adenoids. Studies in adults of oropharyngeal administration of an immune cell-derived cytokine—interferon-a—indicate it might have stimulating effects on an individual's immune response.[23-27] Bocci et al[23-25] have researched therapeutic oropharyngeal administration in adults of interferons from human milk, proposing the oral mucosa is often overlooked as a potential site of action for administration of therapeutic substances.

In 2010 Rodriguez and colleagues evaluated the safety and feasibility of oropharyngeal colostrum administration in the first few days of a preterm infant's life. In this pilot study of five mother-infant pairs, mothers expressed colostrum as soon as possible after delivery. Beginning within 48 hours of life, 0.1 mL of colostrum was administered very slowly by needleless tuberculin syringe to each side of the posterior buccal mucosa (a total of 0.2 mL or approximately 14 drops per treatment). Treatments were done every 2 hours for 48 hours. Infant vital signs and oxygen saturation were continuously monitored. The authors concluded oral administration of colostrum to extremely low birth weight infants was feasible and well tolerated. In addition, all mothers were able to produce enough colostrum (4.8 mL) needed for two days of treatment.

More recently, Seigel et al in 2013[28] evaluated the effectiveness of colostrum administration in a retrospective study of pre- and post-initiation outcomes of a NICU colostrum protocol. In this study, 89 of 369 NICU infants received 0.1 mL of colostrum every 4 hours for 5 days. Again, colostrum administrion was found to be feasible and safe. Although mortality and necrotizing enterocolitis (NEC) rates were not statistically different between the pre- and post-colostrum administration groups, Seigel et al found trends towards reduced risks of surgical NEC (4% vs. 7%) and death (15% vs. 20%) in infants that received early colostrum administration compared to those that didn't receive early colostrum. Additionally, infant average weights at 36 weeks were statistically greater—almost 300 grams greater—in the colostrum cohort than in the pre-colostrum initiation group. The authors hypothesized early colostrum exposure may have improved intestinal absorption via early exposure to growth factors present in mothers' colostrum.

Colostrum and Reduction of the Risk of NEC Progression

Although Seigel and associates[28] did not find a statistically significant relationship between early colostrum intake and the incidence of surgical NEC, Miner and colleagues[29] found an association between early colostrum administration and a reduced risk of NEC progression to Bell's Stage III or surgical NEC. The purpose of this study was to identify factors associated with NEC severity, specifically progression of NEC from medical to surgical management. The authors obtained data from retrospective chart reviews—including radiographic images, surgical and pathology reports—of 220 infants with diagnoses of confirmed NEC, Bell's stages II or III. Four patients had more than one episode of NEC, therefore, 225 episodes of NEC were evaluated:157 episodes of Bell's stage II and 68 episodes of Bell's stage III.

Miner et al[29] found several variables were associated with a higher risk of NEC progression including earlier gestational age, lower birth weight, later post-conceptual age at diagnosis and abnormal labs at the time of NEC diagnosis. Of the factors in this study associated with NEC progression to stage III, one of only two potentially modifiable variables was infant feedings; results suggested early administration of mothers' own (unpasteurized) colostrum seemed to have a protective effect, in a dose-response manner, against the risk of surgical NEC and death. This study by Miner and colleagues is the first to isolate colostrum feedings, defined as early feedings with the first 5 days of life, as potentially protective against progression of NEC from Bells' stage II (medical NEC) to stage III (surgical NEC). As a retrospective data analysis, this study proposes a testable theoretical relationship between early colostrum feedings and reduction in NEC severity. These results suggest early and consistent colostrum feedings possibly abate the progress of a potentially devastating disease in preterm infants. These results do not negate findings of previous studies related to use of human milk and reduction of the incidence of NEC but rather highlight the importance of colostrum in the very early, critical period after birth.

Other Benefits of Colostrum Administration in the NICU

Rodriguez et al[26,27] mentioned in their 2010 study the possibility of colostrum administration reducing ventilator associated-pneumonia (VAP) in NICU infants. As mentioned earlier, colostrum is rich in anti-infective properties as well as immune-modulating cytokines that might boost immunity in the area of the oropharynx and many authors and hospitals now recommend colostrum administration and oral care for preterm infants: Arnold's Human Milk in the NICU: Policy to Practice;[30] Meier and associates';[31] Spatz and Edwards;[32] and Spatz.[33] Given the seriousness of VAP in preterm infants,[34] it seems only a matter of time before research data on colostrum administration on VAP is available. For example, Pinkerton and Wilkinson[35] are progressing research in the area with regard to buccal care with colostrum and VAP (as differentiated from administration) in low birth weight infants.

Oral care or administration of colostrum presents rich opportunities to engage and involve families in the care of their NICU infants. Rodriguez et al[27] briefly mentioned mothers' willingness to participate in their study because mothers wanted their infants to have the opportunity to taste their colostrum weeks or months sooner than they normally would. Involving families in delivery of colostrum to the NICU and allowing them to participate in colostrum administration sends a powerful message regarding the value of—and immediate need for—every drop of mother's milk.[31-33] In addition, knowledge of potential benefits of colostrum may influence breast-pumping decisions in the critical, early days after delivery.

Concluding Remarks

Human colostrum, highly concentrated early milk, assists infant transition from intrauterine to extrauterine life. Although volumes of colostrum are small, high concentrations of antimicrobial agents, antibodies, cytokines and anti-tumor factors (along with many other components) inspire a growing appreciation of the role of colostrum in healthy human growth and development.

In this article we have touched on research related to potential benefits of colostrum. Additional clinical studies are needed on

the potential benefits of colostrum as suggested by the authors highlighted in this essay. Many clinical practices in this area are in the early stages of research and development. However, the science suggests the practices discussed in these studies: 1) do no harm, and 2) are potentially beneficial, especially to preterm infants. A mother's colostrum production after birth lasts just a few days; consequently, clinical studies of its effects are challenged by short exposure periods and commingling of colostral effects with those of transitional and mature milks.

Without a doubt, progression of lactation science from laboratory settings to descriptive studies to clinical trials will uncover and substantiate further advantages of early human milk administration and feeding. These research findings, consequently, will need to coalesce with evidence-based policies, procedures and techniques in the NICU to promote the best possible outcomes for mothers and their infants.

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A Case of Congenital Pseudomonal Sepsis with Cutaneous Manifestation in an Extremely Premature Infant

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Introduction

Pseudomonas aeruginosa (P. aeruginosa) colonization of the genital tract in pregnant women by the end of the third trimester is rare.[1] Even in women with premature rupture of membranes (PROM) and/or chorioamnionitis, colonization or an infection with P. aeruginosa is infrequent.[1,2] In a cohort of 63 women with prolonged rupture of membranes (PROM) before 32 weeks of gestation, 11 subjects (17.4%) acquired P. aeruginosa in the genital tract after admission, and 3 of these newborns died of Pseudomonal sepsis.[2] We found descriptions of only 8 cases of congenital Pseudomonal sepsis, 2 of them with cutaneous manifestation,[3-9] and 1 with Sclerema Neonatorum—a neonatal panniculitis.[4] The fatality rate was 75%. We present a similar case in a premature infant who survived.

Case summary

A premature infant was born by C-section to a mother with PROM >48 hours, diagnosed with chorioamnionitis based on fever, leukocytosis and PROM >48hrs. Ampicillin, Clindamycin and Gentamycin were administered intrapartum. Maternal blood culture was negative, while urine and placenta cultures obtained at the time of delivery grew P. aeruginosa. The infant girl weighed 805 grams. Her gestational age (GA) was 25 weeks by EDD and by Ballard score. Apgar scores were 1/4/5 at 1, 5 and 10mins, requiring resuscitation. She developed RDS, and required intubation /ventilation and Dopamine drip. Initial WBC count was 9.7 x 10^9/L. Blood culture was obtained, lumbar puncture was deferred; and the infant was treated with ampicillin and gentamycin. The initial chest x-ray showed no consolidation. On the second day of life (DOL), she had seizures and developed scattered pustules (yellowish and raised) on the chest, trunk and extremities. The blood culture grew P. Aeruginosa and the culture from the pustules grew P. aeruginosa with sensitivity pattern identical to the ones in maternal isolates. Antibiotic treatment was changed to Meropenem to optimize cerebrospinal fluid (CSF) penetration. On the fifth DOL pustules disappeared, but generalized hard thickening of the skin consistent with sclerema was noted on the trunk anteriorly and posteriorly, including dependent parts. The culture of CSF obtained on the 26th DOL was negative, cell count not available. Head ultrasound (HUS) showed Intraventricular Hemorrhage (IVH) grade III. She was treated with Meropenem for 3 weeks. Eventually, the patient survived and thrived with developmental hydrocephalus and retinopathy of prematurity grade 2. At the time of discharge, at 3 months of life, she passed Auditory Brainstem Response hearing test in both ears, had normal tone /reflexes, and no focal neurological signs. At 6 months of age (corrected GA 3 months) her Head Circumference increased appropriately, but she had global developmental delay. Neurological examination revealed normal muscular tone, Moro and grasp reflexes, and DTRs but no reaction to sounds or visual tracking. At that point HUS showed moderate non-progressing hydrocephalus involving both lateral ventricles.

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Discussion

Both previously described survivors of congenital Pseudomonal sepsis had the following cutaneous presentation: pustules, ecthyma-like lesions, sclerema neonatorum (SN). One of them had severe hearing loss, but another had normal development at 9 months of age.[3-4] Our patient was extremely premature, had IVH grade III and hydrocephalus. We were not able to establish if she had meningitis as well. SN—a rapidly progressive induration of subcutaneous fat is extremely rare. Several studies link SN to neonatal sepsis.[10-12] Its pathogenesis is not clear, but "centralization" of circulation in septic shock might be a plausible explanation. It may also be true that because of advances in early recognition and management of neonatal sepsis in the last decades, it has become a rare entity. In our case Pseudomonal sepsis most likely started in utero, with a perinatal component as well.

Conclusion

The treatment of chorioamnionitis does not and should not target P. aeruginosa. Most of the emphasis in modern newborn medicine is to prevent early onset gram positive, GBS infection. However in extreme cases of fulminant maternal chorioamnionitis, particularly in early gestation, like the infant in our NICU; it is reasonable to have high index of suspicion with other Gram negative organism in women with premature PROM at <37 weeks of gestation, to direct initial antibiotic choice for the newborn. The survival, though rare, will result in severe developmental delay. In our case the mother is very happy with the outcome. The initial developmental assessment is though promising, but long term follow up will define the future growth and development of this infant.

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Temporal Changes of Blood Pressure (BP) Before and After Patent Ductus Arteriosus (PDA) Ligation in Extremely Low Birth Weight (ELBW) Infants

Kimberly Ferguson, Qassim Abid, and Koravangattu Sankaran

Abstract

Health records were retrospectively reviewed on 20 preterm infants one week prior to and two weeks after surgical ligation of PDA. Time wise sequential analysis was made on all components of BP. Contrary to expectation the pulse pressure did not decrease after ligation. As expected there was a steady increase in all components of BP over time (P<.01).We speculate that hemodynamics has only a small role in the control of relatively long standing elevation of pulse pressure in ELBW infants.

Introduction

To our knowledge there is very little information available in the literature on the evolution of pulse pressure in ELBW infants with open PDA and persistent left to right shunt particularly before and after ductal closure. In clinical practice one of the authors (Koravangattu Sankaran) has frequently observed a persistence of elevated pulse pressure in ELBW infants in NICU often triggering unnecessary clinical investigations. It is a common belief in NICU's around the world that pulse pressure contracts significantly after ductal closure. Therefore we hypothesised that after surgical closure of PDA, the pulse pressure will decrease significantly.

Materials and methods

As a part of the Dean of medicine summer research project of University of Saskatchewan, Saskatoon, Canada ethics approval was obtained for chart review. 20 infants who underwent PDA ligation and survived to discharge were selected through health records research analyst. All infants had continuous data on BP at least one week before and two weeks after PDA ligation. All infants had indwelling arterial catheter atleast 3 days prior to and one week after surgery .When the catheter was lost the blood pressure measurements were done using appropriate infant cuffs using standard non invassive blood pressure monitor.The first two authors extracted the following information from the charts. It included date of birth, birth weight in grams, gestational age in weeks, postnatal age in days, age at ligation, need for inotropes, systolic, diastolic, and pulse pressure in mm of Hg. The multiple recordings of BP in the same day were averaged. Each infant acted as his or her own control. Paired t test was used for analyses.

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Results

The table 1 shows the details of the infants studied. The figures 1 and 2 show the BP data. Briefly, the systolic and diastolic BPs increased significantly over time (p<. 01). There was a transient but not sustained decrease in pulse pressure immediately after ligation. However the pulse pressure change was not statistically significant and was not sustained after ligation. All infants received inotropes during and immediately after ligation.

Discussion

The ductus arteriosus is a vascular structure that connects the main pulmonary artery to the proximal descending aorta (fig.3). During foetal life and often immediately after birth, along with the foramen ovale, the ductus arteriosus shunts oxygenated blood to the systemic circulation, bypassing the developing pulmonary circulation (1,2). This structure normally closes spontaneously within the infant's first four days of life and persistence of the ductus arterious beyond the first few weeks of life is considered abnormal (3,4). After birth PDA can persist in 50-70% of extremely low birth weight (<1000g) infants and in many cases the hemodynamics may affect them adversely (5).

In a fetus, the systemic and pulmonary circulations are connected in parallel rather than the series arrangement seen after birth. As a result, both ventricles contribute to cardiac output (CO). The right ventricle contributes approximately 67% while the left ventricle supplies 33%. Because the immature fetal lungs do not play a role in gas exchange, there is not a need to maintain a large blood flow to the pulmonary circulation. Therefore, the majority of the blood ejected from the right ventricle is diverted away from the lungs through the ductus arteriosus. As a result, the lungs receive only 7% of the cardiac output while the systemic circulation receives 93%. This reduction in blood flow to the pulmonary circulation subsequently reduces the amount of blood that returns to the left ventricle from the lungs. Thus, the right-to-left shunt through the ductus arteriosus reduces the total work load on the developing fetal ventricles (4).

After birth, smooth muscle within the wall of the ductus arteriosus contracts, functionally closing of the vessel within 10-15 hours. Over the next 2-3 weeks, the ductus arteriosus becomes permanently closed. If it fails to close within several hours after birth along with decreasing pulmonary vascular resistence blood flow reverses (left to right shunt) through the ductus arteriosus causing left ventricular failure and pulmonary oedema. This interferes with Ventilation of the lungs causing

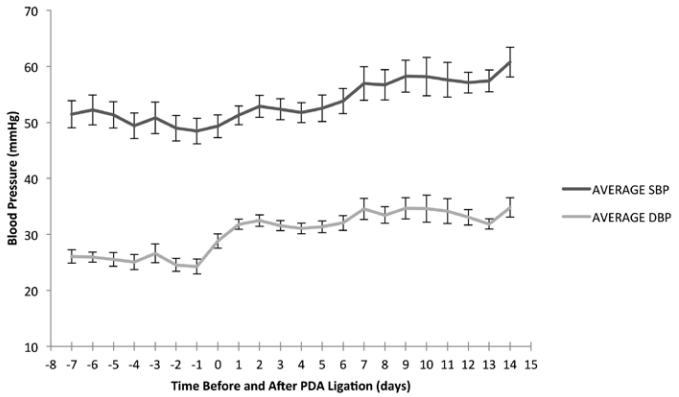

Figure 1. The average systolic and diastolic blood pressures ±SE before and after PDA ligation (day 0).

increase in the pulmonary vascular resistance and hypoxemia. Over time if the situation continues complications such as bronchopulmonary dysplasia ensues.

Blood pressure is determined by cardiac output and systemic vascular resistance. Systolic and diastolic blood pressures are affected by both, but systolic blood pressure is more heavily influenced by cardiac output while diastolic pressure is affected more by systemic vascular resistance (6). According to Strijh et al., systolic and pulse pressures increase linearly during the last half of pregnancy while diastolic pressure does not significantly change with increasing gestational age. In very low birth weight infants, blood pressure is also heavily influenced by birth weight, gestational age and postnatal age. Blood pressure increases with increasing postnatal age and thus, larger, more mature infants typically have a higher blood pressure. In our study we observed similar finding. Blood pressure in mmHg should be maintained at or above the gestational age of the infant in weeks, as recommended by the Joint Working Group of the British Association of Perinatal Medicine (7).However there is no unanimous agreement on the number of either systolic or diastolic BP value in individual cases of preterm newborns.

In infants with a large patent ductus arteriosus, approximately 33-50% of the blood flowing through the descending aorta is shunted back to the left ventricle through the pulmonary circulation (1). In an individual with normal myocardial function and reserve, increased blood flow to the left ventricle by a left-to-right shunt causes the ventricle to increase stroke volume and peak ejection velocity. This prevents the systolic

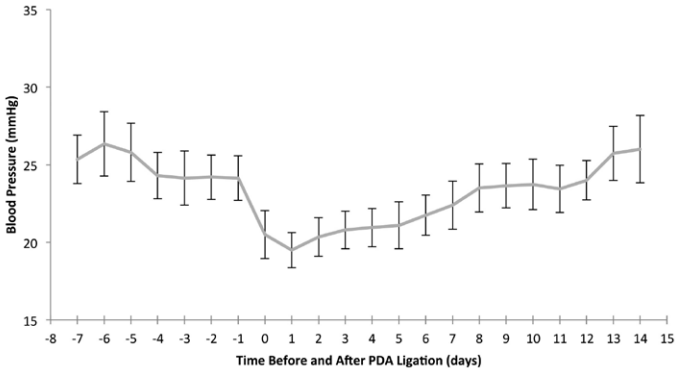

Figure 2. The average pulse pressure ±SE before and after PDA ligation (day 0).

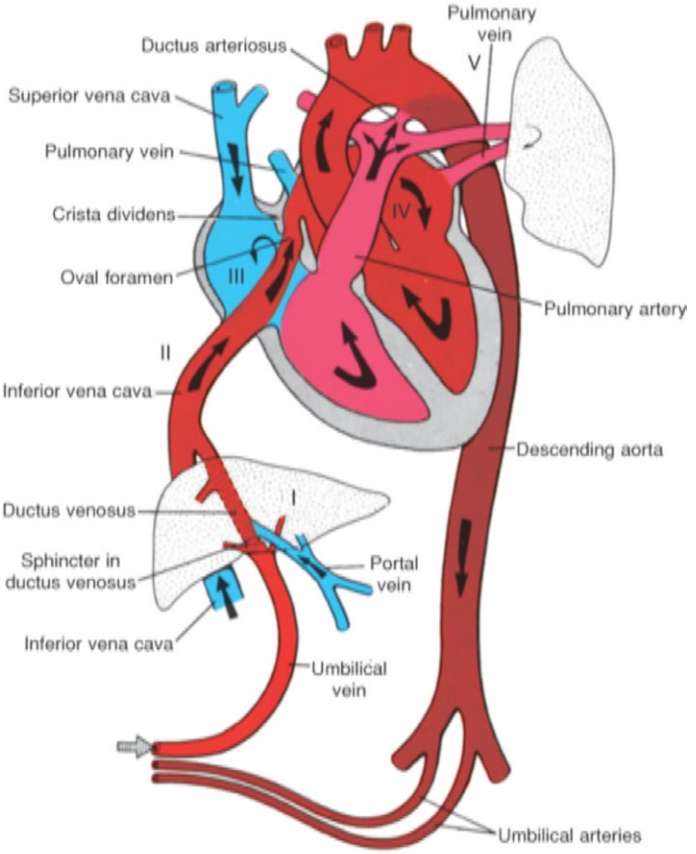

Figure 3. Foetal circulation.

pressure from being significantly affected. However, this shunt causes a reduction in systemic vascular resistance and thus, diastolic blood pressure decreases (6). Conversely, systolic and diastolic blood pressures reduce similarly in preterm infants thus maintaining a wider pulse pressure. On the other hand those infants who appear to have immature myocardium with decreased contractile tissue mass and incomplete innervation their heart cannot adequately cope with the excess volume load and as a result, systolic pressure cannot be maintained. Because both systolic and diastolic pressures decrease relatively equally, the pulse pressure is not significantly affected (6,8,9). This may explain partly why we have observed fluctuating blood pressures in the infants studied and findings that are unexpected.

Patent ductus arteriosus occurs in up to 60% of infants born at less than 28 weeks gestation (10). The PDA may be "silent", meaning it is not evident clinically but diagnosed incidentally by echocardiography, small, moderate or large. In approximately 50% of preterm infants, the PDA closes spontaneously or never becomes symptomatic (11). In symptomatic infants, treatment is provided to ideally prevent short- and long-term effects, such as respiratory decompensation, heart failure, intraventricular hemorrhage, chronic lung disease, necrotizing entercolitis and death (10). Treatment typically involves fluid restriction, diuretics and in up to 80% of cases, indomethacin. Surgical ligation is reserved for infants who fail to respond to medical management (5,10,12). In spite of significant advances in neonatal care over the last two decades, the approach to PDA management remains one of the most controversial topics in neonatal medicine. There is a lack of convincing evidence for the most effective treatment. To our knowledge, there is little information on the evolution of blood pressure and its components before and after PDA closure, especially in ELBW

infants. We hope studies such as this and future prospective studies help to shed more light to this conundrum. It is believed that pulse pressure narrows significantly after PDA closure. In the twenty infants we studied the PDA closure appear to have no effect on the pulse pressure. This is intriguing. Further studies are necessary and important.

