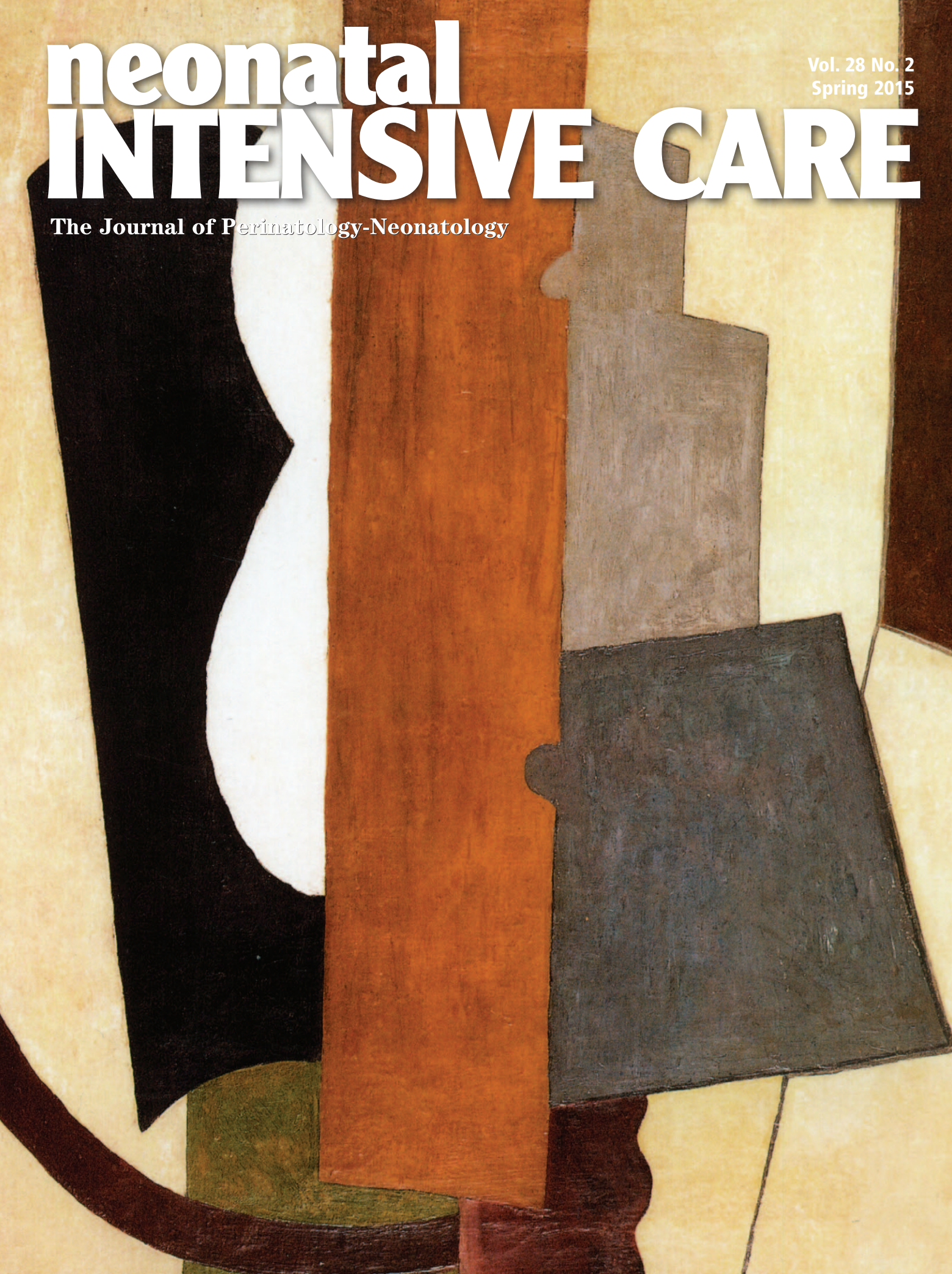
Vol. 28 No. 2
Spring 2015

neonatal INTENSIVE CARE

The Journal of Perinatology-Neonatology



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

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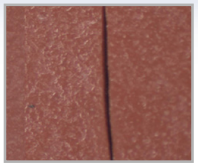

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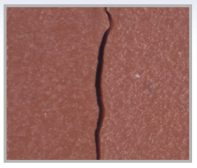

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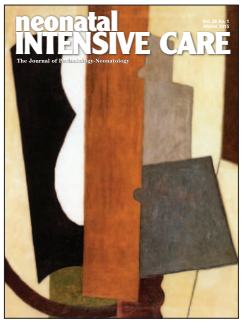

neonatal
INTENSIVE CARE

Vol. 28 No. 2
Spring 2015

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DEPARTMENTS

ARTICLES

Editorial Advisory Board

In term and near-term neonates with hypoxic respiratory failure (HRF)...