Diastolic blood pressure is heavily reliant on systemic vascular resistance. It is believed that ligation of patent ductus arteriosus causes an increase in systemic vascular resistance thus elevating diastolic pressure. In addition, closure of the patent ductus arteriosus, and thus the left-to-right shunt, decreases the amount of blood that returns to the left ventricle from the pulmonary circulation. This results in a decreased preload. In a mature heart, a reduced preload would cause decrease in stroke volume and subsequently, cardiac output. Because cardiac output strongly influences systolic blood pressure, a corresponding decrease in systolic blood pressure is expected. However, this was also not evident in our data.

As reported by Han, U.J., et al, closure of the PDA by indomethacin treatment results in a proportionate increase in both systolic and diastolic blood pressures. Therefore, pulse pressure does not change (13). Because pulse pressure widened after PDA ligation in the 20 infants selected for our study, it is likely that different mechanisms influence blood pressure during medical and surgical treatment of PDA.Again further studies are required. In Summary in our observational study we observed increasing BP consistent with growth. Contrary to expectation Ligation of PDA did not significantly alter Blood pressure. This information may help neonatologists in the management of ELBW infants with significant ductus.

Table 1. Descriptive Statistics

Participant	Gestational Age (SE weeks)	Birth Weight ((SE gm)	Postnatal age at surgery (SE days)
1	24.2	730	5
2	27.5	980	30
3	24.6	770	15
4	24.6	800	17
5	25.2	1000	13
6	26.4	933	4
7	26.4	1065	23
8	25.4	779	29
9	24.5	740	8
10	23.3	870	78
11	24.6	671	17
12	25.6	878	29
13	24.5	710	57
14	26.6	1060	14
15	23.6	660	56
16	27.4	1250	19
17	24.5	770	17
18	25.6	730	25
19	26.1	826	39
20	25.2	800	13
Mean (SE)	25.3	851 .34	25 .4.

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First Nonprofit Partnership of Its Kind Benefits Premature Infants

Carissa Hawkins

Donor blood and breast milk: not a likely combination, yet the two provide a neonate with the best chance for survival.

Two Indiana nonprofits have joined together to ensure that both of these vital resources are readily available to neonatal intensive care units. Starting in July 2014, The Milk Bank and Indiana Blood Center are partners in getting donor resources to hospitals.

The process started In August of 2013, when The Milk Bank approached Indiana Blood Center to ask if they would be interested in serving as milk depot locations for the nonprofit milk bank. With a mission of expanding the safe use of human milk for all babies, especially premature and ill infants, The Milk Bank saw that there was a need to ensure donors have a convenient and easy experience donating their excess breast milk. This led The Milk Bank to look for additional milk depot sites throughout Indiana.

While the initial goal was to provide The Milk Bank's volunteer breast milk donors more convenient locations for dropping off their donations, the ask turned into a full-fledged partnership. As The Milk Bank began working with Indiana Blood Center, the two nonprofits quickly learned that there were several parallels between the two organizations. Serving as a nonprofit community resource organization in Indiana for the past 62 years, Indiana Blood Center views The Milk Bank as a younger version of themselves.

The Milk Bank was founded in 2005 as the Indiana Mothers' Milk Bank. It was established to improve health outcomes for premature and ill infants, foster better health for children and decrease health care expenditures. The Milk Bank receives human milk from carefully screened donors. They then pasteurize, freeze and distribute milk donations throughout the United States from their Indianapolis location. Similarly, Indiana Blood Center serves as a nonprofit community resource organization dedicated to maintaining a stable blood supply for more than 60 hospitals throughout Indiana.

In addition to housing freezers for milk donations and providing use of their distribution network, Indiana Blood Center is also able to perform the mandatory blood draw and testing needed to become a milk donor for The Milk Bank. As the community blood resource draws over 400 units of blood every day, whether it is at one of its eight donor centers or 25 mobile blood drives hosted daily, Indiana Blood Center is best equipped to provide The Milk Bank with that service. Milk Bank donors are also encouraged to donate to Indiana Blood Center when they are six weeks postpartum.

The partnership with The Milk Bank perfectly aligns with Indiana Blood Center's lifesaving mission. Both audiences of donors are literally giving of themselves to ensure patients have the lifesaving components they need. The partnership immediately opened up seven new depot locations for breast milk donors, with an eighth open in August 2014.

As the demand for donated milk and donated blood continues, The Milk Bank and Indiana Blood Center plan to move forward in their relationship, continually working to jointly serve as the community as a resource for patients in need of lifesaving blood and human milk donations.

About The Milk Bank

The Milk Bank is a nonprofit organization that promotes community health by expanding the safe use of human milk for all babies, especially premature and ill infants. The Milk Bank accepts and pasteurizes breast milk from fully screened and approved mothers to be sent to Neonatal Intensive Care Units in hospitals throughout the Midwest. Formerly the Indiana Mothers' Milk Bank, The Milk Bank has depot locations throughout the Midwest and has been in existence since 2005.

About Indiana Blood Center

Indiana Blood Center was founded in 1952 and is a not-for-profit community resource organization dedicated to maintaining a stable blood supply for more than 60 hospitals throughout Indiana. In order to meet patients' blood needs, Indiana Blood Center must see 550 donors every day. This goal is met with the annual help of more than 100,000 volunteer donors and through the dedication of more than 375 Indiana Blood Center staff who live out the lifesaving mission of providing blood components to patients in Indiana hospitals. Indiana Blood Center is the largest independent blood provider in Indiana and among the top 20 nationally, performing some 150,000 procedures annually. Visit www.indianablood.org for more information.

The author is with The Milk Bank.

Urinary and Breast Milk Biomarkers to Assess Exposure to Naphthalene in Pregnant Women: An Investigation of Personal and Indoor Air Sources

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Abstract

Background: Naphthalene exposures for most non-occupationally exposed individuals occur primarily indoors at home. Residential indoor sources include pest control products (specifically moth balls), incomplete combustion such as cigarette smoke, woodstoves and cooking, some consumer and building products, and emissions from gasoline sources found in attached garages. The study aim was to assess naphthalene exposure in pregnant women from Canada, using air measurements and biomarkers of exposure.

Methods: Pregnant women residing in Ottawa, Ontario completed personal and indoor air sampling, and questionnaires. During pregnancy, pooled urine voids were collected over two 24-hour periods on a weekday and a weekend day. At 2-3 months post-birth, they provided a spot urine sample and a breast milk sample following the 24-hour air monitoring. Urines were analyzed for 1-naphthol and 2-naphthol and breast milk for naphthalene. Simple linear regression models examined associations between known naphthalene sources, air and biomarker samples.

Results: Study recruitment rate was 11.2% resulting in 80 eligible women being included. Weekday and weekend samples were highly correlated for both personal ($r = 0.83$, $p < 0.0001$) and indoor air naphthalene ($r = 0.91$, $p < 0.0001$). Urine specific gravity (SG)-adjusted 2-naphthol concentrations collected on weekdays and weekends ($r = 0.78$, $p < 0.001$), and between pregnancy and postpartum samples ($r = 0.54$, $p < 0.001$) were correlated. Indoor and personal air naphthalene concentrations were significantly higher post-birth than during pregnancy ($p < 0.0001$ for signed rank tests); concurrent urine samples were not significantly different. Naphthalene in breast milk was associated with urinary 1-naphthol: a 10% increase in 1-naphthol was associated with a 1.6% increase in breast milk naphthalene (95% CI: 0.2%-3.1%). No significant associations were observed between naphthalene sources reported in self-administered questionnaires and the air or biomarker concentrations.

Conclusions: Median urinary concentrations of naphthalene metabolites tended to be similar to (1-naphthol) or lower (2-naphthol) than those reported in a Canadian survey of women of reproductive age. Only urinary 1-naphthol and naphthalene in breast milk were associated. Potential reasons for the lack of other associations include a lack of sources, varying biotransformation rates and behavioural differences over time.

Background

Naphthalene is an abundant polycyclic aromatic hydrocarbon (PAH) found in urban environments. It is typically present in the gas-phase under usual ambient conditions and is routinely detected in both indoor and outdoor environments. Most naphthalene exposures for non-occupationally exposed individuals occur primarily indoors at home [1]. US ATSDR considers naphthalene as reasonably anticipated to be a human carcinogen [2] while IARC has classified naphthalene as a possible human carcinogen (Group 2B) [3]. Health Canada's long-term (≥24-hour) maximum exposure limit for residential naphthalene indoor air concentrations is 10 $\mu g/m^3$[4].

There are a number of known naphthalene indoor sources and these include pest control products, (i.e., moth balls), incomplete combustion such as cigarette smoke, woodstoves and cooking, as well as some consumer and building products [1,5-7]. Indoor naphthalene concentrations have also been shown to be elevated in homes with smokers versus homes without smokers [8,9] and can off-gas and volatilize from vehicles and stored petroleum products found in attached garages [9-11].

Outdoor sources of naphthalene include exhaust from vehicles, including diesel and gas-powered equipment, as well as vapours from petroleum products. Other sources that are less common include asphalt, forest fires and some industrial processes [9,12,13].

To understand total human exposure to naphthalene a limited

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number of studies have included both personal air sampling along with biomonitoring [14,15]. Uptake, absorption, distribution, and metabolism can be affected by individual physiological differences and behaviours [14]. The overall rate of metabolism of naphthalene by humans is unknown, although it has been suggested that there is a two-phase excretion of 1-naphthol in urine. The first phase exhibits a half-life of approximately 1.2-1.9 hours while the second phase is 14-46 hours [16]. Naphthalene is metabolically activated by forms of cytochrome P450 to naphthalene 1,2-oxide, which can be detoxified by glutathione-S-transferase (GST) to eventually be excreted as mercapturic acids in urine or spontaneously convert to 1- and 2-naphthol and be eliminated in the urine as glucuronides and sulfates. Naphthalene 1,2-oxide can also undergo other transformations to dihydroxydimethylthio and trihydroxymethylthio metabolites and trihydroxymercapturic acid in urine [17].

Biomonitoring can provide insight into the uptake of naphthalene. Only a few studies [18-20] have attempted to measure naphthalene in breast milk and to date, there are no Canadian data available. Conjugates of 1-naphthol and 2-naphthol in urine have been associated with predicted concentrations in the breathing zone but there are limited data available in non-occupationally exposed individuals [14]. Meeker et al. [21] recommended that the ratio of 1-naphthol to 2-naphthol be used to identify the metabolism of naphthalene. They identified situations where discrepancies between concentrations of these metabolites (e.g., ratio >2) were in fact related to the metabolism of the insecticide carbaryl (1-naphthyl methylcarbamate) which is primarily excreted as 1-naphthol.

As pregnancy is associated with a number of physiological changes in women that could affect the toxicokinetics of chemicals [22] and there are critical periods of development during pregnancy for the fetus, it is important to study the exposure of pregnant women to potentially harmful chemicals such as naphthalene. Reports of probable fetal exposure after maternal inhalation or ingestion of naphthalene have been documented in the scientific literature [22,23]. Infants, particularly those with a glucose-6-phosphate dehydrogenase (G6PD) deficiency, may be particularly sensitive to naphthalene exposure. Cases of hemolytic anemia, sometimes leading to more serious outcomes (e.g., kernicterus (irreversible neurological impairment) and death), have been reported in infants exposed to naphthalene-treated household items [24-32]. Authors of a recently published New York study of 5-year old children's urinary naphthalene metabolite concentrations identified an association with chromosomal aberrations (including translocations) which are precancerous changes in adults [33].

We conducted a cohort study in a group of pregnant women residing in Ottawa, Ontario, Canada to assess naphthalene exposure and biomarkers in maternal urine and breast milk. We assessed naphthalene sources and concentrations inside residences, along with personal exposure measures, to assist in determining both source and route-specific information related to naphthalene exposure in a non-occupationally exposed population. This study addresses current knowledge gaps by attempting to measure naphthalene body burdens, identify major sources of naphthalene exposure, and quantify their contribution to an individual's exposure.

Methods

Pregnant women (<20 weeks gestation) from the Ottawa area were recruited to participate in the P4 Study: Plastics and Personal-care Product Use in Pregnancy, a wider study investigating pregnant women's exposure to a range of chemicals. This manuscript focusses on the personal and indoor air exposure to naphthalene and resulting biomarkers. Recruitment was clinic-based and occurred at an obstetrical clinic at The Ottawa Hospital (TOH) and a privately run obstetrical clinic. Posters and pamphlets about the study were placed in the obstetrical and ultrasound clinics of TOH and physician offices. Research nurses from the clinical sites were trained in patient screening, recruitment, obtaining consent, specimen and data collection, and processing, as well as the shipment of biospecimens.

The women completed detailed consumer product diaries along with noting any use of products containing naphthalene for a 48-hour period during the early pregnancy visits and 24-hours prior to the post-partum visit. Typically the biomarker and air collection started at the midpoint of the diary, i.e. 24-hours after the start of the diary.

Biomarker collection and analysis

For the purposes of this analysis, the women provided urine samples on three occasions: twice during pregnancy (<20 weeks) and once at two to three months post-birth. In order to assess activity related differences in exposure, prior to 20 weeks of pregnancy women were asked to collect all voids over two 24-hour periods (multiple spot urines)—once on a week day and again on a weekend day. After collection, a small equal amount from each void during the 24-hour period was pooled to create an aggregated sample for naphthalene biomarker analysis. At two to three months post-birth, the women provided a single spot urine sample at the end of the 24-hour air monitoring period. These samples were stored at -20°C until analyses. One paper has reported that 1- and 2-naphthol in urine was stable for at least 1 month at -20°C [34].

Analyses of 1-naphthol and 2-naphthol were undertaken on the 24-hour aggregated pregnancy urine samples and the post-birth spot sample using the following method. Internal standards (1-naphthol-d_7 and 2-naphthol-d_7) were added to a 1 mL volume of urine. The conjugated forms of 1-naphthol and 2-naphthol were hydrolyzed at 37°C for 16 hours with β-glucuronidase (helix pomatia). The extraction of the analytes was performed on a mixed mode solid-phase extraction (SPE) cartridge Oasis MAX (Waters), 60 mg. The analytes were eluted with methanol, dried and reconstituted in 200 µL of a mixture of mobile phase A and B (72:28) containing gallic acid (200 mg/L). The extracts were analyzed by UPLC-MS-MS (Acquity UPLC system and Xevo TQ-S tandem mass spectrometer, Waters; Milford, MA). The LC separation was performed on a Halo C18 column (2.1 × 50 mm, 2.7 µm, Advanced Materials Technology) with 0.01% NH_4OH in water as mobile phase A and acetonitrile as mobile phase B. The separation was achieved isocratically with 28% of B over 3.5 minutes, and the column was flushed with 100% B for 0.3 minutes at a flow rate of 0.5 mL/min. The total run time was 4 minutes. The limits of detection for 1-naphthol and 2-naphthol were 0.03 µg/L, and the calibration curves were linear up to 50 µg/L. Field blanks for the urinary samples were analyzed and no contamination was found. The analytes were monitored by Multiple Reaction Monitoring (MRM) in the negative mode for the following ions: 1-naphthol and 2-naphthol : m/z 143.0 > 115.1

Table 1 Characteristics of the study population

Participant characteristics and exposures*		Frequency (%) or mean (SD) (N = 80)
Age (years)		32.4 ± 5.0
BMI (Kg/m^2)		24.0 ± 4.3
Education level – college/university degree		71 (89)
Household income	Below $50 k	4 (5)
	Above $100 k	44 (55)
Parity	Primiparous	37 (46)
	Multiparous	43 (54)
Ever smoked more than 100 cigarettes		25 (32)
Smoke currently		2 (3)
Exposed to second hand smoke		18 (23)
Exposed to second hand smoke inside home		3 (4)
Road density in postal code (km/km^2)		2.03 ± 1.32
Highway density in postal code (km/km^2)		0.41 ± 0.37

Home Characteristics#	Pregnancy (N = 56)	Post-birth (N = 53)
Moth balls used	5 (9%)	0 (0%)
House with an attached garage and connecting door	25 (45%)	24 (45%)
Fireplace	33 (59%)	33 (62%)
Wood	16 (29%)	13 (25%)
Natural gas	14 (25%)	17 (32%)
Heating type		
Electric	3 (5%)	6 (11%)
Natural gas	46 (82%)	39 (74%)
Oil	4 (7%)	6 (11%)

*Participant characteristics were asked at the pregnancy visit only.
#Home characteristic questionnaires were not filled out by all participants in each phase of the study. The total number filled out in each period is given in brackets. Models examining these variables were limited to participants with reported data.

(quantifier) and 143.0 > 143.0 (qualifier)1-naphthol-d_7 and 2-naphthol-d_7 : m/z 150.0 > 122.1 (quantifier) and 150.0 > 150.0 (qualifier).

Quality control (QC) materials, including method analytical blanks, were prepared from human urine obtained from volunteers in the analytical laboratory. The urine, previously tested for 1- and 2-naphthol content, was spiked, with a solution of 1- and 2-naphthol from a different supplier, at a concentration of 20 µg/L to obtain a high concentration QC material. The low concentration QC material was composed of the unchanged urine (concentrations: 1.0 µg/L for 1-naphthol and 0.5 µg/L for 2-naphthol). The two QC materials were used in alternation and placed after each set of ten samples in each analytical batch. The INSPQ laboratory participates in the German external quality assessment scheme (http://www.g-equas.de) in which 1- and 2-naphthol are assayed.

As concentrations derived from urine may be affected by the dilution of the urine, concentrations were corrected by the specific gravity (SG) of the sample. The following formula was used (adapted from Just et al., [35]):

$P_c = P_i\ [(SG_m - 1)/(SG_i - 1)]$, where Pc is the specific gravity-adjusted metabolite concentration (ng /mL), P_i is the observed metabolite concentration, and SG_i is the specific gravity of the urine sample and SG_m is the median specific gravity for the cohort.

Breast milk was collected at the two to three month post-birth visit, at the end of the 24-hour air monitoring period. The breast milk sample was collected by either hand or pump in a glass container, kept cool until delivered to the laboratory where it was transferred to 30 mL Nalgene containers and stored at -20°C until analysis as per methods described by other studies [19,20]. Breast milk was analyzed for naphthalene using the following method. Briefly, the internal standard (naphthalene-d_8) was added to a 1 mL volume of breast milk. The extraction of naphthalene was performed with a silicone/PFTE septum, by heating at 80°C for 16 hours. The septum was transferred into a headspace vial, incubated at 145°C for 5 minutes and injected by the headspace technique on a GC-MS-MS (7890A gas chromatograph with 7000B tandem mass spectrometer, Agilent Technologies; Mississauga, Ontario, Canada) equipped with a PAL Combi-xt injector (Leap Technologies; Carrboro, NC, USA). The GC separation was achieved on a DB-5 ms column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies). The temperature of the injector was 250°C and the temperature gradient was: Initial temperature of 100°C for 0.5 minutes, then 40°C/minute until 320°C, then hold for 2 minutes. Carrier gas was helium at a flow rate of 2 mL/min. The limit of detection for naphthalene was 0.03 µg/L. The analytes were monitored by Multiple Reaction Monitoring (MRM) in the positive mode for the following ions:naphthalene : m/z 128 > 128 (quantifier) and 128 > 102 (qualifier)naphthalene-d_8 : m/z 136 > 136 (quantifier) and 136 > 108 (qualifier).

Quality control (QC) materials, including method analytical blanks, were prepared from human milk obtained from volunteers in the analytical laboratory. The milk, previously tested for naphthalene content, was spiked with a solution of naphthalene from a different supplier, at a concentration of 10 µg/L to obtain a high concentration QC material, and a concentration of 0.4 µg/L to obtain a 0.5 µg/L low concentration QC material. The two QC materials were used in alternation and placed after each set of ten samples in each analytical batch. While no studies on the stability of naphthalene in milk stored at -20°C could be found in the literature, one study has reported naphthalene contamination from packaging materials of milk samples stored at room temperature in low-density polyethylene containers [36]. However, our breast milk was collected in glass jars, kept refrigerated until aliquoted in the laboratory into Nalgene containers and immediately frozen at -20°C. The Mendela breast pump provided to participants was tested and no naphthalene was detected.

As naphthalene is fat soluble and concentrations are affected by the lipid concentrations in an individual's breast milk, the naphthalene concentrations were corrected for lipid concentration and are reported in ng/g lipid.