When do you stop the cascade?

Early intervention with INOMAX® (nitric oxide) for inhalation upon confirmation of pulmonary hypertension may help:

- Avoid higher levels of supplemental oxygen
- Improve oxygenation[1]
- Potentially prevent the progression of HRF[2]

Learn more at www.inomax.com

Indication

INOMAX® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery with validated ventilation systems.

Important Safety Information

- INOMAX is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.
- Abrupt discontinuation of INOMAX may lead to increasing pulmonary artery pressure and worsening oxygenation even in neonates with no apparent response to nitric oxide for inhalation.
- Methemoglobinemia and NO_2 levels are dose dependent. Nitric oxide donor compounds may have an additive effect with INOMAX on the risk of developing methemoglobinemia. Nitrogen dioxide may cause airway inflammation and damage to lung tissues.
- In patients with pre-existing left ventricular dysfunction, INOMAX may increase pulmonary capillary wedge pressure leading to pulmonary edema.
- Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOMAX administration.
- Use only with an INOmax DS_{IR}®, INOmax® DS, or INOvent® operated by trained personnel.

Please see Brief Summary of Prescribing Information on adjacent page.

INOmax® (nitric oxide) FOR INHALATION

References: 1. INOMAX [package insert]. Hampton, NJ: Ikaria, Inc.; 2013. **2.** González A, Fabres J, D'Apremont I, et al. Randomized controlled trial of early compared with delayed use of inhaled nitric oxide in newborns with a moderate respiratory failure and pulmonary hypertension. *J Perinatol*. 2010;30(6):420-424.

IKARIA® ADVANCING CRITICAL CARE

INOMAX® (nitric oxide) for inhalation
Brief Summary of Prescribing Information

INDICATIONS AND USAGE

Treatment of Hypoxic Respiratory Failure
INOMAX® is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation.

Utilize additional therapies to maximize oxygen delivery with validated ventilation systems. In patients with collapsed alveoli, additional therapies might include surfactant and high-frequency oscillatory ventilation.

The safety and effectiveness of INOMAX have been established in a population receiving other therapies for hypoxic respiratory failure, including vasodilators, intravenous fluids, bicarbonate therapy, and mechanical ventilation. Different dose regimens for nitric oxide were used in the clinical studies.

Monitor for PaO_2, methemoglobin, and inspired NO_2 during INOMAX administration.

CONTRAINDICATIONS
INOMAX is contraindicated in the treatment of neonates known to be dependent on right-to-left shunting of blood.

WARNINGS AND PRECAUTIONS

Rebound Pulmonary Hypertension Syndrome following Abrupt Discontinuation
Wean from INOMAX. Abrupt discontinuation of INOMAX may lead to worsening oxygenation and increasing pulmonary artery pressure, i.e., Rebound Pulmonary Hypertension Syndrome. Signs and symptoms of Rebound Pulmonary Hypertension Syndrome include hypoxemia, systemic hypotension, bradycardia, and decreased cardiac output. If Rebound Pulmonary Hypertension occurs, reinstate INOMAX therapy immediately.

Hypoxemia from Methemoglobinemia
Nitric oxide combines with hemoglobin to form methemoglobin, which does not transport oxygen. Methemoglobin levels increase with the dose of INOMAX; it can take 8 hours or more before steady-state methemoglobin levels are attained. Monitor methemoglobin and adjust the dose of INOMAX to optimize oxygenation.

If methemoglobin levels do not resolve with decrease in dose or discontinuation of INOMAX, additional therapy may be warranted to treat methemoglobinemia.

Airway Injury from Nitrogen Dioxide
Nitrogen dioxide (NO_2) forms in gas mixtures containing NO and O_2. Nitrogen dioxide may cause airway inflammation and damage to lung tissues. If the concentration of NO_2 in the breathing circuit exceeds 0.5 ppm, decrease the dose of INOMAX.

If there is an unexpected change in NO_2 concentration, when measured in the breathing circuit, then the delivery system should be assessed in accordance with the Nitric Oxide Delivery System O&M Manual troubleshooting section, and the NO_2 analyzer should be recalibrated. The dose of INOMAX and/or FiO_2 should be adjusted as appropriate.

Heart Failure
Patients with left ventricular dysfunction treated with INOMAX may experience pulmonary edema, increased pulmonary capillary wedge pressure, worsening of left ventricular dysfunction, systemic hypotension, bradycardia and cardiac arrest. Discontinue INOMAX while providing symptomatic care.

ADVERSE REACTIONS
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from the clinical studies does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

Controlled studies have included 325 patients on INOMAX doses of 5 to 80 ppm and 251 patients on placebo. Total mortality in the pooled trials was 11% on placebo and 9% on INOMAX, a result adequate to exclude INOMAX mortality being more than 40% worse than placebo.

In both the NINOS and CINRGI studies, the duration of hospitalization was similar in INOMAX and placebo-treated groups.

From all controlled studies, at least 6 months of follow-up is available for 278 patients who received INOMAX and 212 patients who received placebo. Among these patients, there was no evidence of an adverse effect of treatment on the need for rehospitalization, special medical services, pulmonary disease, or neurological sequelae.

In the NINOS study, treatment groups were similar with respect to the incidence and severity of intracranial hemorrhage, Grade IV hemorrhage, periventricular leukomalacia, cerebral infarction, seizures requiring anticonvulsant therapy, pulmonary hemorrhage, or gastrointestinal hemorrhage.

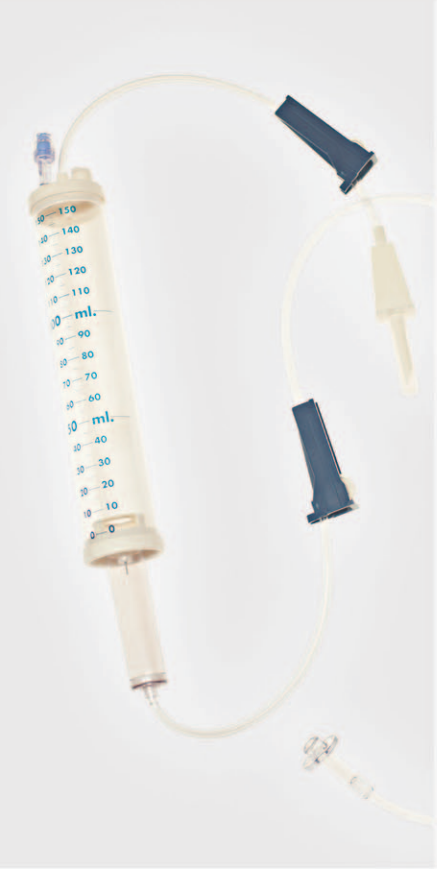
In CINRGI, the only adverse reaction (>2% higher incidence on INOMAX than on placebo) was hypotension (14% vs. 11%).

Based upon post-marketing experience, accidental exposure to nitric oxide for inhalation in hospital staff has been associated with chest discomfort, dizziness, dry throat, dyspnea, and headache.

OVERDOSAGE
Overdosage with INOMAX will be manifest by elevations in methemoglobin and pulmonary toxicities associated with inspired NO_2. Elevated NO_2 may cause acute lung injury. Elevations in methemoglobin reduce the oxygen delivery capacity of the circulation. In clinical studies, NO_2 levels >3 ppm or methemoglobin levels >7% were treated by reducing the dose of, or discontinuing, INOMAX.

Methemoglobinemia that does not resolve after reduction or discontinuation of therapy can be treated with intravenous vitamin C, intravenous methylene blue, or blood transfusion, based upon the clinical situation.