Air monitoring and analysis

Personal and indoor air measures of naphthalene were completed concurrently with the 24-hour urine collection and prior to the spot urine and breast milk collection. Personal air monitoring was completed in the women's breathing zone by attaching the sampler to their collar, while indoor air monitoring required the women to place a sampler in their living rooms at a height of approximately 1.5 m, away from any sources of heat. Each passive sampler measured 24-hour air samples

Table 2 Descriptive statistics of measured naphthalene and metabolite concentrations in environmental and biological samples in the P4 Study

Sample	Pregnancy - weekday							Pregnancy - weekend							Weekday vs weekend day	2-3 months post-birth							Pregnancy vs. post-birth
	N	Q1	Median	Q3	GM (GSD)	95th% ile	Min – Max	N	Q1	Median	Q3	GM (GSD)	95th% ile	Min – Max	Sign rank test	N	Q1	Median	Q3	GM (GSD)	95th% ile	Min – Max	Sign rank test
Air (µg/m^3)																							
Personal	56	0.38	0.73	1.03	0.7 (2.18)	3.14	0.2 –6.37	58	0.37	0.79	1.21	0.79 (2.41)	3.9	0.2 - 14.85	0.10	61	1.09	1.74	2.46	1.68 (1.86)	4.51	0.3 - 12.31	<0.0001
Indoor	57	0.5	0.68	1.06	0.8 (2.01)	3.51	0.27 –5.97	58	0.45	0.73	1.05	0.76 (2.05)	3.56	0.22 -4.79	0.55	60	1.14	1.83	2.48	1.79 (1.82)	4.94	0.31 - 11.71	<0.0001
Urine (µg/L)																							
1-naphthol	62	0.73	1.14	2.28	1.32 (2.80)	6.06	0.13 - 126.08	67	0.67	1.05	1.91	1.16 (2.44)	3.85	0.23 - 81.57		62	0.65	1.06	1.62	1.04 (2.69)	6.16	0.14 - 11.62	
1-naphthol – SG* corrected	62	0.7	1.19	2.22	1.34 (2.72)	6.3	0.2 - 140.7	67	0.78	1.15	1.74	1.23 (2.41)	3.68	0.22 -133.4	0.34	62	0.73	1.09	1.67	1.23 (2.08)	4.46	0.38 - 12.48	0.4
2-naphthol	62	1.7	2.73	5.09	2.92 (2.08)	9.57	0.81 -16.41	67	1.72	2.56	4.29	2.72 (2.14)	12.4	0.68 - 19.93		62	1.56	2.86	5.85	2.86 (2.86)	13.47	0.19 - 32.8	
2-naphthol – SG* corrected	62	1.73	2.53	4.7	2.94 (1.99)	9.47	0.87 - 14.43	67	1.97	2.84	3.95	2.92 (1.93)	11.56	0.77 - 17.53	0.44	62	2.09	3.33	5.66	3.39 (2.16)	12.19	0.74 - 25.39	0.15
Breast Milk (ng/g lipid)																							
Naphthalene															N/A	52	6.06	7.55	13.05	9.12 (1.92)	40.17	3.86 – 79.36	N/A

*Specific gravity.

for naphthalene (OVM 3500, 3 M, St. Paul, MN). As the air monitoring was participant-based, replicate sampling was not attempted due to the complexity of conducting this additional monitoring.

Naphthalene was extracted using toluene, which was previously demonstrated to have a recovery of 72%. The analysis protocol has been described previously [37]. Briefly, this involved extracting the samples with 2 mL of toluene for one hour on a mechanical shaker. The toluene extraction solvent was spiked with 1,2-dichlorobenzene-d_4 (1.34 ng/µL). The extraction solvent was then transferred to a 1.5 mL autosampler vial and analyzed via GC-MS (HP5890 II GC & HP5792 MS). The GC was equipped with a capillary column (J&W 123-1364 DB-624, 60 m × 0.32 mm × 1.8 µm). The carrier gas (helium) head pressure was 6.0 PSI and injector and detector temperatures were kept constant at 220°C and 260°C, respectively. The temperature program yielded a 12.9 min retention time (initial temperature, 80°C for 1 min, 80°C to 260°C at 15°C/min, hold for 1.5 min). The MS was configured to quantify the following 3 characteristic ions of naphthalene: 128, 102 and 64 amu. The ion ratios and peak integration were verified manually for each sample.

The naphthalene concentrations were calculated using the mass adsorbed on each sampler, the specific uptake rate for naphthalene, exposure times to the nearest one minute and laboratory blank PSDs analyzed at the same time as the samples. The method detection limit (MDL), including handling and extraction was determined by the CFR 40 method. The MDL was 0.1 µg/m^3[37].

To calculate concentrations, the laboratory results were merged with log sheet data. Concentrations were calculated based on sample mass, sampling duration, sampling rate and recovery efficiency. All samples were coded as valid, flagged, or invalid, based on the sampling period and field technician comments. Samples with a sampling period ± 25% of the target duration (24-hours) were deemed invalid. If the sampling period was ± 12.5 to 25%, the samples were flagged. Samples with technician comments such as container not sealed on time, unknown sampling location, were also flagged.

Statistical methods

Given the naphthalene exposures were not normally distributed, Spearman correlations were conducted to determine correlations between the air and urine naphthalene measurements within visits and across visits. The non-parametric signed rank test was used to test differences between levels measured at different visits. Intra-class correlation coefficients (ICC) were calculated using a one way random effects model (Proc Mixed) on air and biomarker concentrations that were transformed using the natural logarithm. ICC measures the ratio of between-subject variance to total variance ranging from 0 (meaning no within person reproducibility) to 1 (meaning perfect reproducibility). We defined 0.75 as high; 0.40 to 0.75 as moderate; below 0.40 as poor reproducibility [38]. Simple linear regression models were used to examine associations between a number of known naphthalene sources and measured concentrations in log transformed air and biomarker samples. As there were no significant differences between air or urinary biomarker concentrations measured on the weekday and weekend pregnancy visit, these were averaged for the pregnancy models; separate models were created for the pregnancy and the post-partum visits.

Table 3 Seasonal simple linear regression results for naphthalene in air (µg/m^3)

Exposure	Season	N	Median	Mean	GM	*p*-value*
Weekday Indoor	Fall	19	0.947	1.254	1.029	0.341
	Spring	13	0.827	1.349	0.903	0.728
	Summer	15	0.780	1.005	0.827	
	Winter	15	0.489	0.626	0.524	0.063
Post-Partum Indoor	Fall	9	1.024	1.532	1.258	0.006
	Spring	22	1.798	1.923	1.815	0.074
	Summer	8	2.355	3.164	2.781	
	Winter	19	1.849	2.223	1.639	0.031
Weekday Personal	Fall	20	0.950	1.423	1.083	0.583
	Spring	13	0.801	1.156	0.840	0.695
	Summer	15	0.734	1.449	0.940	
	Winter	13	0.295	0.477	0.338	0.001
Post-Partum Personal	Fall	9	1.023	1.183	1.153	0.002
	Spring	22	1.739	1.742	1.665	0.031
	Summer	8	2.501	3.219	2.819	
	Winter	20	1.556	2.236	1.538	0.015

*Simple Linear Regression with Summer as the reference category.

The independent variables examined were: age, body mass index (BMI) (calculated from pre-pregnancy weight), season, moth ball use, exposure to smoke (current smoker, previously a smoker, exposure to second hand smoke (SHS) and exposure to SHS in the home), exposure to traffic pollutants including the presence of an attached garage, density of roads and highways in neighbourhood (total road or expressway segment length in a participant's 3-digit postal code divided by the area of the 3-digit postal code), exposure to indoor combustion (presence of wood-burning fireplace), and type of ventilation and heating in the home. Naphthalene in indoor and personal air, 1-naphthol and 2-naphthol in urine, and naphthalene in breast milk were examined as the dependent variables in turn. In biomarker models, personal and indoor air naphthalene concentrations were also entered as independent predictors in turn.

Since 1-naphthol in urine can also originate from exposure to carbaryl, we examined the ratio of 1-naphthol to 2-naphthol as an indicator of its source. Ratios above 2 may indicate that a portion of the 1-naphthol originated from carbaryl rather than naphthalene exposure [21,39]. Where this occurred, a sensitivity analysis excluding these individuals was conducted. All data processing and analyses were conducted using SAS Enterprise Guide 4.2 (SAS Institute, Inc.).

Results

A total of 1307 potential research participants were approached to enter the study from November 2009 to December 2010. 769 potential participants were eligible for the study of whom 86 were recruited during this time period, with an acceptance rate of 11.2%. The reasons for the low recruitment included significant participant burden, no interest in participating, too busy, and unease about wearing the air monitors in public. There were a total of 86 participants recruited, six participants agreed to participate and signed the consent form but then shortly afterwards withdrew leaving 80 participants who completed the first visit in early pregnancy. 70 participants completed visit 2, 71 completed visit 3, 73 had completed chart reviews at delivery and 63 completed the final post-partum visit. A total of 7 participants withdrew from the study, 7 were lost to follow-up and three had early outcomes (miscarriage, stillbirth, neonatal death). Initially recruitment of the women was aimed at before the 14th week of pregnancy; however, this had to be expanded to include the window of 19 weeks 6 days gestation in the winter of 2010 due to low recruitment as women seemed hesitant to participate early in pregnancy. This change dramatically increased recruitment.

Table 1 includes details of the participant characteristics. The average age of participants at time of delivery was 33 years, with a range from 20 to 47 years. This was the first pregnancy for 37 of the participants. Fifty-five percent of the participants had a household income exceeding $100,000CDN and 89% had a college or university degree.

For the air monitoring component, a total of 375 participant days were completed. These included 322 valid samples, 28 flagged samples due to sampling times being 12.5 to 25% greater or less than the targeted 24-hours, 24 invalid samples due to sampling times being beyond the targeted 24-hours ± 25% or due to damage of the sampler, and 1 sample below detection. Blank corrections were not required as no blanks had detectable concentrations of naphthalene. There were no duplicates collected due to the complexity of having participants complete their own data collection.

A total of 191 urine samples (all samples were included from both of the pregnancy visits) were analyzed for 1-napthol and 2-napthol (62 from weekday and 67 from weekend visits during pregnancy, and 62 from the post-delivery visit). Fifty-two breast milk samples were analysed for naphthalene.

Descriptive statistics for the naphthalene in air and breast milk, and the biomarker urinary metabolites are presented in Table 2. Results for the urinary biomarkers are presented with and without adjustment for specific gravity (SG), which accounts for differences in individual hydration status. SG-adjusted geometric mean urinary concentrations tended to be higher than the corresponding unadjusted concentrations. Median SG-adjusted urinary concentrations of 1-naphthol collected on a weekday and weekend day were 1.19 and 1.15 µg/L, respectively. SG-adjusted median concentrations of 2-naphthol in urine varied from 2.53 to 3.33 µg/L, depending on when the sample was collected. Concentrations of SG-adjusted 1- and 2-napthol in urine did not significantly differ between the three collection periods.

Indoor and personal air naphthalene concentrations did not significantly differ between the weekday and weekend visits (Table 2). However, both indoor and personal air measurements were significantly higher at the post-partum visit than at the pregnancy visits ($p < 0.0001$ for signed rank tests). The air samples were analysed throughout the course of the data collection with some samples from the pregnancy visits and post-partum visit being analysed at the same time, therefore reducing the likelihood of bias resulting from differences in timing of analyses.

Personal and indoor air naphthalene concentrations collected during pregnancy were highly correlated, within each visit and across visits with Spearman Correlation Coefficients ranging from 0.83 to 0.91 (p-value = <.0001). The average of the two pregnancy measures and the postpartum concentrations were also significantly correlated, personal $r = 0.4$ (p-value = 0.0026) and indoor $r = 0.46$ (p-value = 0.0004).

Significant correlations were observed in the urinary SG-adjusted 2-naphthol concentration in samples collected during pregnancy on weekdays and the weekend ($r = 0.78$, $p < 0.001$) and when comparing pregnancy and postpartum samples ($r = 0.54$, $p < 0.001$). There were no significant correlations seen with the postpartum breast milk samples with any of the air or urine measures.

The ICC analysis suggested moderate reproducibility for SG-adjusted 2-naphthol across the study period (weekday, weekend and post-partum samples) (ICC = 0.66). The ICCs were low for indoor air (ICC = 0.31), personal air (0.32) and 1-naphthol (0.24).

Simple linear regression models examined associations between various naphthalene sources (e.g., moth ball use, exposure to smoke, attached garage, density of roads and highways in neighbourhood, presence of a wood-burning fireplace, type of ventilation and heating in the home) and measured concentrations of naphthalene in air and biomarkers; no significant associations were found (all p-values greater than 0.05, see Additional file 1: Table S1). Similarly, no consistent associations were seen with age or BMI. The data suggested that samples collected during the winter had lower indoor and personal air naphthalene concentrations compared to summer. This was significant at the 0.05 level for indoor air

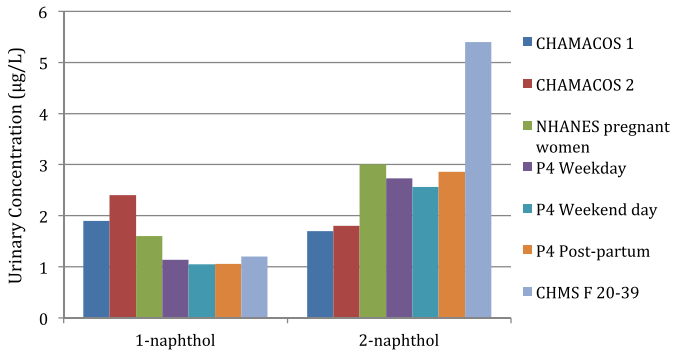

Figure 1. Median urinary concentrations of 1- and 2-naphthol from the CHAMACOS 1stand 2nd prenatal samples [39], NHANES pregnant women [39], the Canadian Health Measures Survey (CHMS) (females 20-39 years of age) [50] and our P4 Study.

concentrations post-partum, and for personal air concentrations during pregnancy on both sampling days (see Table 3). The weekday 2-napthol concentrations were significantly different in the fall and winter compared to the summer. We did observe a small association between urinary 1-naphthol concentrations and naphthalene in breast milk despite there being no correlations: a 10% increase in 1-naphthol in urine was associated with a 1.6% increase in naphthalene in breast milk (95% CI: 0.2% - 3.1%). Two observations were removed from this analysis due to their high influence on the association, as indicated by Cook's distance.

There were 4 women in the pregnancy period and 2 women in the post-birth period with 1-naphthol to 2-naphthol ratios above 2 which could be indicative of exposure to carbaryl as opposed to naphthalene. Excluding these individuals from the simple linear regression models did not change the results, with the exception of the association between urinary 1-naphthol and naphthalene in breast milk; this association was reduced to a 1.1% (95% CI: -0.3% - 2.6%) increase in naphthalene with a 10% increase in 1-naphthol. This association should be interpreted with caution due to the small sample size measured, and the fact that the association was reduced when we removed individuals suspected of having alternative sources of 1-naphthol.

Discussion

The measured indoor air concentrations of naphthalene (medians across the 3 sampling periods ranging from 0.68 to 1.83 µg/m^3) are comparable to findings from previous studies. Jia and Batterman [9] summarized residential indoor air naphthalene concentrations for studies conducted from 1986 to 2006. The authors reported a median concentration range for homes without smokers of 0.18 to 1.7 µg/m^3. Only one indoor air measurement from the three monitoring sessions in this study was found to be above the Health Canada guideline of 10 µg/m^3[4] at 11.71 µg/m^3. This measurement was taken at the post-birth visit; the source for this higher level was not clear but the pregnancy-period measurement at the same home was well below the guideline (3.03 µg/m^3) suggesting that this was not the result of an ongoing source.

Canadian levels of indoor air naphthalene have been measured in several studies. The median naphthalene level in Quebec City homes without smokers was 1.12 µg/m^3 for a seven day integrated sample [40]. Measurements made in a population-based study of Canadian homes in 1991 over multiple seasons had a range of 24-hour mean concentrations of 1.10 – 8.10 µg/m^3[41]. An Ottawa based study of 75 residences had a 24-hour mean of 3.87 µg/m^3. These studies included both homes with and without smokers [42]. Indoor air naphthalene was also measured in homes without smokers in Edmonton, Alberta, where 7-day median concentrations were 0.32 and 0.29 µg/m^3, for winter and summer seasons respectively [43]. This contrasts with our findings where summer indoor concentrations were significantly higher than both fall and winter. The sampling time frame for the different studies may explain some of the differences in concentrations of naphthalene in indoor air.

Very little data exists on personal monitoring for naphthalene exposure; however, the concentrations we measured among pregnant women (medians ranging from 0.73 to 1.74 µg/m^3) are slightly lower than concentrations found in other populations. An Italian study of non-occupationally exposed adults living and working in Milan and the surrounding areas included 108 subjects, 18 of whom completed both personal air sampling and urine samples which were analysed for naphthalene. Median personal naphthalene air samples taken during the 5-hour work period was 3.4 µg/m^3 (interquartile range: 1.4 – 4.9 µg/m^3), with no differences between smokers and non-smokers. They also measured concentrations of un-metabolized naphthalene in urine (median: 46 ng/L, interquartile range: 41 – 56 ng/L) but found no associations between the personal air and urine naphthalene concentrations. They were also unable to identify any predictors for either the air or urine samples [44].

In an Atlanta, GA-based study of 8 non-occupationally exposed individuals who completed a personal air sample along with urine samples, median exposure levels to naphthalene ranged from a low of 0.13 µg/m^3 at work (interquartile range: 0.095-0.22 µg/m^3) to a high of 0.92 µg/m^{3}indoors at home (interquartile range: 0.37-3.27 µg/m^3). Indoor home concentrations were higher than concentrations measured while driving, which reflect the importance of residential indoor sources for naphthalene. A comparison of personal air measurements and urinary excretion of naphthalene between days with high and low PAH diets suggested that inhalation is the primary route of exposure for naphthalene [14].

The study by Bouchard et al., [45] asked participants to record habits and activities involving potential PAH exposures. They were only able to identify passive smoking with exposure associated with higher 2-naphthol excretion. The authors felt that the absence of a link between most of the variables from the questionnaire and the urinary excretion of PAH biomarkers was due to the low reporting of exposure to these variables.

Indoor air measurements of naphthalene have previously been recognized to be a good proxy for personal naphthalene air exposures [46]. The high correlations we observed between indoor and personal naphthalene concentrations confirm this, see Additional file 2: Figure S1.

There are a number of sources of naphthalene in the indoor environment, including moth balls and deodorizers, smoking, attached garages, construction and wood products, indoor combustion, and heating systems [9,47]. Although we examined a number of known indoor sources, we were unable to confirm associations with measured indoor and personal concentrations, as has been the case for previous studies [45,46], (see Additional file 1: Table S1 for the univariate analyses). Moth balls are available with two different formulations in Canada (para-dichlorobenzene or naphthalene) and we did not collect data

on which type our study participants used therefore limiting our ability to interpret the influence of this particular source. In a larger study of 288 homes in Michigan, Batterman et al. [8] identified pest repellant use, presence of an attached garage, cigarette smoke, and outdoor sources as contributing to indoor naphthalene concentrations. This may indicate that for many homes, the total naphthalene level reflects a number of smaller sources and cannot easily be attributed to distinct sources without significant statistical power. An exception is the improper use of moth balls, which can be associated with extremely high indoor concentrations of naphthalene [48]. Only 4 homes in the current study reported using moth balls, and they did not have elevated indoor naphthalene concentrations. Health Canada's Pest Management Regulatory Agency re-evaluated naphthalene pest-control products in 2010 and concluded that they do not present unacceptable risks to human health when used according to label directions. In this study, there were also a limited number of smokers and exposure to SHS was also limited so it was not possible to evaluate the impact of smoking as a source of naphthalene.

We observed that indoor and personal naphthalene concentrations in air were significantly higher at the post-birth visit than the two pre-birth visits. This may be a result of new products being introduced into the home during this period, as well as possible home renovations. Consumer product uses of naphthalene include some commercially available coatings and paints. As we did not specifically ask about these, we are unable to confirm this hypothesis.

Monitoring was quite evenly distributed throughout the year; some seasonality was seen for the indoor air concentrations but not for the biomarkers. In studies in California there was evidence that PAH concentrations increased as temperatures decreased. However, this trend in seasonality was most pronounced for particle phase PAHs. Vapour phase PAHs (i.e., 99% of naphthalene) did not demonstrate any dramatic seasonality [49]. Naphthalene data collected in the Halifax and Edmonton indoor air studies conducted by Health Canada demonstrated that homes sampled during both the winter and summer had similar concentrations to one another [37,43].