DRUG INTERACTIONS
No formal drug-interaction studies have been performed, and a clinically significant interaction with other medications used in the treatment of hypoxic respiratory failure cannot be excluded based on the available data. INOMAX has been administered with dopamine, dobutamine, steroids, surfactant, and high-frequency ventilation. Although there are no study data to evaluate the possibility, nitric oxide donor compounds, including sodium nitroprusside and nitroglycerin, may have an additive effect with INOMAX on the risk of developing methemoglobinemia. An association between prilocaine and an increased risk of methemoglobinemia, particularly in infants, has specifically been described in a literature case report. This risk is present whether the drugs are administered as oral, parenteral, or topical formulations.



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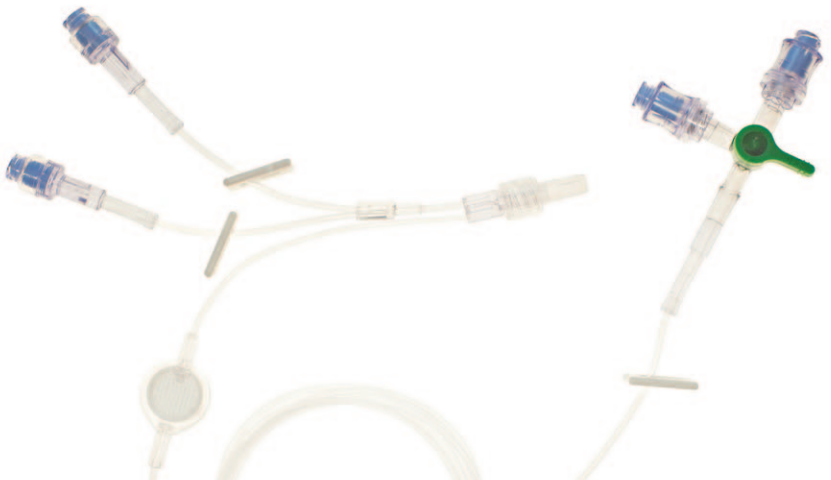

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In Response to: 'Quality and Safety Indicators for Human Milk and Donor Milk Use in the NICU'

Dear Editor: Given the importance of donor human milk to the health of very low birth weight and other fragile infants and the track record of HMBANA member mothers' milk banks in meeting these needs, I am responding to the advertorial placed on pages 28 and 29 in the Winter 2015 issue of Neonatal Intensive Care (Vol. 28, No. 1).

HMBANA values clinical excellence, ethical practice, community education, and research for the purpose of ensuring that all individuals with a medical need for it have access to pasteurized donor human milk.

The three areas in the advertorial I wish to address are safety, supply, and legislation and regulation. As I begin each section, I will cite specific references from the advertorial which I will subsequently address.

Safety

"In the desperate race to procure human milk, the question of quality and safety is not given sufficient scrutiny."

"Given the lack of consistent quality and safety standards, hospitals must begin to demand transparency and the detailed information needed to qualify vendors of donor milk products, regardless of whether they are commercial processors or tax exempt community milk banks."

Related to the issues of quality and safety standards, HMBANA's Guidelines1 are recognized by the FDA as safe for donor human milk. These Guidelines are updated annually based upon advice from the AABB, Canadian Blood Services, CDC, Health Canada, and FDA, as well as published clinical studies.

HMBANA's Guidelines address three layers of recipient protection from disease transmission. First, donors are screened for medical and lifestyle risk factors, and serum screened for HIV, HTLV, syphilis, and Hepatitis B and C. Then, milk is pasteurized, a process that kills HIV, cytomegalovirus, as well as other viruses and bacteria. Lastly, no milk is dispensed after pasteurization until a culture is negative for bacteriological growth.

Analogous to hospital practices, member milk banks conduct an annual self-assessment prior to the visit of an external accreditor charged with reviewing compliance with these Guidelines.

HMBANA members are committed to growing the body of research regarding safe handling and storage of human milk and the benefits of human milk in clinical situations.

Supply

"The chronic shortage of donor milk has triggered widespread rationing to such a degree that many preterm babies are now being denied access because they don't fit into the narrow definition of babies who "qualify".

"In an attempt to maintain a steady supply, hospitals are now forced to place numerous small orders at multiple milk banks across the country. Even so, they often receive only a fraction of what they ordered."

HMBANA dispensed over 3.1 million ounces of pasteurized donor milk in in 2013, half a million ounces more than in 2012 and nearly one million more than 2.18 million ounces dispensed in 2011.

As a system of member mothers' milk banks, at any point where demand outpaces supply at any hospital receiving donor mothers' milk, members coordinate with each other to address the need. True to our vision, they work to meet the needs of the most vulnerable infants first without deviation from compliance with the HMBANA Guidelines.

In the US alone, the annual need for donor human milk for babies born weighing less than 1,500 grams is estimated to be 9 million ounces2.

HMBANA members dispense milk to all 50 U.S. states and several Canadian provinces. Membership now numbers 18 banks -3 in Canada and 15 in the U.S. -with the most recent additions of the Mothers' Milk Bank of Montana, in Missoula, and the Northwest Mothers' Milk Bank in Portland, Oregon.

We also have several banks in our "developing" stages, so our capacity to provide safe donor milk continues to grow.

Legislation and Regulation

"While they are unable to meet the growing demand, community milk banks are asking legislators to create new laws that could very well violate anti-trust rules. These new laws would attempt to prevent mothers from sharing their milk outside the tax exempt milk banking system while

refusing to acknowledge the legitimate role of commercial milk banks."

HMBANA members are committed to advocating for legislative and regulatory changes supporting breastfeeding.

HMBANA is a long-established leader in human milk banking and the HMBANA Guidelines for the Establishment and Operation of a Donor Human Milk Bank are referenced worldwide. Human milk service or selling milk products in North America is composed of the Internet sale and sharing of raw breast milk and the activities of non-profit milk banks and commercial human milk processing companies. We encourage health care providers, prospective milk donors and families seeking donor human milk to consider carefully the societal benefits, costs, and risks of these several approaches.

We stand on our record and our commitment to safety, science, and saving babies.

Respectfully,
John M. Honaman, CFRE Executive Director

HMBANA (the Human Milk Banking Association of North America) is a non-profit organization of nonprofit donor human milk banks committed to the highest standards in the provision of safely processed human milk to preterm and otherwise fragile infants. Established in 1985, HMBANA sets standards for and facilitates establishment and operation of mothers' milk banks in North America.

1 Human Milk Banking Association of North America. Guidelines for the Establishment and Operation of a Donor Human Milk Bank, 2013 Edition. Fort Worth, TX.
2 Human Milk Banking Association of North America. "How much is enough?", HMBANA Matters. 2008 March;5 (7). As cited in the Surgeon General's Call to Action to Support Breastfeeding, Office of the Surgeon General (US); Centers for Disease Control and Prevention (US); Office on Women's Health (US).Rockville (MD): Office of the Surgeon General (US); 2011.

In Response to John M. Honaman

Dear Editor: I am pleased HMBANA has responded to my article "Quality and Safety Indicators for Human Milk and Donor Milk Use in the NICU." Unfortunately, Mr. Honaman fails to address any of the real issues raised in the article, instead sidestepping the quality and safety indicators with the same rhetoric that has become standard fare for HMBANA. Readers may notice that the bulk of my article cited the questions that hospitals need to ask about their source of donor milk. I made no judgments about the relative safety of any particular source.[1] In his response, Mr. Honaman cites the history of the HMBANA guidelines and the involvement of several regulatory bodies, including the FDA in the development of them but those guidelines seem to have been subject to a change by the association at some point after they were issued in 1990 by removing pre-processing microbiological screening of donor milk as a requirement.[2]

Since the HMBANA guidelines are reviewed annually, "based on advice from the AABB, Canadian Blood Services, CDC, Health Canada, and the FDA, as well as published clinical studies", it begs the question as to when the pre-process microbiological screening was dropped and what was the clinical evidence used to justify the change. Was the change approved by all of those regulatory bodies cited by Mr. Honaman and by HMBANA? Additionally, where is the scientific evidence to support Mr. Honaman's claim that the pasteurization method used "kills HIV, cytomegalovirus as well as other viruses and bacteria?"

The publication cited in my article by Landers and Updegrove (the former Executive Director of HMBANA prior to Mr. Honaman) contradicts Mr. Honaman's claim as they state that Holder pasteurization does not eradicate the spores of b. cereus, a heat resistant pathogen that can also produce toxins. I reiterate their declaration and would encourage hospitals to insist on an answer to the following question, "What is the milk bank's strategy to reduce the risk of b. cereus contamination?" I also stand by my original statement that a negative culture after pasteurization is not sufficient to safeguard premature infants from potentially dangerous heat stable toxins resulting from high bacterial levels in raw milk prior to processing.