The urinary results for this study indicated that there were no significant differences between the three visits with the medians across the 3 sampling periods, ranging from 1.05 to 1.14 for 1-naphthol and 2.56 to 2.86 µg/l for 2-naphthol. The results are similar to those identified by Bouchard et al., [45] where the geometric mean urinary concentrations of 1-naphthol in first morning voids varied from 0.99 to 1.23 µmol/mol creatinine over an 8-month period; the corresponding figures for 2-naphthol were 1.37 to 2.39 µmol/mol creatinine. This indicates that there are some small differences in concentrations by urine collection days which may be lost in our sampling approach of using 24-hour samples in the pregnancy period. Our results for 1-naphthol are also comparable with those from another pregnancy cohort study and with population-based Canadian data, but lower than the Canadian data for 2-naphthol. In the CHAMACOS study of pregnant women in California, the median 1st trimester urinary concentrations of 1- and 2-naphthol were 1.9 and 1.7 µg/L, respectively [39]. For females 20-39 in the Canadian Health Measures Survey (2009–2011), the median 1- and 2-naphthol concentrations in urine (measured by the same laboratory as this study) were 1.2 and 5.4 µg/L, respectively [50], (Figure 1).

Median concentrations of naphthalene in breast milk were significantly lower than observed in previous studies at 7.55 ng/g lipid. The study by Tsang et al. [18] in Hong Kong showed mean naphthalene concentrations of 786 ng/g lipid in breast milk. The researchers found a positive correlation between PAH concentrations in milk and maternal age; our study was unable to reproduce this result. The Turkish study of Çok et al., [19] found that naphthalene was one of the most abundant PAH (contributing 42.6% to the total PAH) identified in human milk from 47 women (mean = 45.75 ng/g lipid). When they separated the analyses by smoking status some of the non-smoking mothers did not have detectable naphthalene concentrations. Similarly, Zanieri et al.,[20] found that human milk derived from non-smoking women had approximately half the concentrations of naphthalene compared to smokers (5.56 vs. 10.54 µg/kg of fresh weight milk). The participants in our study were overwhelmingly non-smokers (97%), which may contribute to their low naphthalene concentrations.

Limitations of this study include the relatively small sample size measured and the lack of detailed information on possible indoor sources including the use of deodorizers, sanitizers and air fresheners. Another limitation of the low recruitment rate is the ability to generalise the findings to other pregnant women and populations. Methods for measuring naphthalene in air may be inferior compared to other volatile organic compounds (VOCs) due to methodological limitations (e.g., recovery and reproducibility) [51] although this may be less problematic as the analytical approach used in this study included a naphthalene specific extraction to ensure maximum recovery [37]. In general, very few studies have attempted to identify predictors of naphthalene concentrations in indoor air. The studies that did examine predictors found that indoor naphthalene concentrations were positively and significantly associated with the presence of forced air heating systems with filtration [40], attached garages [40,52], bathroom cleaners/deodorizers [53], and moth control products [9,54].

This study provides valuable information on personal exposure to naphthalene as measured in air, urine and breast milk in the prenatal and post-natal windows. Suspected naphthalene transfer across the placenta has been documented in case studies at high maternal exposure concentrations, with the fetus apparently more vulnerable to naphthalene toxicity than the mother [22,23]. In a pilot study conducted in California, 70% of the amniotic fluids tested positive for 1- or 2-naphthol indicating direct exposure of the young fetus to these phenols; however as the authors did not distinguish between the two metabolites and the study was conducted in an agricultural area, exposure to carbaryl as opposed to naphthalene cannot be ruled out [55]. Since the rate of metabolism of naphthalene in humans is not well characterized, information on the urinary biomarker concentrations may be useful for future attempts to characterize the rate of metabolism in humans in general, or in specific populations (e.g., pregnant women). One possible reason for the lack of association between the biomarkers and the indoor and personal air samples may be the different excretion rates identified by Heikkila et al., [16]. They also found that in workers exposed to occupational concentrations of naphthalene that there were poor associations between air concentrations and the urinary metabolite 1-naphthol with correlation coefficients less than 0.5. Kuusimaki et al., [13] also failed to observe any associations between air samples and urinary metabolites which they attributed to the fact that the workers' exposures were a

combination of diet and occupational exposures. The lack of association between air concentrations and urinary metabolite concentrations could also be due to genetic differences. P450 isoform screening of naphthalene metabolism, performed with human P450 isoforms expressed in baculovirus-infected insect cells, identified CYP1A2 as the most efficient isoform for producing dihydrodiol and 1-naphthol, and CYP3A4 as the most effective for 2-naphthol production [56]. Genetic variations in these enzymes as well as those involved in forming naphthol conjugates (SULT1A1 and UGT1A9) could lead to differences in biotransformation capacity among participants [57,58].

Conclusions

The results from this study suggest that indoor air monitoring of naphthalene provides a good indication of personal air exposures for pregnant women. Potential sources of biomarker concentrations of naphthalene could not be identified, which is consistent with several other studies. While urinary 1-naphthol and naphthalene in breast milk were associated there were no other associations found for the personal and indoor air concentrations and biomarkers. Potential reasons for this include a potential lack of significant sources, physiological (excretion rates) and behavioural differences that were not captured.

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The Use of Single Prong Nasal Cannula to Deliver High Flow Therapy on Neonates <1000 g

Jorge Rojas, MD and Thomas Miller, PhD

Abstract

Objective: This study compares the effectiveness of high flow nasal cannula therapy (HFT) using a single prong nasal cannula compared to a conventional two prong cannula for the extubation support of infants <1000 g. A single prong cannula is presumed to prevent significant pressure from building in the nasopharynx while supporting nasopharyngeal flush of carbon dioxide.

Study Design: Data was collected on all infants < 1000 g admitted to the St. Thomas Midtown NICU that were on a respirator and were extubated to HFT during two sequential five month periods. The primary outcome measure was failed extubation (re-intubation) within 48 hrs.

Result: There were two failed extubations in each group. All infants survived to discharge and there were no differences in Length of Stay, Discharge Weight, Discharge Head Circumference, Discharge Gestational Age, Days on Oxygen, Days on Respirator, or Days on HFT.

Conclusion: No significant difference in outcomes was observed between the two cannulas.

Introduction

The use of nasal cannulas to deliver high flows of humidified respiratory gases (High Flow Therapy; HFT) to neonatal patients has seen a marked increase in use during the last few years.[1] Two large, recently published trials, showed it to be comparable to nasal Continuous Positive Airway Pressure (nCPAP).[2,3] One of the fears of using HFT has been the possibility of generating significant airway pressure.[4] The manufacturers of HFT devices recommend that the cannula prongs should only occupy 50% of the nares. This requirement is not always possible in infants of <1000 g since their nares are commonly 3 mm or less and the smallest cannula available is 2.5 mm at the base. A single prong nasal cannula (SPC), compared to a conventional double prong cannula (DPC) would guarantee at least 50% open nares.

Following this rational, we hypothesized that a SPC (SOLO, Vapotherm Inc.) should be as effective as the standard DPC without violating the 50% open nares recommendation and therefore lowering the risk of generating significant pressure.

Dr Rojas is a consultant for Vapotherm, Inc., Dr Miller is employed by Vapotherm, Inc.

To test this hypothesis, we studied a series of infants using both types of nasal cannulas.

Materials and Methods

Data was collected on all infants < 1000 g admitted to the St. Thomas Midtown NICU, that were on a respirator and were extubated to HFT during two sequential periods: from 3/1/2012 to 8/31/2012 when using a standard DPC and from 9/1/2012 to 2/28/13 when using SPC. The SPC was used alternating nares every six hours. The primary outcome measure was failed extubation (re-intubation) within 48 hrs. Secondary outcomes included arterial PCO_2 and pH, fractional inspired oxygen requirement (FIO2) titrated to optimized arterial oxygen saturation, infant growth metrics (Discharge Weight, Head Circumference and Gestational Age), duration of HFT and oxygen therapy use and length of stay. Statistical analysis was done using the Chi-square test or the Students to test to compare data between groups. The study was approved by the Saint Thomas Research Institute, Institutional Review Board.

Results

There were 31 infants that met the inclusion criteria, 15 during the first period who were extubated to DPC and 16 during the second period who were extubated to SPC. Both groups were comparable in birth weight, Gestational Age, Head Circumference and blood gas parameters before extubation (arterial PCO_2, pH and required FIO_2). Age at extubation was also comparable (Table 1). All infants were extubated to a flow rate between 4-6 L/min (5.2 ± 0.7 L/min vs. 5.5 ± 0.6 L/min). There were 2 failures within 48 hr. of extubation in each group (13% vs. 13%). On HFT, both groups were comparable in arterial PCO_2 and pH as well as required FIO_2 (Table 1).

All infants survived to discharge and there were no differences in Length of Stay, Discharge Weight, Discharge Head Circumference, Discharge Gestational Age, Days on Oxygen, Days on Respirator, or Days on HFT (Table 2). Other variables such as number of infections, incidence of apnea, retinopathy of prematurity, intraventicular hemorrhage, and use of medications were also not different between the groups.

Discussion

With both groups identical in terms of size and gestational age, there was no difference in any of the measured parameters between the neonates treated with SPC versus DPC. The incidence of extubation failure was the same as well as the length of HFT and days of oxygen. Long term outcomes were

Table 1

	Standard Cannula n=15		Solo Cannula n=16		
	Mean	S.D.	Mean	S.D.	p
Birth Weight	829.33	131.83	754.38	128.48	0.120
Gestational Age	27.48	1.54	26.61	1.75	0.154
Birth H.C.	24.08	1.60	23.38	1.25	0.180
Age of Extubation	3.67	3.56	4.88	4.30	0.409
Respirator					
PCO2	42.79	9.86	39.78	7.71	0.176
pH	7.34	0.05	7.36	0.06	0.397
FIO2	22.73	3.47	23.03	3.11	0.720
H.F.T.					
PCO2	39.62	7.06	42.12	8.20	0.173
pH	7.36	0.04	7.34	0.06	0.106
FIO2	23.56	5.18	23.50	3.61	0.955

All values are expressed as Mean ± S.D.

Table 2

	Standard Cannula n=15		Solo Cannula n=16		
	Mean	S.D.	Mean	S.D.	p
L.O.S.	62.20	10.43	73.25	12.41	0.120
Days on Respirator	4.47	4.00	6.63	7.25	0.323
Days on HFNC	36.87	17.55	40.88	12.25	0.464
Days on Oxygen	46.73	19.64	56.88	16.10	0.126
Discharge Weight	2017.87	223.75	2155.00	197.61	0.080
Discharge G.A.	36.68	1.44	36.93	1.69	0.659
Discharge H.C.	31.03	1.41	31.28	1.15	0.595

All values are expressed as Mean ± S.D.

also not different; babies in both groups grew adequately and went home at similar times.

HFT delivered by nasal cannula is intended to be an open system as opposed to nCPAP which requires a closed, pressure tight system.[8,9] By using a single prong cannula in infants of <1000 g an open system is guaranteed and the generation of significant pressure is minimized. Research has shown that during HFT pressures generated are less than those seen with nCPAP[5,6] and mechanistic research has indicated that HFT works primarily by purging extra-thoracic dead space, thus improving the efficiency of respiration.[7] The size of the leak may actually be an important factor by which HFT works to support respiration. Frizzola et al.[7] showed in lung injured neonatal piglets that HFT likely provides other mechanisms to support respiratory efficiency beyond the application of distending pressure. PaCO2 and PaO2 improved in a somewhat flow dependent manner independent of tracheal pressure, consistent with nasopharyngeal dead space washout. The Frizzola et al model also included a double prong (low leak) versus a single prong (high leak) comparison where the partial pressure of carbon dioxide was lower for all flow rates under the single prong condition.

In the current evaluation, the arterial blood gases and required FIO2 were identical in both groups, as well as the length of therapy and final outcomes. We concluded that a single prong nasal cannula is as effective as a standard double prong cannula to provide HFT in neonates of <1000 g.

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High Dose Anakinra for Treatment of Severe Neonatal Kawasaki Disease: A Case Report

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Abstract

We report an 11-week-old female who presented with Kawasaki disease (KD) complicated by macrophage activation syndrome (MAS). The infant presented to the hospital with persistent fever, cough, diarrhea, and emesis, among other symptoms. Her condition quickly began to decompensate, and she developed classic features (conjunctivitis, rash, cracked lips, distal extremity edema) prompting a diagnosis of acute KD. The patient was treated with standard therapy for KD including three doses of intravenous immunoglobulin (IVIG), aspirin, and high dose glucocorticoids with no change in her condition. Due to a high suspicion for MAS, high dose anakinra therapy was initiated resulting in dramatic clinical improvements. She also received one dose of infliximab for concern for coronary artery changes, and over the course of several months, anakinra and high dose glucocorticoids were tapered. Nearly complete reversal of echocardiogram changes were observed after 8 months, and the infant is now off all immunosuppressive therapy. In this case report, we briefly review the importance of early recognition of MAS in pediatric patient populations with rheumatic diseases, and we suggest early initiation of anakinra therapy as a rapid and effective treatment option.

Background

Kawasaki disease (KD), a condition of unknown etiology, is one of the most common acute inflammatory vasculitides in children and infants. Diagnosis of KD is based on clinical findings, resulting from diffuse inflammation of small and medium sized vessels. The most common acute inflammatory manifestations in KD include fever, bilateral nonexudative conjunctival injection, erythema of oral mucosa, peripheral edema, polymorphous rash, and cervical lymphadenopathy [1]. Though no specific laboratory test exists for the diagnosis of KD, laboratory studies reveal typical findings, including: leukocytosis with neutrophilia, elevated erythrocyte sedimentation rate (ESR), elevated C-reactive protein (CRP), normocytic anemia, hyponatremia, hypoalbuminemia, and thrombocytosis [1,2]. Less commonly seen but associated with KD are elevated transaminases (30%), mild hyperbilirunemia (10%), and sterile pyruia (33%) [3,4]. Early identification and initiation of treatment is critical in improving the outcome of KD. Approximately 15-25% of children left untreated, or with a severe delay in treatment, develop coronary artery aneurysms, which can lead to myocardial ischemia, infarction, and death [5]. Initial treatment of KD involves administration of high dose intravenous immunoglobulin (IVIG) and aspirin. In cases of refractory KD, retreatment with IVIG, corticosteroids, or infliximab, a tumor necrosis factor inhibitor (TNF-i), have been shown to be effective treatment [1,6,7].

A life-threatening complication of KD that may easily remain undiagnosed is macrophage activation syndrome (MAS), also known as secondary hemophagocytic lymphohistiocytosis (HLH). Though the pathophysiology is not completely understood, it is thought that MAS results from an uncontrolled immune response to certain immunologic stressors such as infections, malignancies, and autoimmune or autoinflammatory states. Undiagnosed and untreated, MAS results in a systemic inflammatory reaction characterized by excessive cytokine production, activation of macrophages, and hemophagocytosis. Patients diagnosed with MAS have been shown to exhibit decreased natural killer (NK) cell number and function [8-11].

Unfortunately, since many of the clinical and laboratory findings of acute KD, including fever, rash, and elevated transaminases, are shared with MAS, it can be difficult to distinguish between the two processes and requires a high degree of suspicion for diagnosis [1,8]. An important, available, timely, and affordable diagnostic marker of MAS is an elevated serum ferritin level. Other laboratory values highly characteristic of MAS include elevated triglycerides, elevated liver enzymes, and decreased fibrinogen [12]. In a retrospective review of 638 children with acute KD, the 12 (1.9%) children found to also have a diagnosis of MAS all exhibited hyperferritinemia. Hypofibrinoginemia, cytopenia in 2 or more cell lines, hypertriglyceridemia, and hepatosplenomegaly were seen in 11 of the 12 children with concomitant MAS. Treatment beyond the standard protocol for acute KD, IVIG and aspirin, was required in all children except 1 [13]. Recently, several case reports have successfully demonstrated the use of the interleukin-1 (IL-1) receptor antagonist, anakinra, in the treatment of KD (1-2 mg/kg/day) complicated by MAS, as well as other pediatric and adult diseases complicated by MAS [14-18]. In this case presentation, we report the use of high dose (9 mg/kg/day) anakinra in the treatment of severe neonatal KD complicated by MAS.

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Table 1 Laboratory values

Hospital Day	CRP, mg/dl (normal <10)	ESR, mm/hr (normal <15)	Hb, g/dl (normal 12.5-19)	WBC × $10^3/mm^3$ (normal 6–18)	Neutrophil (%) (normal 19–45)	PLTs × $10^3/mm^3$ (normal 140–400)	AST, U/l (normal 15–60)	ALT, U/l (normal 10–40)	Triglyceride, mg/dl (normal <149)	Fibrinogen, mg/dl (range 220–450)	Ferritin, ng/ml (normal 50–200)	GGT, U/l (normal 8–80)	Other labs and important events related to clinical course:
1			9.6	14.3	57	229							Serum sodium 130 mEq/L Total bilirubin 3.5 mg/dL
3	133.4	40	6.9	12.12	59	32	25	18				138	Serum sodium 131 mEq/L Hypoalbuminemia of 1.8 g/dL (reference range: 3.4-5.4 g/dL) IVIG dose #1
4			10.1	16.8		121	24	19	82		801		IVIG dose #2/Transfusion
6	191.6	24	9.9	27.5		66	22	17		239	255		IVIG dose#3 Methylprednisolone 30 mg/kg × 3 days Flow cytometry
													Anakinra started
7	234.1	113	8.2	16.6		52				273	207		(3 mg/kg BID) (increased to 3 mg/kg TID after 3 days)
8	122.9	64	7.8	21.5		133	16	14			213		Slight clinical improvement
9	65	37	12.7	22.6		156			165				Methylprednisolone (1 mg/kg BID) Marked clinical improvement Extubated day 9 Tranfusion
12	98.3	67	13.2	19.6		607	32	21	288		574		Echocardiogram: diffuse enlargement of the entire coronary artery system Infliximab 5 mg/kg ×1 Methylprednisolone increased (4 mg/kg TID)
16	1.6	25	11.3	12.9		963	27	28	201	239	700	108	Methylprednisolone decreased (2 mg/kg TID)
20	0.3	5	13	20.9		972			269	212	754	134	Methylprednisolone decreased (2 mg/kg Daily)
27	<0.2	8	11.3	16.7		427			293		406	58	Anakinra decreased (4 mg/kg BID) Methylprednisolone stopped Prednisolone started 1.5 mg/kg Daily (tapered over 10 days)

CRP: C-reactive protein; *ESR*: erythrocyte sedimentation rate; *Hb*: hemoglobin; *WBC*: white blood count; *PLTs*: platelets; *AST*: aspartate aminotransferase; *ALT*: alanine aminotransferase; *GGT*: gamma-glutamyl transpeptidase; *IVIG*: intravenous immunoglobulin treatment.

Case presentation

An 11-week-old Caucasian female fraternal twin, from non-consanguineous parents who were not from a genetically isolated population, presented to the hospital with two days duration of cough, congestion, rhinorrhea, diarrhea, emesis, decreased oral intake, reduced urine output, and fever of 103 °F. She was admitted for dehydration and begun on IV fluids. Initial laboratory studies revealed neutrophilia, anemia, normal platelet count, hyponatremia, and mild hyperbilirubinemia (Table 1). Urine studies revealed sterile pyuria, and blood cultures were reassuringly negative after 48 hours. Work up for CMV, HHV8, parvovirus B19, adenovirus, and HSV infections were all negative.

On hospital day 3, the infant developed a diffuse, erythematous, macular-papular blanching rash on the face, trunk, and extremities. She was also noted to have peripheral edema, non-purulent injected bilateral conjunctiva, dry and cracked desquamating rash on her lips and eyelids, mild hepatomegaly, and a few small (<1 cm) bilateral cervical lymph nodes. Progressive respiratory distress developed requiring intubation. Repeat laboratory studies showed persistent neutrophilia, worsening anemia, new onset thrombocytopenia, normal transaminases, mild hyponatremia, hypoalbuminemia, and elevated acute phase reactants (Table 1). A cerebral spinal fluid (CSF) analysis revealed pleocytosis with mononuclear cell predominance, normal glucose, and mildly elevated protein. An echocardiogram showed a right coronary artery diameter at the upper limits of normal with no evidence of aneurysm. Due to a high suspicion for KD, an abdominal ultrasound was performed showing hydrops of the gallbladder with circumferential edematous thickening of the gallbladder wall, further supporting the clinical diagnosis. The patient was subsequently started on aspirin and 2 g/kg IVIG on days 3, 4, and 6 of hospitalization (Table 1). Echocardiogram of the coronary arteries on day 4, revealed the following measurements and associated Z-scores: 0.16 cm (Z 0.91), 0.17 cm (Z – 1.4), and 0.19 cm (Z – 0.25) for the left anterior descending (LAD), right (RCA), and left main (LM) coronary arteries, respectively.

On day 6 of hospitalization, flow cytometry was obtained due to concern for MAS (elevated ferritin and thrombocytopenia). Results revealed few NK cells (<1%) and decreased CD8 T cells compared to the reference range for age making it difficult to analyze cytolytic function; however, there were normal perforin and granzyme B levels in both cell lines. Genetic analyses detected no mutations in familial HLH genes (STXBP2, STX11, MUNC13-4, RAB27A). Subsequently, the patient was given a three-day course of high-dose methylprednisolone, 30 mg/kg/dose. Despite treatment with IVIG and methylprednisolone, the patient's conditioned remained unchanged. A repeat echocardiogram was obtained revealing that the right coronary artery and the left anterior descending artery were mildly dilated, and the left main coronary artery was at the upper limits of

normal, all significantly larger than on the initial echocardiogram four days prior. The infant was started on therapy with high dose anakinra (3 mg/kg/dose, twice daily for 3 days, then increased to three times a day for rash and rise in CRP). There was an immediate reduction noted in pulmonary resistance by mechanical ventilation, and she stabilized in terms of her vital signs. By day 3 of treatment the patient was displaying a dramatic improvement in her rash and an impressive reduction in her overall inflamed appearance, as well as decreased levels of CRP and an increasing platelet count (Table 1). A second rise in ferritin occurred despite a remarkable rise in platelet count and fall in CRP coincident with anakinra treatment. Perhaps this was a reflection of liver and bone marrow recovery as she dramatically improved clinically.

Despite noted clinical improvements with the use of anakinra and methylprednisolone, a third echocardiogram revealed interval diffuse enlargement of the entire coronary artery system [LAD – 0.36 cm (Z – 11), RCA – 0.32 cm (Z – 6.5), LM – 0.37 cm (Z – 5.6)] without discrete aneurysms. Due to concerns about progressive coronary changes, therapy was supplemented with one dose of infliximab (5 mg/kg) and increased methylprednisolone (4 mg/kg/dose, three times a day). The patient remained clinically stable and slowly tapered off prednisolone (1.5 mg/kg/day) over the 10 days following discharge, and then off anakinra (8 mg/kg/day, divided twice daily) slowly over the next 5 months. A repeat flow cytometry study, approximately 6 months after initial laboratory studies, revealed reduced perforin and granzyme B in CD8 T cells, normal perforin and granzyme B in NK cells, and normal absolute levels of both CD8 T cells and NK cells. An echocardiogram 8 months after initial coronary artery changes revealed normal coronary artery origins, with only mild dilation of the proximal right coronary artery, and a left main coronary artery at the upper limits of normal in size [LAD – 0.18 cm (Z – 0.84), RCA – 0. 24 cm (Z – 2.8), LM – 0.26 cm (Z – 1.6)]. The patient is now off all immune suppressive therapy and is maintained on low dose aspirin and propranolol, with regular follow-up appointments with her cardiologist and rheumatologist.

Discussion

Our case highlights the importance of anakinra, an IL-1 receptor antagonist, in children with KD complicated by MAS. Our patient was rather young for typical KD and may not be universally representative, but she did meet KD criteria and had evidence of coronary artery abnormalities. Although our patient did not have sCD25 measured and did not meet restrictive familial HLH criteria, she had a >88% chance of having reactive HLH/MAS as per the newly developed HScore [19]. Though only 1.9% of children with acute KD are reported to develop overt MAS, it is unclear whether there is a greater percentage with subclinical MAS as in children with systemic juvenile idiopathic arthritis (sJIA) [13,20]. It is also unknown whether KD complicated by MAS is being labeled as Kawasaki shock syndrome (7% of KD patients studied), refractory KD, or recurrent KD, as the processes may appear to have similar clinical features [21,22]. At present it is unclear whether Kawasaki shock syndrome is on the MAS spectrum or whether a subset of children with Kawasaki shock syndrome have frank MAS. Recently, a retrospective survey was conducted in Korea to determine differentiating factors between recurrent KD and KD complicated by MAS. The study concluded that the most important distinguishing factor was the time course of symptoms and signs. The onset of MAS following KD was approximately 13.3 days (range, 3–22 days); however, recurrent KD typically occurred much later at a mean of 17.9 months (range, 1–60 months) [21].

It is critically important to identify MAS early, as the potentially devastating complication carries a high mortality rate and may require a more aggressive treatment approach than the traditional treatment protocol for KD [23]. The initial treatment strategy for MAS involves supportive therapy and aggressive immunosuppressive treatment to reduce the severe systemic inflammation, which causes the life threatening symptoms. While it is highly important to control hyperinflammation, suppressing the patient's remaining immune system can also pose significant risks. Traditionally, immunosuppressive therapies used in the treatment of MAS include IVIG, high dose glucocorticoids, and cyclosporine [24]. Etoposide has also been suggested for treatment of MAS but carries high morbidity and mortality rates [25].

As an alternative to traditional therapy for MAS, we suggest a more targeted therapy with anakinra as an effective and rapid treatment for MAS in children and very young infants. Additionally, our case presentation demonstrates that anakinra used with infliximab can be an effective combination therapy in treating MAS and KD associated complications. Several case reports have shown successful use of the IL-1 receptor antagonist, anakinra, in treating children with various rheumatologic conditions complicated by MAS [14-16]. In addition to treating a case of KD associated MAS [15], low dose (1-2 mg/kg/day) anakinra has also been shown to successfully treat refractory KD [26]. Because infants with autoinflammatory disorders [27] and cases of severe MAS may require higher dosing of anakinra [14], it may be prudent to treat with higher doses of anakinra until the inflammation can be controlled. Recently, a retrospective case series demonstrated the therapeutic role of anakinra in 8 children in the intensive care unit that were diagnosed with secondary HLH or MAS. The study showed promising results with dramatic decreases in ferritin, fibrinogen, and CRP levels following initiation of anakinra. Importantly, no infections were attributed to the use of anakinra [18]. Infliximab may also play a role in lowering inflammation in KD and potentially reducing coronary artery abnormalities [28,29].

Conclusions

It appears that early intervention with high dose anakinra may provide a safe and effective alternative to treatment of life-threatening MAS in children. As further knowledge of MAS, or secondary HLH, comes to the forefront, it is imperative to consider this diagnosis in critically ill children, especially those with underlying rheumatic conditions. Further study of anakinra in the treatment of MAS, as well as combination therapy of anakinra and infliximab, as a therapeutic alternative in KD associated MAS with coronary artery dilation is warranted.

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Staphylococcus Aureus in a Neonatal Care Center: Methicillin-Susceptible Strains Should be a Main Concern

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Abstract

Background: In the context of a methicillin-susceptible *Staphylococcus aureus* (MSSA) outbreak, we aimed to improve our knowledge of *S. aureus* (SA) epidemiology in the neonatal care center (NCC) of a tertiary care teaching hospital.

Methods: We performed a complete one-year review of SA carrier, colonized or infected patients. Monthly prevalence and incidence of SA intestinal carriage, colonization and infection were calculated and the types of infection analysed. During the MSSA outbreak, strains were studied for antimicrobial resistance, content of virulence genes and comparative fingerprint in Pulsed-Field Gel Electrophoresis. Hand hygiene and catheter-related practices were assessed by direct observational audits. Environmental investigation was performed in search of a SA reservoir.

Results: Epidemiological analyses showed 2 or 3 prevalence peaks on a background of SA endemicity. In the NCC, during 2009, overall MSSA prevalence did not decrease below 5.5%, while mean MRSA prevalence was about 1.53%. Analysis of infection cases revealed that the outbreak corresponded to the emergence of catheter-related infections and was probably related to the relaxation in infection control practices in a context of high colonization pressure. Health care workers' white coats appeared as a potential environmental reservoir that could perpetuate SA circulation in the ward.

Conclusion: This report emphasizes the importance of integrating MSSA along with methicillin-resistant SA in a program of epidemiological surveillance in the NCC.

Background

The high incidence of hospital-acquired infections (HAIs) in neonatal intensive care units (NICUs) is related to the immaturity of patients who are also subjected to many invasive procedures. Coagulase negative staphylococci (CoNS) and *Staphylococcus aureus* (SA) are the main and often sole bacteria colonizing the digestive tract of low birth-weight infants during the 3 first weeks of life [1]. Furthermore, CoNS and SA are responsible for most infections in hospitalized preterm infants [2-4].

As in other hospital units, methicillin-resistant *S. aureus* (MRSA) outbreaks have often been reported in NICUs [5-8] whereas, at first glance, methicillin-susceptible *S. aureus* (MSSA) outbreaks seem less frequent. Indeed, a PubMed database search returns 1108 papers for "MRSA outbreak" versus 52 for "MSSA outbreak" (January 2014). In second analysis, the scarcity of MSSA outbreaks could be due to a bias in detection or reporting, MRSA being one of the most threatening pathogens as well as the principal indicator of nosocomial risk. Patients' screening and outbreak alert systems in most hospitals focus on MRSA, while MSSA infections are generally treated piecemeal with little or no insight into molecular typing and epidemiology. However, a study of 358 *S. aureus* strains (2,007,681 days of hospitalization in 32 healthcare institutions) showed there is a significant increase of bloodstream HAIs largely due to MSSA strains [9,10].

The published MSSA outbreaks concerned merely NICUs [11-13], burns units [14] and multi-resistant MSSA [14,15]. The spread of MSSA clonal strains in NICUs seems to be very successful. For instance, in a 5-year outbreak affecting 202 neonates, classical control measures failed to end the outbreak [13] but atypical reservoirs near the patient, such as skin protectant [13] and ultrasound gel [12] were found. Intestinal carriage of SA seems to be neglected in NICUs, although it frequently occurs in infants [1,16,17]. Furthermore, it is associated with a high risk of skin colonization which can in turn increase the risk of infections, environmental contamination and cross transmission [17].

The aim of this study is to describe the epidemiology of SA in the neonatal care center (NCC) of the Montpellier Academic Hospital. An increase of MSSA colonization and infection cases in the NCC led to a complete investigation: (i) analysis of cases, (ii) assessment of hygiene practices (hand hygiene and catheter-related care) (iii) search of SA environmental reservoir, (iv) molecular typing. The outbreak was confirmed and related to the spread of a common strain with a probable environmental reservoir.

Methods

Settings, patients and infection surveillance policies

The NCC of Montpellier is organized in 3 sectors: the paediatric reanimation and intensive care unit or PRICU (14 beds in 9 boxes for neonates including very preterm neonates and 6 beds in 5 rooms for infants), the NICU (24 beds in 10 boxes), and a mother-cum-child or "kangaroo" ward (9 beds in individual rooms, and 3 beds in a nursery).

Hospitalized patients in the PRICU are low birth-weight (<1500 g) preterm infants and newborns aged < 1 month with diseases or unstable states. The PRICU also hosts newborns having surgery and older children requiring intensive care. Newborns are transferred to the NICU once their clinical state is stabilised or improved. As soon as their condition allows, patients are transferred to the kangaroo ward before returning home.

The medical and paramedical teams from the PRICU, the NICU and the kangaroo ward, a microbiologist and a member of the infection control (IC) team meet weekly to discuss HAI cases, differentiating true HAIs from bacterial carriage or colonization by confronting microbiological data, biological and clinical contexts and initiation of antimicrobial treatment [18]. Infants are considered as colonized if a positive culture is obtained from a non-sterile site, and infected if a pathogen is isolated from a normally sterile site or if cultures are obtained for clinical purposes. In addition, in the NICU sector, the digestive carriage of SA and multi-drug resistant bacteria (MDRB) is screened for each patient upon admission and once a week thereafter. A positive culture from a digestive sample is considered as SA carriage [17]. Stool samples are cultured to determine methicillin and ceftazidime resistance in staphylococci and gram-negative bacilli, respectively. MDRB detection leads to supplementary infection control measures to prevent cross-contamination among patients, and to further environmental investigations. A real-time surveillance is implemented by a daily account of all newly MDRB colonized or infected patients by the microbiologist, using an antibiotic resistance information system (Sirweb, i2a, Montpellier, France). Surveillance data is transmitted to the IC team via the hospital information system. This automatic surveillance system operates for MDRB only; hence outbreaks involving susceptible microorganisms can be detected only through clinical observation of an increased number of cases over a given time span.

Outbreak investigation of MSSA infections

This clinical surveillance thus detected an increase in HAIs involving MSSA, leading to an outbreak investigation by the IC team including analysis of patients' medical records, environmental investigation, and assessment of healthcare practices. Clinical strains were tested for their antimicrobial susceptibility by disk diffusion assay according to the French Committee for Antimicrobial Susceptibility Testing (Members of the Société Française de Microbiologie Committee, 2003). After extraction of the DNA performed according to Predari et al. [19], isolates were screened for genes encoding staphylococcal enterotoxins A (sea), toxic shock syndrome toxin 1 (tst),

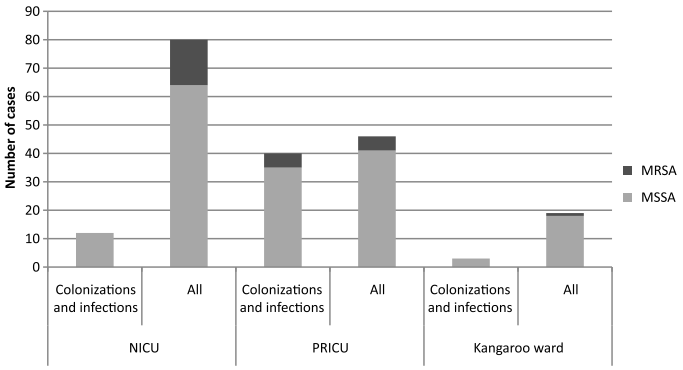

Figure 1 Distribution of SA carriage, colonisation and infection for each sector in 2009. All = carriage, colonization and infection

Table 1 Typological analysis of colonization- and infection-associated clinical SA positive samples by care sector

	NICU		PRICU		Kangaroo ward		Total	
	n	%	n	%	n	%	n	%
Sites of isolation								
Respiratory	4	20	39	63.9	2	40	45	52.3
Ophthalmic	9	45	4	6.6	2	40	15	17.5
Blood	2	10	8	13.1	0	0	10	11.6
Catheter	2	10	5	8.2	0	0	7	8.1
Other	3	15	5	8.2	1	20	9	10.5
Total	20		61		5		86	

Panton-Valentine leukocidin (PVL; luk-PV) [2-4]. Clinical MSSA strains were explored by genomotyping to determine genetic links between them. For this purpose, intact genomic DNA was extracted in agarose plugs and digested by the endonuclease SmaI, as described [20]. Macrorestriction fragments were separated by pulsed-field gel electophoresis (PFGE) by a ramp of pulses of 20s to 5 s at 6 V/min during 24 h on CHEF-DRII apparatus (Biorad).

Dry and humid surfaces were sampled, as well as health-care workers' clothes (i.e.: white over-gowns used for the manipulation of central venous lines, especially the front part which comes into contact with patients); the cotton swabs used for sampling served to inoculate trypticase-soy, Chapman and McConkey agar plates (bioMérieux, France) which were incubated at 37 °C for 48 h. Water was sampled at different points of use in the units in 250 mL sterile bottles containing sodium thiosulfate for chloride inhibition. Water samples were passed through a 0.22 µm nylon filters, which were then incubated at 30 °C during 48 h on Chapman agar. Healthcare practices in the NCC were audited by direct observation of hand hygiene opportunities (a minimum of 30 observations) and central venous catheter manipulations (a minimum of 5 observations).

Epidemiology

Monthly prevalence and incidence of SA colonizations and infections were calculated for each sector, as well as the prevalence of MSSA and MRSA for all the NCC. Prevalence was defined as number of patients with SA per total number of hospitalized patients in the month, reflecting the endemicity of SA in the NCC. The incidence was the ratio of new cases on new admissions during the month. Duplicate cases in a same sector (repeat admission) or between sectors (transfer of a previously known case) were excluded from prevalence and incidence calculations.

Ethical considerations

We studied bacterial isolates obtained during the daily care of preterm infants in our NICU. Therefore, this observational study was fully in line with the routine care of preterm infants and did not require the agreement of the ethical committee of our institution.

Results

SA epidemiology in the NCC

One hundred and thirty nine patients admitted to the NCC were included in the epidemiological study. Distribution of carriage,

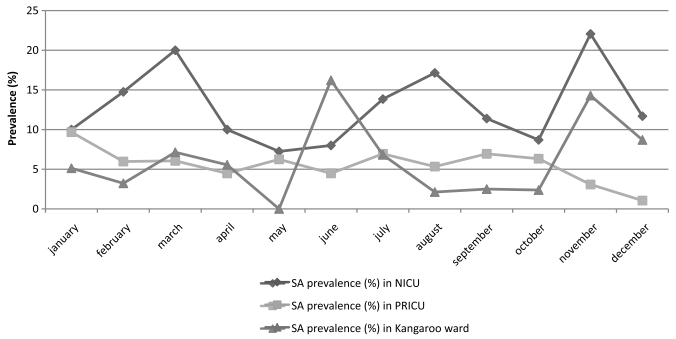

Figure 2 Monthly prevalence of SA carriage, colonization and infections by care sector in 2009

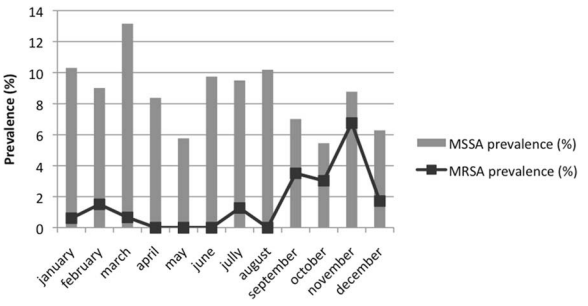

Figure 3 MSSA and MRSA monthly prevalence in 2009 for NCC

colonization and infection cases for each sector is summarized in Figure 1. Fifty-four patients were carriers, colonized or infected by SA, 22 in the summer period (4, 6, 4 and 8 respectively in June, July, August and September). In the NICU and kangaroo ward, most SA isolates were involved in carriage with a low frequency of colonization and/or infection. By contrast, patients in the PRICU with more instable clinical status were more frequently colonized and/or infected.

Distribution of the different types of SA positive clinical samples corresponding to colonization and/or infection by care sector is summarized in Table 1. Most of them occurred in the PRICU (70.9% of all positive samples), followed by the NICU (23.3%) and the kangaroo ward (5.8%). Respiratory samples were predominant in the NCC, overall representing 52.3% of all clinical samples, especially in the PRICU (63.9%). Ophthalmic samples were the second most frequent with about 17.5%, followed by blood cultures (10%) and catheter samples (7%). By care sector, ophthalmic colonization and infections predominated in the NICU, and came equal with respiratory samples (40% each) in the kangaroo ward. SA positive catheter samples and blood cultures were mainly present in the PRICU (8 of the 10 samples), 2 other cases were found in the NICU, and none in the kangaroo ward where patients are less often perfused.

The evolution of SA monthly prevalence for each sector is represented on Figure 2. In the NICU, where digestive carriage of SA is routinely screened, average prevalence reached 12.9%, with increasing rates in March (20%), August (17%) and November (22%). In other sectors, because of the absence of systematic screening, prevalence rates obtained were certainly underestimated and did not reflect the real presence of SA. In the PRICU, average rates were around 5.5% and ranged generally between 5 and 10%, except in November and December when they fell to 3.1% and 1.1% respectively. In the kangaroo ward, rates were more variable, roughly around 6.2% with 2 peaks in June (16.2%) and November (14.3%). Monthly incidences of SA clinical samples and infections by care sector were also calculated. Evolution of incidences over the year was very similar to prevalence curves, especially in the NICU with increased incidence in March (26.1%), July (20%) and August (17.4%), and November (28.9%) (data not shown). Finally, epidemiological analyses showed several epidemic episodes on a background of SA endemicity: each care sector presented 2 or 3 prevalence peaks.

Monthly MSSA and MRSA prevalence rates in the entire NCC are summarized in Figure 3. The overall MSSA prevalence did not decrease below 5.5% (in May and October), and reached 13.2% in March. Concerning MRSA, the mean prevalence in 2009 for all of the NCC was around 1.53%, with increasing rates in September (3.7%) and November (6%). These results show that MSSA endemicity largely exceeded MRSA endemicity in the NCC. Incidence rates confirmed that new cases of MSSA were also more frequent than MRSA in the NCC (data not shown).

Monthly analysis of infections showed an emergence of SA bacteraemia and catheter-linked infections in summer. For one of them, positive blood-culture was associated with a positive catheter. The origin of the other bacteraemia was not found.

MSSA infections outbreak

The epidemic curve of SA infection cases between January and December 2009 is shown in Figure 4. Among the 17 SA infections, 15 were caused by MSSA strains versus 2 by MRSA. Outbreak alert was sounded because of an increase in MSSA case numbers observed in July (4 cases) and August (2 cases), contrasting with the average rate of one case every 2 months until then. Six other SA infections occurred in September and October with respectively 3 cases including one MRSA infection each month. Another MSSA infection in November and one in December were also reported.

The 6 cases of July and August led to the outbreak investigation. All 6 patients were hospitalized in the PRICU and 5 of them were preterm neonates. Principal characteristics of these patients and associated strains are given in Table 2. Antimicrobial susceptibility was tested for the 12 MSSA strains from the 6 patients (Table 2). The 12 strains were mostly penicillinase producers (n = 11) and susceptible to most antibiotic classes except one strain resistant to macrolides. Most strains lacked virulence factors since only one carried tst, the gene encoding

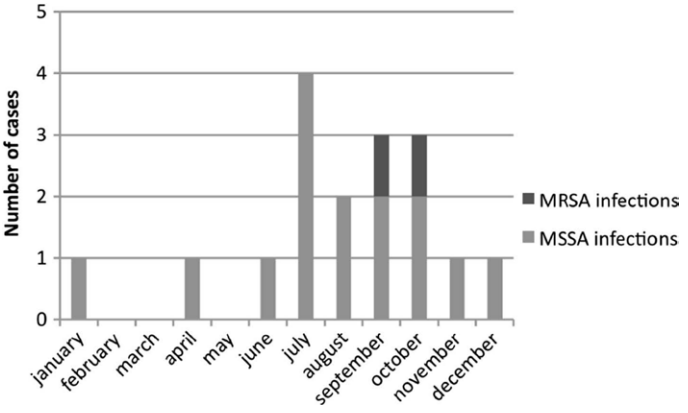

Figure 4 Evolution of SA infections in 2009 in NCC.

Table 2 Principal clinical characteristics of outbreak patients and strains

Patient	Reason of admission	Samples	Nature of sample	NCC sector	PFGE profile	MSSA/MRSA	*luk-PV*	*sea*	*tst*	Penicilline G	Oxacilline	Kanamycin
1	Prematurity and NEC	1A	BSI	NICU	A	MSSA				S	S	S
		1B		PRICU	B	MSSA				R	S	S
2	Severe epileptic encephalopathy	2A	BSI	PRICU	C	MSSA				R	S	S
		2B	Conjunctivitis		C	MSSA		+		R	S	S
3	Prematurity and NEC	3A	Insertion point of KT	PRICU	C	MSSA				R	S	S
		3B	KT		C	MSSA	ND	ND	ND	R	S	S
		3C			C	MSSA	ND	ND	ND	R	S	S
4	Prematurity	4A	BSI	PRICU	Untypable	MSSA				R	S	S
		4B			Untypable	MSSA	ND	ND	ND	R	S	S
5	Prematurity	5A	Tracheal Asp.	PRICU	C	MSSA				R	S	S
		5B	BL		C	MSSA			+	R	S	S
6	Severe epileptic encephalopathy	6A	Tracheal Asp.	PRICU	C	MSSA			-	R	S	S

Table 2 Principal clinical characteristics of outbreak patients and strains *(Continued)*

Patient	Gentamicin	Tobramycin	Tetracycline	Macrolides	Cotrimoxazole	Fluoroquinolones	Rifamycin	Fusic acid	Fosfomycin	Glycopeptides	Linezolid
1	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S	ND
2	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S	ND
	S	S	S	S	S	S	S	S	S	S	ND
	S	S	S	S	S	S	S	S	S	S	ND
4	S	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S	ND
	S	S	S	R	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S	S

NEC: necrotizing enterocolitis, BSI: bloodstream infection, KT: catheter, Asp.: aspiration, BL: bronchial liquid, *luk-PV*, gene coding for Panton-Valentine leukocidin; *sea*, genes coding for staphylococcal enterotoxins A; *tst*, gene coding for toxic shock syndrome toxin 1, ND, non determinate.

for toxic shock syndrome toxin 1 and one other carried sea coding for enterotoxin A. PFGE-typing for the 12 strains revealed a predominant profile named pulsotype C and shared by 8 strains isolated from 4 patients (Table 2). Interestingly, some isolates with the same pulsotype displayed different content in virulence genes.

NEC: necrotizing enterocolitis, BSI: bloodstream infection, KT: catheter, Asp.: aspiration, BL: bronchial liquid, luk-PV, gene coding for Panton-Valentine leukocidin; sea, genes coding for staphylococcal enterotoxins A; tst, gene coding for toxic shock syndrome toxin 1, ND, non determinate.

The synopsis of the IC team's interventions and investigations is presented in Table 3. During the environmental investigation, a high level of surface contamination (109 surfaces sampled) in all areas (average rate of 82.6%) of the NCC was observed with the presence of pathogens such as *Enterococcus faecalis*, *Pseudomonas aeruginosa* and MSSA. MSSA were isolated from a rack of electric syringes, medical records of patient, 2 spare beds stored in the hallway, and an incubator. All of these environmental MSSA strains were typed by PFGE and differed from the profile C. Water samples (n = 19) at various points of use were also analyzed, but none was positive for SA.

The clinical audit of hand hygiene practices revealed compliance rates of 60% in the NICU (14 persons assessed) and 76% in the PRICU (16 persons assessed). Non-compliance with recommendations was mostly due to the concatenation of multiple care sequences for the same patient, for example the absence of hand hygiene between a contaminated care (nap change) and a clean one (catheter manipulation). The assessment of central venous catheter manipulations in the PRICU revealed failures in catheter monitoring: catheter insertion points were covered with opaque dressings preventing visual control, and the frequency of dressing changes was insufficient. Furthermore, health-care workers always wore white over-gowns whenever tending to a catheter or coming into direct contact with perfused patients. These gowns were stored in a drawer of the incubator until re-use. During the observations, these white gowns were pointed out as a potential bacterial reservoir or source of cross-contamination. Thereby, 35 of them were sampled in December: 25 gowns in the PRICU and 10 in the NICU. Many of them were positive (15/35) for several pathogens including SA, which was found on 8 separate gowns. Overall these gowns showed a high level of contamination (on average 50 CFU/25 cm^2). SA strains were not typed due to the delay since the summer outbreak but the level of contamination suggested that misuse of these gowns could promote the transmission of pathogens.

Discussion and conclusion

The study of SA epidemiology in the Montpellier NCC showed an MSSA infection outbreak in the context of SA endemicity. The overall prevalence of SA in 2009 was consistent with other published studies [11,21]. The SA outbreak consisted mostly of bloodstream and respiratory tract infections, mainly caused by the same clone in PFGE. Observations by the IC team suggested that slack healthcare practices could be directly linked to the outbreak. Poor practice concerned mainly the manipulation and monitoring of central venous catheters, and the use of non-disposable white gowns. Aside from constant attention to hand

Table 3 Outbreak investigation and IC interventions

Actions	Dates
Case study	August 07, 17 and 18, 2009
- Clinical records review and strains typing	
Environmental investigation	August 05, 12, September 15 and October 13, 2009
- Surface and water sampling	
- Gowns	December 2009 (retrospectively)
Hygiene practices assessment	October 01, 2009
- Hand hygiene	October 19, 24 and November 09, 2009
- Catheter-linked practices	

hygiene [22,23], the complexity of care in neonatology requires a perfect knowledge of infection control principles, enabling healthcare professionals to cluster interventions by risk level so as to limit self-contamination of the patient or contamination of his environment [22,23]. Some studies have confirmed the progressive contamination of hands or gloves [24] and identified key opportunities for hand hygiene during routine cares, even if wearing gloves, because of possible hand contamination during removal [22]. Moreover, health care workers' white coats remain a controversial subject, supposedly protecting the patient during central venous catheter manipulations, but highly contaminated by pathogens for most of them (about a quarter positive for SA). As previously described by Treakle et al. in 2009, we could imagine that they were a vector of patient-to-patient transmission, relaying SA circulation and outbreak in the NCC [25]. However, we did not investigate the possibility of SA chronic carriage by a health care worker which could also have relayed the outbreak [8,26].

The typological analysis of colonized sites and infections occurring in 2009 showed a majority of cases in the PRICU, where patients are most susceptible to infections. The occurrence of catheter-related infections in the summer was consistent with the increased SA prevalence (colonization pressure), and slack catheter monitoring. Catheter-related infections are the most common health-care associated infections in NICUs [22,27,28]. The general strategy for their prevention is based on good practice recommendations concerning (i) insertion and maintenance of indwelling lines, (ii) administration of prophylactic antibiotics e.g.: antibiotic lock therapy, (iii) use of skin emollients to reduce bacterial penetration, and (iv) health-care workers and visitors donning of single-use gowns [22]. However in our NCC, points (ii) and (iii) were not applied and compliance with points (i) and (iv) was not optimal.

SA is a significant pathogen in neonatology and an important cause of morbidity [7]. Epidemiological studies of MRSA in NICUs have reported widely varying prevalence rates, ranging from 0.6 to 53% [6,7,21,29,30], and MRSA outbreaks are often described [6,31]. Far less attention is given to MSSA. In the Montpellier NCC, MRSA prevalence appeared rather low (1.53% in 2009, ranging from 0 to 6.1%), and quantitatively a less important problem than MSSA. We believe the commonly encouraged focus on MRSA surveillance [22,27,28] may lead to unrecognized or underestimated spread of MSSA. This is particularly worrying for infection control in wards where MSSA is the most prevalent SA type, as we observed for the NCC.

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Compliance of Mothers Following Recommendations to Breastfeed or Withhold Breast Milk During Rotavirus Vaccination in North India: A Randomized Clinical Trial

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Abstract

Background: Neutralizing antibodies in breast milk may adversely influence the immune response to live oral vaccines. Withholding breastfeeding around the time of vaccine administration has been suggested for improving vaccine performance. However, we do not know whether mothers find withholding breastfeeding around the time of vaccination acceptable and how they perceive this recommendation.

Methods: In a clinical study designed to examine predictors of poor immune response to rotavirus vaccine in infants in India, Rotarix was administered to infants at 6 and 10 weeks with other childhood vaccines. For the study, 400 mother-infant pairs were randomized into two groups in a 1:1 ratio. Mothers were either recommended to withhold breastfeeding or were encouraged to breastfeed half an hour before and after administration of Rotarix. The mother-infant pairs were observed and the breastfeeding intervals were recorded during this period. Mothers were administered a questionnaire about their perception of the intervention after the infants received the second dose of Rotarix.

Results: Almost 98% (391/400) of the infants received both doses of Rotarix. Adherence to the recommendations was high in both groups. All mothers in the group who were asked to withhold breastfeeding did so, except one who breastfed her infant before the recommended time after the first dose of Rotarix. Of the mothers, 4% (7/195) reported that the recommendation to withhold breastfeeding was difficult to follow. All mothers in this group reported that they would withhold breastfeeding at the time of vaccination if they were asked to by a health-care provider. Only one mother responded that withholding breastfeeding would be a reason for not giving rotavirus vaccine to her infant.

Conclusions: Withholding breastfeeding half an hour before and after vaccination appears to be acceptable to mothers in this setting. If withholding breastfeeding produces an improvement in the performance of the vaccine, it could be used to increase the public health impact of rotavirus immunization.

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Background

Rotavirus is the most common cause of severe dehydrating diarrhea worldwide in infants and young children, killing approximately 453,000 children under the age of 5 each year [1]. Rotavirus is particularly threatening in India, causing around 100,000 deaths in young children every year [2]. Vaccination remains a cornerstone in the prevention of rotavirus-associated morbidity and mortality.

The two oral rotavirus vaccines commercially available, Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq (Merck and Co, PA, USA), have been shown to be safe and effective [3]. Overall, rotavirus vaccines are associated with 74% and 61% reductions in very severe and severe rotavirus infections, respectively, and a 47% reduction in rotavirus-related hospital admissions [4-8]. However, in low-income countries, these vaccines have a lower efficacy compared to other oral vaccines like polio and cholera [9]. In impoverished high-mortality settings, host factors, including maternal antibodies, interfering bacterial and viral agents, and child and maternal malnutrition, may affect immune responses [10].

In vitro studies of the neutralizing effect of breast milk have suggested that withholding breastfeeding around the time of rotavirus vaccine administration may impact vaccine performance [11]. Efficacy trials have reported no difference between breastfed and nonbreastfed infants. However, in these studies the breastfeeding practices were self-reported and the interval between breastfeeding and vaccine administration was not adequately evaluated [10,12,13]. Breast milk from Indian mothers is reported to have much higher concentrations of rotavirus-neutralizing antibodies than breast milk from mothers in industrialized countries [11]. In phase I/II studies of a recently developed rotavirus vaccine (116E) in India, breast milk was withheld for half an hour before and after each vaccine dose, and the seroconversion rate was almost 90% [14].

Other studies are also being conducted to assess the modifying effect of breast milk on vaccine efficacy [15,16]. If these studies demonstrate an improvement in the immune response by withholding breastfeeding around the time of vaccination, this practice could be used to increase the public health impact of rotavirus vaccination. However, little is known about mothers' perceptions of withholding breastfeeding and whether such an intervention would be feasible and acceptable to mothers in low-income settings.

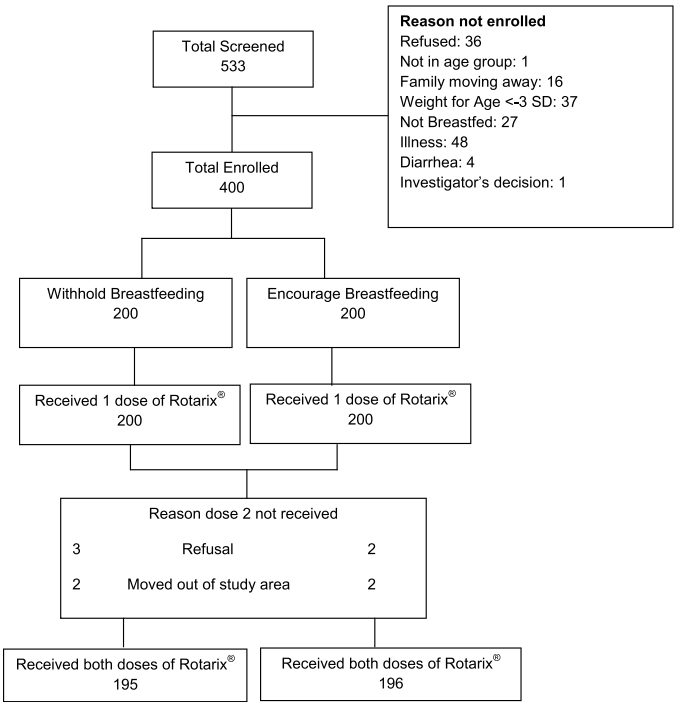

Figure 1 Trial profile.

The primary aim of the study was to assess the impact of withholding breastfeeding compared to encouraging breastfeeding on the immune response to Rotarix in infants. The results of the primary aim are not presented here. This paper describes the study methodology and the mothers' ability to adhere to the breastfeeding recommendation as well as their perception of the recommendation.

Methods

Study setting

The trial was conducted in the urban resettlement neighborhoods of Govindpuri-Tigri-Dakshipuri, Tuglakabad and Sangam Vihar in South Delhi, India. These areas are typical urban resettlement neighborhoods.

Randomization

The randomization list was generated by a statistician independent of the study team. Subject ID allocation for each participant was through serially numbered, opaque sealed envelopes.

Sample size

The sample size was based on the primary aim of the study. Assuming 60% seroconversion in the infants whose mothers were encouraged to breastfed and 80% in the group in whom breastfeeding was withheld, at 90% power and alpha level of 5%, 200 infants were required in each group. This sample also accounted for 30% dropouts and 10% who might be excluded from the analysis because of high levels of antibodies at baseline.

Enrollment and intervention delivery

Participants were enrolled into the study from October 2012. Infants aged less than 7 weeks were identified through a household survey. The families of infants aged 6 to 7 weeks were called to the study clinic for screening and enrollment. Infants were enrolled if their parents gave consent for participation, were aged 6 to 7 weeks, had a weight-for-age Z score that was not ≤ –3 [17] and were from a family with no plans to move out of the study area in the next 4 months. Infants were excluded if they were not breastfed, had already received a rotavirus vaccination, had a chronic enteric disease and illness requiring hospital referral or causing diarrhea on the day of enrollment or had a condition that the investigator judged to warrant exclusion, or if the mother or infant had an immune deficiency disease.

During consent, families were informed that if they agreed to take part in the study, their baby would be randomly selected for either the withhold breastfeeding or encourage breastfeeding group. All information was provided in the local dialect. The verbatim information in the informed consent form regarding allocation to either group was:

You are being asked permission for your baby to be screened for this study. This is to check if your baby is healthy enough to receive the rotavirus vaccine and the childhood vaccines. If your baby is assessed to be well enough to receive the vaccine, your baby can take part in this study. In case you agree to allow your baby to participate, your baby will be randomly (like tossing a coin) selected to either receive breastfeed or not be breastfed 30 minutes before and after receiving the Rotarix.

It was also explained that the purpose of the study was to test: "The effect of not giving and giving breast milk on the antibody response of the two doses of Rotarix." The rationale for asking mothers to withhold or encourage breastfeeding was: "Your baby's participation in this study may help in generating information about the usefulness of giving or not giving breast milk before and after the Rotarix vaccine."

After obtaining written informed consent, 400 eligible mother-infant pairs were enrolled and randomized into one of the two study groups.

Group 1: Mothers were advised to withhold breastfeeding 30 min before and 30 min after each dose of the vaccine.

Group 2: Mothers were encouraged to breastfeed immediately before and after each dose of the vaccine.

The recommendation was given by a trained study team nutritionist in both groups. The Group 1 mothers were told: "You have been selected to be in the group where breastfeeding needs to be withheld. Do not breastfeed your child for half an hour before and after receiving the rotavirus vaccine." Group 2 mothers were told: "You have been selected to be in the group where you are encouraged to breastfeed your child in the half an hour duration before and after receiving the rotavirus vaccine." At the study clinic there were two separate designated areas for the two groups. Each area was supervised by clinical coordinators.

Mother-infant pairs were required to wait in the designated area as per their group allocation. All activities, including specimen collection and administration of vaccines, were conducted in these areas. The team members were present in the same area to observe the mother-infant pairs during this time. After the 30 min of observation following Rotarix administration, the infants were administered the other childhood vaccines. In line with usual practice, infants remained in the study clinic for another 30 min after administration of the childhood vaccines to allow observation, management and documentation of any immediate

Table 1 Baseline characteristics of participants in the study

	Breastfeeding withheld (*n* = 200)	Breastfeeding encouraged (*n* = 200)
Infant characteristics		
Age at enrollment (days) (mean and SD)	48 (4.0)	49 (3.8)
Birth weight* (kg) (mean and SD)	2.80 (0.4)	2.84 (0.5)
Weight at screening (kg) (mean and SD)	4.41 (0.6)	4.43 (0.5)
Sex:		
Boys	103 (51.5)	105 (52.5)
Girls	97 (48.5)	95 (47.5)
Exclusively breastfed	150 (75.0)	160 (80.0)
Socioeconomic characteristics		
Home birth	61 (30.5)	52 (26.0)
Type of family:		
Nuclear	112 (56.0)	115 (57.5)
Joint	88 (44.0)	85 (42.5)
Number of siblings (mean and SD)	0.95 (0.96)	1.1 (1.1)
Maternal age (years) (mean and SD)	24.4 (3.5)	24.8 (3.9)
Mother has not attended school	48 (24.0)	45 (22.5)
Father has not attended school	22 (11.0)	22 (11.0)
Family owns color television, cooler or scooter	182 (91.0)	179 (89.5)
Annual family income (rupees) (median and interquartile range)	84,000 (60,000, 120,000)	84,000 (72,000, 120,000)

All values are *n* (%) except when otherwise indicated. SD, standard deviation.
*Information on birth weight was available for 137 (68.5%) infants in the withholding breastfeeding group and 143 (71.5%) infants in the group encouraged to breastfeed.

Table 2 Adherence to breastfeeding recommendations in the two groups

	Breastfeeding withheld	Breastfeeding encouraged
Dose 1	*n* = 200	*n* = 200
Time since last breastfeed (min) (median and IQR)	39 (33, 57)	100 (45, 152)
Pre-vaccine observation		
Number breastfed during observation period	0	200
Total breastfeed duration (min) during observation period (mean and SD)	–	11.8 (3.4)
Post-vaccine observation		
Number breastfed during observation period	1	200
Total breastfeed duration (min) during observation period (mean and SD)	0.01 (0.1)	7.7 (3.6)
Dose 2	*n* = 195	*n* = 196
Time since last breastfeed (min) (median and IQR)	56 (34, 70)	68 (43, 105)
Pre-vaccine observation		
Number breastfed during observation period	0	196
Total breastfeed duration (min) during observation period (mean and SD)	–	10.7 (2.8)
Post-vaccine observation		
Number breastfed during observation period	0	195
Total breastfeed duration (min) during observation period (mean and SD)	–	6.5 (2.4)

All values are *n* (%) except when otherwise indicated. IQR, interquartile range; SD, standard deviation.

adverse events. In this observation period, the women were not given any specific breastfeeding instructions, although breastfeeding practices were recorded by the project team members.

Each enrolled infant was given two doses of the Rotarix vaccine along with a pentavalent vaccine (diphtheria, pertussis, tetanus, hepatitis B and Haemophilus influenzae) and an oral polio vaccine. The vaccines were administered at the ages of 6 to 7 and 10 to 14 weeks, maintaining a minimum interval of 4 weeks between the two doses. The third dose of the pentavalent vaccine was offered to all infants when they came to the study clinic for the end of study activities 4 weeks after the second dose of Rotarix.

After enrollment, participants were contacted weekly after each dose of Rotarix to ascertain whether there were any signs or symptoms of suspected intussusception or whether they had suffered from an illness requiring hospital referral or had been hospitalized. Severe adverse events were reported to the Society for Applied Studies, Ethics Review Committee (SASERC). The follow-up of the last child was completed in May 2013.

Data collection

Baseline information on maternal and infant characteristics was collected at the time of enrollment. During the observation period, details, such as the time the observation started and ended and the duration of any breastfeeding, were documented in a form. After an infant received the second dose of Rotarix, its mother was asked a set of structured questions about how she perceived the intervention (the recommendation to withhold or encourage breastfeeding). Mothers were also prompted to comment on their answers.

Biological specimens from mothers and infants were collected to assess immunogenicity. Baseline maternal blood and breast milk specimens, and infant blood, saliva and stool specimens were obtained. Before the second dose of Rotarix, maternal breast

Table 3 Mothers' perception of breastfeeding recommendations

	Yes	No
Breastfeeding withheld (*n* = 195)		
Found it difficult to withhold breastfeeding for 30 min before and after Rotarix®	7	188
She would withhold breastfeeding around time of vaccination if health-care provider asked her	195	0
Withholding breastfeeding would be a reason not to give rotavirus vaccine to her baby	1	194
Breastfeeding encouraged (*n* = 196)		
Found it difficult to breastfeed for 30 min before and after Rotarix®	1	195
She would breastfeed around time of vaccination if health-care provider asked her	196	0
Breastfeeding would be a reason not to give rotavirus vaccine to her baby	2	194

milk specimens and infant saliva specimens were obtained. Four weeks after the second dose of Rotarix, blood, saliva and stool specimens were collected from the infants.

Presentation of data

Descriptive measures of continuous variables are presented as means and standard deviations (SDs) for symmetrical data, and as medians and interquartile ranges for skewed data. Descriptive measures of categorical data are presented as frequencies and percentages. An independent-samples t-test was used to explore the relationship between continuous variables.

Ethical clearance

Ethical clearance was obtained from SAS-ERC (SAS ERC/43/2012) and the South-East Regional Ethical Committee of Norway (2012/193/REK). This study was conducted in compliance with the protocol Good Clinical Practices and other relevant regulatory guidelines.

Results

Of the 533 infants screened for eligibility, 400 were enrolled and randomized (Figure 1). Baseline infant characteristics and socio-economic factors were comparable between the two groups (Table 1). Nine subjects did not receive the second dose of Rotarix; five refused further participation and four moved out of the study area, leaving 391 subjects who received both doses of Rotarix and whose mothers completed the questionnaire on their perception of the intervention.

The number of years in school varied greatly among both mothers and fathers, from 0 to 17 and 0 to 19, respectively. These distributions were similar in the two study groups. Mothers in the group withholding breastfeeding had a mean of 7.2 (SD, 4.8) years of education and those in the group encouraged to breastfeed had a mean of 7.0 (SD, 4.9) years. Similar figures were obtained for men (8.9 (SD, 4.1) and 9.1 (SD, 4.4) years, respectively).

With the exception of one mother, all mothers who were advised to withhold breastfeeding adhered fully to this recommendation (Table 2). Similarly, all mothers who were encouraged to breastfeed, except one, breastfed at least once (range one to four times) in each of the periods before and after vaccine administration. One woman did not breastfeed following the second dose of Rotarix, but breastfed in the period before vaccine administration.

The interval between the last breastfeed and the beginning of the intervention period varied widely among subjects, with maximum times of 491 and 388 min and minimum times of 6 and 9 min for the first and second doses, respectively. Mean intervals were significantly longer for infants who were given supplementary nutrition compared with those who were breastfed exclusively (mean difference, 40 min; P = 0.002).

Almost 78% of the infants were not given any other foods and fluids except breast milk; the mean number of breastfeeds per day was ten times. It was found that 75% of the infants in the group withholding breastfeeding were being exclusively breastfed. Infants were not breastfed for about an average of 49 and 46 min after receiving the first and second doses of Rotarix, respectively.

Adherence to the breastfeeding recommendations was high in both groups. Half of the mothers in each group made additional comments on how they perceived the intervention. The main emerging theme in both groups was that the mothers found withholding breastfeeding around the time of vaccination acceptable and feasible, understanding that the vaccines were important and beneficial for their child and by withholding breastfeeding they could potentially improve the vaccine effect (Table 3).

Found it difficult to breastfeed for 30 min before and after Rotarix 1 195 She would breastfeed around time of vaccination if health-care provider asked her 196 0 Breastfeeding would be a reason not to give rotavirus vaccine to her baby 2 194

Among mothers who were asked to withhold breastfeeding for 30 min before and after vaccine administration, seven (4%) reported that this practice was difficult; five found withholding breastfeeding stressful when their infants cried, one found the interval to be too long and one made no comment. Three of these seven infants were breastfed exclusively. Only one mother reported that withholding breastfeeding would be a reason for not giving the rotavirus vaccine to her infant, commenting that the duration of the non-breastfeeding period was too long.

All mothers withholding breastfeeding reported that they would adhere to this practice if asked to do so by health-care professionals. Thirteen of them said that they would do so since they understood the importance of vaccination. Two mothers said that they did the same when the health workers in the immunization center asked them to do so during oral polio vaccine administration.

Of the mothers who did not find withholding breastfeeding difficult, nine commented that their baby was calm and slept during the observation period, eight commented that the observation period was not too long and five reiterated that they did not find it difficult to withhold breastfeeding.

Discussion

This study assessed the feasibility of asking mothers to withhold breastfeeding. Mothers did not have any difficulty in complying with this request. The fact that almost all mothers adhered to the recommendations is encouraging and this practice can potentially be adopted into policy. It was also observed that the recommendation appeared to be acceptable to mothers as they perceived it to be beneficial for their children.

The time of 30 min was chosen since this was assumed to be a reasonable time limit for withholding breastfeeding. Studies show that the half gastric emptying time varies between 47 and 61 min [18-20]. Withholding breastfeeding for an hour before and after may not have been feasible in this setting. Many infants would likely have been offered supplementary food or water and the intervention could inadvertently have interfered with the World Health Organization's recommendation of exclusive breastfeeding for the first 6 months of life. The 30 min time interval was used in the rotavirus vaccine 116E trials in Delhi [14], which demonstrated good immunogenicity for the vaccine.

At least two other studies are underway to assess the importance of withholding breast milk to improve the immunogenicity of oral vaccines [15,16]. Advising mothers to withhold breast milk around the time of vaccination may be contemplated if there is clear benefit. It is essential that children get the maximum effect from their life-saving vaccines and at the same time it is essential to ensure that the benefits of breastfeeding are not undermined. Clear messages should be developed and tested further before being used in a program setting. It is important that the mothers understand that withholding breastfeeding around the time of vaccination may be required not because there are harmful substances in breast milk but because the beneficial substances may work against the effect of the vaccine.

This study was conducted with a limited population and the investigators did not measure the mothers' understanding of the breastfeeding recommendations. It is likely that the high compliance seen in this study is an artifact of the study setting for several reasons. Firstly, the recommendations were given by trained study team members with a background in nutrition and skilled in delivering the message. In this setting, it is well known that mothers are more likely to listen to health workers whom they perceive to be of a higher position and qualification: advice given by physicians or nutritionists is more likely to be adhered to. Secondly, the study team members who gave the recommendations also observed the mothers and were present in the same area as the mother-infant pairs. Thirdly, it is possible that the group of mothers who consented to participate in this study were inherent compliers. It is also likely that the mothers' perceptions of the intervention may have been different in other settings.

The study was conducted in urban resettlement neighborhoods of South Delhi. Though the participants in this study represent an important group, the generalizability of the study is limited since all the participants were from one area of Delhi. Nevertheless, reporting good quality data generated from smaller studies like this is important before considering larger trials in the population.

Conclusions

In conclusion, mothers in this setting complied with the recommendations given by the study team to withhold breastfeeding or breastfeed half an hour before and after vaccination. It is likely that the mothers perceived the recommendation to have potential benefits to the health of their infants, therefore resulting in the high compliance.

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Determinants of Neonatal Mortality in Pakistan: Secondary Analysis of Pakistan Demographic and Health Survey 2006-07

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Abstract

Background: Globally 7.6 million children died in 2010 before reaching their fifth birthday and 40% of these deaths occur in the neonatal period. Pakistan has the third highest rate of neonatal mortality globally. To implement evidence-based interventions for the reduction of neonatal mortality, it is important to investigate factors associated with neonatal mortality. The aim of the current study was to identify determinants of neonatal mortality in Pakistan.

Methods: Data was derived from the Pakistan Demographic and Health Survey 2006-07. All singleton live births between 2002 and 2006 were selected for the current analyses. Data was analysed by using STATA 13 and adjusted for the cluster sampling design. Multivariate Cox proportional hazard models were performed using step-wise backward elimination procedures to identify the determinants of neonatal mortality.

Results: A total of 5,702 singleton live births in the last five years preceding the survey were selected. Multivariate analyses showed that living in Punjab province (Adj HR = 2.10, p = 0.015), belonging to the poorest household wealth index quintile (Adj HR = 1.95, p = 0.035), male infants (Adj HR = 1.57, p = 0.014), first rank baby (Adj HR = 1.59, p = 0.049), smaller than average birth size (Adj HR = 1.61, p = 0.023) and mothers with delivery complications (Adj HR = 1.93, p = 0.001) had significantly higher hazards of neonatal death in Pakistan.

Conclusions: To reduce neonatal mortality, there is a need to implement interventions focusing on antenatal care, effective referral system and retraining of healthcare providers to manage delivery complications and smaller than average birth size babies in resource poor communities of Pakistan.

Background

Of 7.6 million under-five deaths in 2010 globally, 3.072 million of these deaths occurred in the neonatal period (first four weeks of life) [1] and most of these deaths (99%) arise in low and middle income countries [2]. Globally, neonatal deaths account for 40% of under-five deaths [1], while in South Asia these deaths account for slightly over half of under-five deaths [3]. The fourth Millennium Development Goal (MDG-4) target to reduce under-five deaths by two-thirds by 2015, with a global target of 32 per 1,000 live births [2]. Substantial efforts have been made to reduce the under-five mortality over the last two decades and a global decline of 35% has been achieved, from 87.6 per 1,000 live births in 1990 to 56.7 per 1,000 live births in 2010, with an annual rate of reduction of 2.2% [4]. However, there has been limited progress in reducing neonatal mortality over the same time period and a drop of 31% with an annual rate of reduction of 1.8% has been achieved globally [4]. Given that the current global neonatal mortality rate, is 30 per 1,000 live births, the burden of deaths in the neonatal period alone approximates the entire MDG 4 target [5]. To reduce under-five mortality considerably, therefore, it is pertinent to pay attention to neonatal mortality, especially in low and middle income countries. Previous research has shown that many neonatal deaths are preventable with existing low-cost interventions [6,7]. However, before implementing these innovations, country-specific factors which influence the neonatal mortality in specific population should be examined.

Pakistan comprises a total land mass of 796,096 square kilometres and is divided into four provinces and federally administrative areas. The population is estimated around 170 million in 2011 [8]. Pakistan has the third highest rate of neonatal mortality globally. Despite having made significant progress in incorporating newborn care into national policies and programs and improvement in coverage of several interventions relevant to newborn survival during the last decade, neonatal mortality has declined slowly with an annualized decline of 0.9% during the same period [8]. This shows that the current rate of decline will be insufficient for the country to reach its child survival MDG. The latest Pakistan Demographic and Health survey (PDHS) 2012-13 reported a neonatal mortality of 55 per 1,000 live births [9].

In Pakistan, the maternal and child health (MCH) related services are provided by a mixed public-private healthcare delivery system with the conventional three tiers of primary, secondary and tertiary healthcare facilities. The public sector includes basic health units, rural health centres, referralh ospitals and tertiary level hospitals with trained doctors and staff and subsidized medicines. At present, there are 965 tertiary and secondary hospitals, and 13,051 first-level care facilities in the public sector [10]. However, the use of these facilities remains low in Pakistan for several reasons such as long distances to facilities, restricted hours of operations, poor facility infrastructure, lack of staff,

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Table 1 Percent distribution of women age 15–49 who had a live birth in the five years preceding the survey by receiving antenatal care (ANC) from a skilled provider, delivered by a skilled provider, neonatal mortality rate and maternal mortality ratio by background characteristics and time period, Pakistan 1990–91 to 2012-13

Background characteristics	Percentage receiving ANC from a skilled provider			Percentage delivered by a skilled provider			Neonatal mortality rate (per 1,000 live-births)			Maternal Mortality ratio (per 100,000 births)		
	1990-91[14]	2006-07[15]	2012-13[9]	1990-91[14]	2006-07[15]	2012-13[9]	1990-91[14]	2006-07[15]	2012-13[9]	1990-91[16]	2006-07[15]	2012[17]
Place of residence												
Urban	58.3	78.1	87.8	60.6	60.1	71.0	40.8	48.0	47.0		175	
Rural	12.6	53.5	66.7	24.1	29.8	44.4	58.6	55.0	62.0		319	
Province												
Punjab	22.1	60.9	77.8	36.2	37.7	52.5	58.4	58.0	63.0		227	
Sindh	45.9	70.4	78.2	39.6	44.4	60.5	44.4	53.0	54.0		314	
Khyber Pakhtunkhwa	18.0	51.3	60.5	20.4	37.9	48.3	48.2	41.0	41.0		275	
Balochistan	24.2	40.7	30.6	52.6	23.0	17.8	46.1	30.0	63.0		785	
Total	**26.7**	**60.9**	**73.1**	**35.4**	**38.8**	**52.1**	**51.4**	**54.0**	**55.0**	**533**	**276**	**250**

equipment and drugs, and financial restrictions [11,12]. Hence, with more than 73,000 private health facilities across the country, about 71% of the population of Pakistan obtain health services from these facilities [10]. In addition, in rural areas community health workers, such as lady health workers and community midwives also provide MCH services. At present, around 93,000 lady health workers and 6,000 community midwives are working in rural communities of Pakistan [8]. Lady health workers were not initially mandated to provide newborn care [13] but were progressively involved in newborn care during the last decade [8]. To cover almost all rural areas of Pakistan 30 times more community midwives are required than are presently working in the rural areas [8]. Therefore, at present these services are inadequate to cater for the large rural population of Pakistan.

Table 1 presents the trends of percentage of women receiving antenatal care from a skilled provider, delivery by a skilled provider, neonatal mortality rates and maternal mortality ratios by background characteristics in Pakistan [9,14-17]. Over the last two decades there has been a gradual increase in the percentage of women receiving antenatal care and being delivered by a skilled provider. Nevertheless, these indicators have shown substantial variation between urban and rural communities, and between provinces. Neonatal mortality rates have slightly increased from 51.4 per 1,000 live births in 1990-91 to 55.0 per 1,000 live births in 2012-13, with regional variation [9,14,15]. On the other hand, the maternal mortality ratio has been reduced by half from 1990-91 [16] to 2012 [17]. Therefore, it could be argued that improvements in antenatal care and delivery care have had an impact on maternal mortality but not on neonatal mortality.

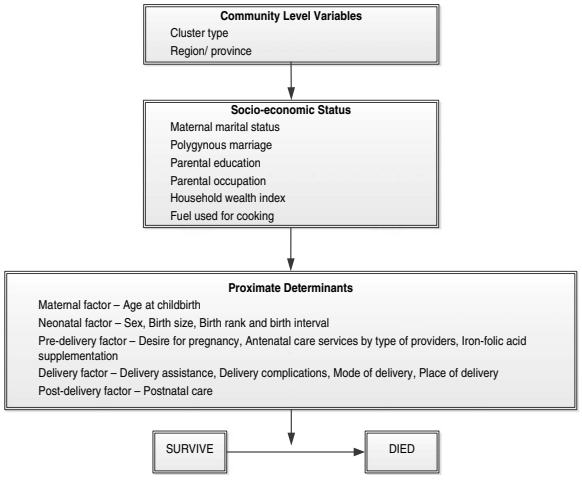

Figure 1 Conceptual framework for determinants of neonatal mortality in Pakistan.

Keeping in mind the slow progress in reducing neonatal mortality during the last decade in Pakistan, there is a need to understand the epidemiology, causes and risk factors for neonatal mortality. The aim of the current study was to investigate determinants of neonatal mortality in Pakistan by using the nationally representative data. The findings of the current study will help stakeholders to implement evidence-based interventions for newborn survival and improve the targeting of the program to the most at risk populations. We conducted the secondary analyses of PDHS 2006-07 to identify the determinants of neonatal mortality for all singleton live births between 2002 and 2006 in Pakistan.

Methods

Data source

Data used for the current study were derived from the PDHS 2006-07, which used a stratified, multistage cluster sampling method to ensure a sample representative of the population of Pakistan excluding the federally administered northern and tribal areas and restricted military and protected areas. Urban and rural samples were drawn separately and in proportion to the population of each province. At the first stage 1,000 clusters with probability proportional to size (390 in urban and 610 in rural areas) were selected. In Punjab, Sindh, Khyber Pakhtunkhwa and Baluchistan provinces, 440, 260, 180 and 100 clusters were selected, respectively. In urban areas, the clusters were selected from 26,800 enumeration blocks, each including 200-250 households. A list of 50,588 villages enumerated in the 1998 population census was used to select clusters in rural areas. In the second stage, 105 households were selected randomly and 10 households in each sampled household were selected using a systematic random sampling technique to conduct interviews with married women of reproductive age [15].

PDHS 2006-07 used six types of questionnaires, the Community, the Short Household, the Long Household, the Women's, the Maternal Verbal Autopsy, and the Child Verbal Autopsy Questionnaires. The contents of the Household and Women's Questionnaires were based on model questionnaires developed by the Measure DHS program. The Household Questionnaire listed all the usual members and collected basic information such as age, sex, marital status, education, relationship to the head of the household and household level characteristics. The Women's Questionnaire collected information from ever-married women 12-49 years of age on background characteristics

Table 2 Description of variables used in the analysis

Variables	Description and categorization
Community level factors	
Cluster type	Type of cluster (1 = urban; 2 = rural)
Region/province	Region (1 = Baluchistan; 2 = Khyber Pakhtunkhwa; 3 = Sindh; 4 = Punjab)
Socioeconomic factors	
Maternal marital status	Marital status of respondents (1 = currently married; 2 = formerly married including widows, divorced, separated)
Polygynous marriage	Polygynous union (1 = No; 2 = Yes)
Parental education	Maternal and paternal education status (1 = both had secondary and above education; 2 = both were illiterate; 3 = at least one or both with primary education; 4 = father had secondary and above education and mother illiterate; 5 = father had secondary and above education and mother had primary)
Parental occupation	Maternal and paternal employment status (1 = mother without a job outside the home and father employed; 2 = mother and father both employed; 3 = father unemployed)
Household wealth index	Composite index of household amenities (1 = riches; 2 = richer; 3 = middle; 4 = poorer; 5 = poorest)
Fuel used for cooking at home	Fuel used for cooking at home (1 = natural gas/ electricity; 2 = biomass)
Proximate factors	
Maternal age at childbirth	Maternal age at childbirth (1 = 20-29 years; 2 = less than 20 years; 3 = 30 years and more)
Baby's gender	Gender of neonate (1 = female; 2 = male)
Birth size	Subjective assessment of the respondent on the birth size (1 = average; 2 = smaller than average; 3 = larger than average)
Birth weight	birth weight of neonate (1 = less than 2500 grams; 2 = 2500-3500 grams; 3 = more than 3500 grams; 4 = not weighed)
Birth rank and birth interval	Birth rank and birth interval of neonate (1 = 2nd or 3rd birth rank, birth interval > 2 years; 2 = 1st birth rank; 3 = 2nd or 3rd birth rank, birth interval ≤ 2 years; 4 = ≥4th birth rank, birth interval > 2 years; 5 = ≥4th birth rank, birth interval ≤2 years)
Desire for pregnancy	Intention to become pregnant (1 = wanted then; 2 = wanted later; 3 = wanted no more)
ANC services by type of providers	ANC services by type of providers (1 = health professionals; 2 = untrained providers; 3 = no services used)
Antenatal iron/folic acid supplements used	Use of iron/folic acid supplements during pregnancy(1 = yes; 2 = no)
Delivery complications	Complications during delivery (1 = none; 2 = prolonged labour; 3 = other)
Delivery assistance	Birth attendance during delivery (1 = health professional; 2 = traditional birth attendant/others)
Mode of delivery	Mode of delivery (1 = non-caesarean; 2 = caesarean section)
Place of delivery	Place of delivery (1 = home; 2 = health facility)
Postnatal care	Postnatal service received by the neonate (1 = no; 2 = yes)

ANC: Antenatal care.

(education, literacy, native language, marriage characteristics etc.), full birth history, history of antenatal care (ANC) for the most recent birth within five years preceding the survey, delivery and postnatal care for all births as well as the survival of live-birth infants.

In the current PDHS 2006-07, 98% of the 97,687 available households were successfully interviewed, and 10,023 women were interviewed, which was 95% of the 10,601eligible women. Multiple pregnancies (n = 65) were excluded from the analysis due to higher odds of newborn deaths associated with prematurity and pregnancy complications among them [18]. Only last live births in the last five years (2002-2006) preceding the survey were selected for the current study to avoid the violation of the independence assumption and reduce potential recall bias. Further, for last live births in the last five years preceding the survey, antenatal care, delivery, and postnatal care services details were also recorded. A verbal informed consent was taken from each respondent before the interview. The study protocol was approved by the ethics committee of the University of Sydney, Australia.

Conceptual framework

The conceptual framework proposed by Mosley and Chen [19] with modifications based on the limitations and structure of the DHS data was used (Figure 1).

Description of variables

We defined the death of a live born baby in the first month of life (birth to 30 days) as neonatal mortality. The neonatal mortality rate was calculated as the number of deaths in the first month of life per 1,000 live births. The outcome was neonatal deaths recorded as a binary variable. The age of neonatal death was measured in days and for deaths within 24 hours a value of 0.01 days was used. A list of explanatory variables along with their definitions and categories is presented in Table 2.

Two variables were included at the community level, which were cluster type and region. Six variables were included in the socio-economic status, which were maternal marital status, polygynous marriage, parental education, parental occupation, household wealth index and fuel used for cooking at home. The household wealth index was calculated using an inventory of households assets including presence of television, radio, refrigerator, electricity, type of toilet, condition of housing, and ownership of vehicles, which were weighted using principal components analysis method [20].

The proximate determinants at the individual level were maternal age at childbirth (maternal factor), baby's gender, mother's perception of birth size, birth weight and a combined variable of baby's birth rank and birth interval (neonatal factor), mother's desire for pregnancy (pre-delivery factor), ANC services by type of providers, antenatal iron-folic acid (IFA) supplements used, delivery complications, delivery assistance, mode of delivery, place of delivery (delivery factor), and postnatal care as a post-delivery factor.

Statistical analysis

Data analyses was conducted by using STATA 13.1 (Stata-Corp, College Station, TX, USA) with 'Svy' commands to allow for adjustments for the cluster sampling design used in the survey. The frequencies along with weighted percentage were calculated for the selected variables. The neonatal mortality rate was calculated for each category, followed by Cox proportional hazard analysis to identify potential determinants of neonatal mortality without adjusting for other covariates.

All variables with a p-value ≤ 0.2 were included in a multivariate Cox proportional hazard models, which were constructed using step-wise backward elimination procedures. However, variables like ANC services by type of providers and delivery assistance were considered as a priori in the models due to their strong association with neonatal mortality. Hazard ratios (HR) were estimated as the exponential of the regression coefficients, and

Table 3 Characteristics of variables (n = 5,702)

Variables	n	n*	%*	NMR*
Community level factors				
Cluster type				
Urban	1988	1705	30.1	43.9
Rural	3714	3954	69.9	46.2
Region/province				
Balochistan	678	264	4.7	26.6
Khyber Pakhtunkhwa	1108	823	14.5	25.8
Punjab	2302	3176	56.1	51.7
Sindh	1614	1396	24.7	46.9
Socioeconomic factors				
Maternal marital status				
Currently married	5634	5579	98.6	45.2
Formerly married	68	80	1.4	66.1
Polygynous marriage				
No	5235	5225	92.3	45.5
Yes	394	350	6.2	39.0
Missing	73	84	1.5	
Parental education				
Both had secondary and above education	949	976	17.2	43.0
Both were illiterate	1870	1781	31.5	53.1
At least one or both with primary education	1077	1097	19.4	50.3
Father had secondary and above education and mother illiterate	1263	1216	21.5	42.8
Father had secondary and above education and mother had primary	463	504	8.9	21.3
Missing	80	85	1.5	
Parental occupation				
Mother and father both employed	965	988	17.5	72.9
Mother without a job outside the home and father employed	4173	4111	72.6	36.9
Father unemployed	193	175	3.1	39.4
Missing	371	385	6.8	
Household wealth index				
Richest	1010	1021	18.0	30.1
Richer	1060	1063	18.8	38.6
Middle	1115	1093	19.3	45.3
Poorer	1233	1193	21.1	54.3
Poorest	1284	1288	22.8	55.7
Fuel used for cooking at home				
Natural gas/electricity	3,857	3934	69.5	32.1
Biomass	1,614	1454	25.7	47.4
Missing	231	271	4.8	
Proximate factors				
Maternal age at childbirth				
20-29 years	2966	2976	52.6	42.5
Less than 20 years	2549	2504	44.2	48.9
30 and more	187	179	3.2	49.2
Baby's gender				
Female	2633	2601	46.0	38.5
Male	3069	3058	54.0	51.5
Birth size				
Average birth size	2333	2505	44.3	32.0
Smaller than average birth size	1924	1810	32.0	52.2
Larger than average birth size	1371	1265	22.4	50.7
Missing	74	79	1.4	
Birth weight (in grams)				
Less than 2500	155	144	2.5	37.5
2500-3500	304	322	5.7	30.9
More than 3500	196	215	3.8	63.3
Not weighed	4362	4294	75.9	43.6
Missing	685	684	12.1	
Birth rank and birth interval				
2nd or 3rd birth rank, birth interval >2 yrs	1157	1167	20.6	44.0
1st birth rank	992	970	17.1	80.6
2nd or 3rd birth rank, birth interval ≤2 yrs	735	740	13.1	42.0
≥4th birth rank, birth interval >2 yrs	1981	1953	34.5	33.4
≥4th birth rank, birth interval ≤2 yrs	836	829	14.6	39.3
Desire for pregnancy				
Wanted then	4143	4078	72.1	48.2
Wanted later	751	739	13.1	34.4
Wanted no more	737	763	13.5	29.5
Missing	71	79	1.4	
ANC services by type of providers				
Health providers	2646	2649	46.8	43.7
Untrained providers	977	962	16.9	57.5
No services used	1997	1961	34.7	41.6
Missing	82	87	1.5	
Antenatal iron/folic acid supplements used				
Yes	2536	2427	42.9	40.6
No	3095	3158	55.8	45.7
Missing	71	74	1.3	
Delivery complications				
None	3,277	3422	60.5	36.2
Prolonged labour	484	409	7.2	47.3
Other	1,871	1752	31.0	56.4
Missing	70	75	1.3	
Delivery assistance				
Health professional	2039	2026	35.8	48.0
Traditional birth attendant	1946	1982	35.0	46.2
Other untrained	1595	1533	27.1	35.4
Missing	122	118	2.1	
Mode of delivery				
Non-caesarean	5224	5151	91.0	43.5
Caesarean section	433	464	8.2	74.4
Missing	45	44	0.8	
Place of delivery				
Health facility	2076	2061	36.4	46.9
Home	3563	3528	62.3	41.9
Missing	63	70	1.2	
Postnatal care				
Yes	2852	2822	49.9	35.5
No	739	733	13.0	60.5
Missing	2111	2104	37.2	

*Weighted for the sampling probability.
ANC: antenatal care.

95% confidence interval (CI) for the HRs were calculated. Two variables, birth weight and postnatal care, because of higher proportions of missing values were excluded from the analysis.

Results

For this study, 5,702 (5,659 weighted) singleton live births in the last five years preceding the survey were selected. We found that between 2002-2006, 68% of infants deaths occurred during the first month of life, of which 33% occurred on the first day and 73% occurred in the first week of life.

The basic characteristics and neonatal mortality rates are shown in Table 3. The majority of mothers belonged to rural areas (70%) and 56% of the mothers were living in Punjab province. Almost all (99%) mothers were currently married. One third of both parents were illiterate. In about three quarters of parents (73%),

Table 4 Factors associated with neonatal mortality: unadjusted and adjusted hazard ratio

Factors	Unadjusted				Adjusted†			
	HR	95% CI		p	HR	95% CI		p
Community level factors								
Cluster type								
Urban	1.00							
Rural	1.07	0.73	1.57	0.715				
Region/province								
Balochistan	1.00				1.00			
Khyber Pakhtunkhwa	0.88	0.49	1.59	0.670	1.02	0.50	2.08	0.952
Sindh	1.58	0.93	2.67	0.088	1.59	0.85	2.96	0.145
Punjab	1.78	1.07	2.95	0.025	2.10	1.15	3.82	0.015
Socioeconomic factors								
Maternal marital status								
Currently married	1.00							
Formerly married	1.53	0.47	5.02	0.481				
Polygynous marriage								
No	1.00							
Yes	0.78	0.40	1.52	0.474				
Parental education								
Both had secondary and above education	1.00							
Both were illiterate	1.27	0.77	2.08	0.347				
At least one or both with primary education	1.15	0.67	1.97	0.604				
Father had secondary and above education and mother illiterate	1.00	0.63	1.59	0.995				
Father had secondary and above education and mother had primary	0.51	0.22	1.16	0.107				
Parental occupation								
Mother and father both employed	1.00				1.00			
Mother without a job outside the home and father employed	0.47	0.32	0.69	<0.0001	0.46	0.32	0.66	<0.0001
Father unemployed	0.51	0.18	1.47	0.210	0.50	0.17	1.53	0.226
Household wealth index								
Richest	1.00				1.00			
Richer	1.26	0.62	2.55	0.518	1.04	0.52	2.09	0.904
Middle	1.57	0.89	2.77	0.117	1.60	0.85	3.01	0.143
Poorer	1.81	1.07	3.08	0.028	2.09	1.16	3.75	0.014
Poorest	1.86	1.10	3.16	0.021	1.95	1.05	3.63	0.035
Fuel used for cooking at home								
Natural gas/electricity	1.00							
Biomass	1.50	0.99	2.27	0.056				
Proximate factors								
Maternal age at childbirth								
20-29 years	1.00							
less than 20 years	1.18	0.86	1.63	0.307				
30 and more	1.25	0.60	2.64	0.549				
Baby's gender								
Female	1.00				1.00			
Male	1.38	0.98	1.94	0.067	1.57	1.09	2.26	0.014

only fathers were employed. At the time of childbirth, slightly over half of the mothers (53%) were aged between 20 and 29 years. Fifty-four percent of the live births in our sample during 2002-2006 were males. About one third of the mothers perceived that their babies were smaller than average birth size.

Analyses of utilization of health services showed that 47% of mothers received ANC services from health professionals and slightly over one third of mothers did not use any ANC services. About 43% women took IFA supplements. Slightly over one third of mothers (36%) delivered at health facilities and 31% had complications during delivery. Only 13% of respondents stated that their babies received postnatal care.

The unadjusted and adjusted Cox proportional HRs of the probable factors associated with neonatal mortality are presented in Table 4. Infants who were living in Punjab province had a significantly higher risk for neonatal mortality compared to Baluchistan province after adjusting for all other factors. Similarly, infants of mothers belonging to the poorest household wealth index quintile also had a significantly higher risk of neonatal mortality compared to mothers in the richest quintile after adjusting for other factors. Male infants had 60% higher risk of neonatal mortality than female infants in our sample. Compared to average sized babies, smaller than average birth size babies had 61% and larger than birth size babies had 68% higher risk of neonatal mortality. First rank infants had a 59% higher risk of neonatal mortality than 2nd or 3rd rank with birth interval of equal or more than 2 years. Infants whose mother had complications during delivery, the risk of neonatal mortality was 93% higher compared to those who did not have delivery complications after adjusting for all other potential factors for neonatal mortality. Compared to parents who were both working, the risk of neonatal mortality was reduced significantly by 54% in infants whose mothers were not doing any work outside home while father was employed.

Household wealth index and fuel used for cooking at home were found to be highly correlated to each other ($p < 0.0001$). Therefore, when we replaced the household wealth index with the fuel used for cooking at home in the multivariate modelling, infants whose mothers used the biomass fuel for cooking had significantly higher risk of neonatal mortality compared to those who used the natural gas/electricity for cooking after adjusting for other factors (aHR 1.62, 95% CI 1.02, 2.55, $p = 0.039$).

Discussion

Main findings

The current study found that male infants, smaller than average birth size and delivery complications were significantly associated with higher risk of neonatal mortality in Pakistan. Other factors which were associated with neonatal mortality were: infants who were living in province Punjab, first rank baby and belonging to the poor household wealth index quintile. Our

Table 4 Factors associated with neonatal mortality: unadjusted and adjusted hazard ratio *(Continued)*

Birth size								
Average birth size	1.00				1.00			
Smaller than average birth size	1.64	1.14	2.37	0.008	1.61	1.07	2.42	0.023
Larger than average birth size	1.64	1.07	2.50	0.022	1.68	1.02	2.75	0.040
Birth rank and birth interval								
2nd or 3rd birth rank, birth interval >2 yrs	1.00				1.00			
1st birth rank	1.85	1.23	2.78	0.003	1.59	0.99	2.53	0.049
2nd or 3rd birth rank, birth interval ≤2 yrs	0.95	0.56	1.60	0.847	0.79	0.41	1.50	0.470
≥4th birth rank, birth interval >2 yrs	0.79	0.51	1.22	0.284	0.66	0.40	1.11	0.116
≥4th birth rank, birth interval ≤2 yrs	0.91	0.50	1.68	0.768	0.51	0.28	0.94	0.032
Desire for pregnancy								
Wanted then	1.00							
Wanted later	0.75	0.47	1.22	0.244				
Wanted no more	0.61	0.34	1.08	0.090				
ANC services by type of providers								
No services used	1.00							
Untrained providers	1.37	0.72	2.64	0.335				
Health providers	1.03	0.73	1.47	0.841				
Antenatal iron/folic acid supplements used								
Yes	1.00							
No	1.15	0.82	1.61	0.429				
Delivery assistance								
Health professional	1.00							
Traditional birth attendant/other untrained	0.88	0.60	1.27	0.479				
Delivery complications								
None	1.00							
Prolonged labour	1.45	0.84	2.51	0.185	1.69	0.88	3.26	0.114
Other	1.62	1.14	2.30	0.007	1.93	1.31	2.85	0.001
Mode of delivery								
Non-caesarean	1.00							
Caesarean section	1.71	1.07	2.72	0.024				
Place of delivery								
Health facility	1.00							
Home	0.91	0.63	1.32	0.616				

748 missing cases were excluded from the analysis. †Adjusted for community level, socioeconomic status and proximate determinants of neonatal mortality.
ANC: antenatal care. HR: Hazard ratio.

findings are important because the level of neonatal mortality in Pakistan is unacceptably high, 54 per 1,000 live births, and the current rate of decline will be insufficient for the country to reach its child survival MDG. Detection of these risk factors for neonatal deaths will help to formulate strategies and program innovations to improve neonatal survival.

Strength and limitations

We used the nationally representative sample for the current study, which was considered as the major strength of our study findings. Further, PDHS 2006-07 had high response rates at household and individual levels. We used infant survival data for the last five years to reduce recall errors about dates for births and deaths by interviewed mothers [21-23]. Moreover, we performed Cox proportion hazard models to identify determinants of neonatal mortality, which is a standard method for dealing with censored failure time data and has been widely used in biomedical research [24].

However, in PDHS 2006-07 only surviving mothers were interviewed, which was one of the limitations of the current study. It may have led to an underestimate of the neonatal mortality rate, because of the association of neonatal deaths with maternal deaths, and could also have led to an underestimate of the effect of some of the associated factors, such as delivery complications [22]. Further, some variables, such as parental occupation which represented the employment status within the last 12 months preceding the survey, were not infant specific because these only presented the most recent conditions. We did not consider the initiation of breastfeeding variable because of the low number of neonatal deaths in the late neonatal period (8-30 days), which was hypothesized as the time when colostrum would start to provide protection to the infant for infectious diseases. The variables like birth weight and postnatal care were excluded from the multivariate analysis due to high proportions of missing values. Genetic and some environmental factors which are also possible determinants of neonatal mortality were not available in the PDHS dataset. Nevertheless, these limitations were unlikely to have had an important influence on the validity of our findings.

Comparison with other studies

The current study revealed that smaller than average birth size was an independent risk factor for neonatal mortality in Pakistan. We did not use the birth weight variable in the analyses as only 12% of infants were reported to have been weighed at the time of birth in the survey. We considered smaller than average birth size as a proxy variable for low birth weight. Our findings agree with several other studies which have identified low birth weight as a risk factor for neonatal mortality [2,25-29]. An in-depth analysis of 2006-07 PDHS data by National Institute of Population Studies (NIPS) Pakistan reported a strong association between small birth size and neonatal mortality. However, the authors did not adjust the analysis for antenatal, delivery and postnatal care variables [30]. Low birth weight arises through preterm birth or in-utero growth restriction, or both [31]. In a hospital based retrospective study from Pakistan, 68% of mortality in newborn was contributed by low birth weight, 74% of them being preterm, suggesting high mortality among low birth weightpreterm infants [32]. Preterm births with complications are considered as the leading cause of neonatal deaths in Pakistan [1,8].

Infants whose mothers had delivery complications had a higher risk of neonatal death in our sample. The delivery complications included vaginal bleeding, presence of fever or convulsions, and these complications need to be managed by a skilled birth attendant in a well equipped health facility. However, only 36% of deliveries were conducted by health professionals in our sample. Research has shown that deliveries in a health facility with a skilled birth provider reduces early neonatal deaths [33,34].

Male gender was identified as a predictor of neonatal mortality in Pakistan. Several studies have identified male gender as a risk factor for neonatal deaths [25,35-38]. Biologically male children have higher risk of getting infectious diseases due to higher prevalence of immune deficiency [37], higher prevalence of respiratory illnesses and congenital malformations of urogenital system due to late maturity [38], probably all these lead to higher neonatal mortality among males.

Our study highlighted an association between first birth order and neonatal mortality in Pakistan. Previous studies have reported a U-shaped curve relationship between birth order and neonatal mortality as neonatal mortality tends to be higher for the first born child, and higher order births of order 4 and above compared with second and third order births [39-41]. First birth infants were at a 33% increased risk of dying during the neonatal period than births of second through third order reported by others [41]. There is a biological basis for the poor survival experience of first births during the neonatal period. First births in developing countries take place before a woman has reached full physical and reproductive maturity. Furthermore, it could be due to poor preparation by first-time mother to handle new roles and responsibilities in her life [40].

The poorest household wealth index quintile was identified as a risk factor for neonatal mortality in our analyses. Similar to our finding, secondary analysis of Sudan DHS has also found lower household wealth index as a risk factor for neonatal mortality [42]. Household poverty has been reported to increase neonatal mortality either by increasing the prevalence of risk factors like maternal infections or through reducing access to effective care [2].

Our sample also showed regional variation in neonatal mortality in Pakistan. In this context, living in Punjab province was identified as a predictor of neonatal mortality in Pakistan. Punjab is the most populated province of Pakistan with rural and urban population. In the Punjab, there are about 2,800 primary health facilities (basic health units and rural health centres) in the public sector serving the rural communities with trained staff and heavily subsidized medicines. Nevertheless, these facilities are distributed unevenly compared to the population catchment area. Hence, there are more doctors per facility in some areas at the expense of others. In 2011 a survey [43] was conducted to evaluate the current status of basic health units in Punjab. Out of 850 selected basic health units, 7.2% were closed, and 52.4% did not have the essential staff at the time of survey. Further, the monitoring system of these facilities was found to be weak and non-availability of drugs at these facilities were also considered a major problem [43]. The Punjab has had the highest neonatal mortality rates over the last two decades (Table 1) despite an increase in the percentage of women receiving ANC and being delivered by a skilled provider. There could be several reasons to explain this discrepancy, such as lack of staff at the primary level health facilities, and shortages of essential drugs. However, we also analysed the subset of the sample from PDHS 2006-07 of babies whose mother were living in the Punjab and found that smaller than average birth size and delivery complications were significantly associated with higher risk of neonatal mortality (data not shown). Hence, there is a need to conduct further research to examine factors associated with neonatal mortality in the Punjab.

Infants whose mothers were not working and father was employed had lower risk of mortality in neonatal period in the current study compared to both working. Involvement of mothers in work outside home may adversely affect the care provided to the newborn. Others have reported an increased odds of neonatal deaths due to lack of personal and timely care including infrequent breastfeeding experienced by infants born to working mothers [44]. However, parental employment status in PDHS only showed working during the last 12 months preceding the survey.

Program implications

The current study findings have implications for the maternal and newborn health programs in Pakistan. To achieve national MDG-4 target, it is important to reduce neonatal deaths substantially as 57% of under-five child deaths are in neonatal period in Pakistan [15]. In the light of the current analyses, there is a need to implement cost effective interventions through community based trials to see their effectiveness in the Pakistani context. However, at the same time, further research in various regions of Pakistan is required to indentify the obstacles, such as socio-cultural issues, practices etc., for poor newborn health and survival.

Conclusions

In conclusion, there is a need to formulate antenatal interventions to educate pregnant women, especially those who are becoming mothers for the first time, a timely referral system, and retraining of healthcare providers to mange delivery complications and antenatal care programs such as IFA supplementation to reduce the risk of having smaller than birth size babies. The impact of these interventions should be tested through community based trials in various settings of Pakistan.

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