Human donor milk should be subject to the same scrutiny as other infant nutritional products used in the neonatal intensive care unit and I refer readers back to my original set of unanswered safety and quality questions particularly for use by hospital staff responsible for assessing standards of clinical care and risk management.

With only a fraction of preterm infants having access to donor milk at present, we all have much more to do to close the gap, including asking questions about how currently accepted milk banking practices and models affect those outcomes.

Elena Medo, Chairman and CEO
Medolac Laboratories, A Public Benefit Corporation

1 Readers may wish to revisit the study by Brownell, et al "Donor Human Milk Bank Data Collection in North America: An Assessment of Current Status and Future Needs" from J Hum Lact February 2014 vol. 30 no. 1, 47-53. Without making further comment, readers will find this article and the subsequent letters to the editor quite informative, especially in light of the current debate.
2 The "three layers" of protection cited in Mr. Honaman's HMBANA letter are: "First, donors are screened for medical and lifestyle risk factors and serum screened for HIV, cytomegalovirus, as well as other viruses and bacteria. Then, milk is pasteurized, a process that kills viruses and bacteria. Lastly, no milk is dispensed after pasteurization until a culture is negative for bacteriological growth." The layers of protection cited by Lois Arnold, PhD on page 208 of the federal transcript of her testimony at the FDA Pediatric Advisory Meeting on December 6, 2010 were different than those cited by Mr. Honaman. Dr Arnold served as the Executive Director of HMBANA for many years and stated that, "The first set of guidelines was published in 1990, with subsequent editions released on an annual or biannual basis. The guidelines instituted a triple level of protection for the consumer, with thorough screening by history as well as serology, bacteriological screening of the milk, and pasteurization of all milk prior to distribution. (emphasis added)."

News

☐ Spring 2015

Intelligent Ventilation During MRI Procedures

Hamilton Medical has unveiled its HAMILTON-MR1 ventilator, which allows medical professionals to take patients from the ICU to the MRI suite and back without having to change anything about the ventilation, even when they are on an advanced mode. This diminishes the risks for lung de-recruitment and a patient setback, which would keep patients in the hospital longer and make their stay more uncomfortable. The new Neonatal option now also optimizes the HAMILTON-MR1 for MR-compatible neonatal ventilation. With tidal volumes as low as 2 ml, safe, and lung-protective ventilation of even the most fragile patients is guaranteed. The intelligent leak compensation function IntelliTrig automatically adjusts the inspiratory and expiratory trigger sensitivity to potential leaks. This enables optimal synchronization with the neonate's breathing pattern. The small and rugged housing, an integrated high-performance turbine, and powerful internal batteries make the HAMILTON-MR1 ideal for patient transport and easy to handle for the caregiver. The wide range of modern and classic ventilation modes guarantees that before, during, and after the MRI procedure, all of your patients from neonates to adults receive the same high level of ventilation care as at the bedside. The HAMILTON-MR1 is the first ventilator able to be used at a magnetic field strength of 50 mT, equivalent to 1 m distance for a 3T static magnetic field scanner, without creating any MR image artifacts. Positioning a medical device too close to the MRI scanner can have fatal consequences. For maximum safety, the HAMILTON-MR1 continuously monitors the magnetic field with TeslaSpy, an integrated gaussmeter, and gives you an audible and visual signal if you are getting too close.

Positive Pregnancy Outcomes of Bariatric Surgery

Weight-loss surgery may help obese women have safer pregnancies and deliver healthier babies, compared with women who do not undergo surgery, according to a major new Swedish study through the Karolinska Institute. While the study found some risks for women who had surgery, including more babies born too small and a greater likelihood of stillbirths, experts said that overall the results were better. The findings have implications for an increasing number of women and children, especially in the United States, where nearly a third of women who become pregnant are obese. Obese women have more problems in pregnancy, including gestational diabetes, pre-eclampsia, and stillbirth. Their babies are more likely to be premature, overweight or underweight at birth, have certain birth defects, and develop childhood obesity. Researchers evaluated records of 2,832 obese women who gave birth between 2006 and 2011, comparing women who had bariatric surgery before becoming pregnant with women who did not. They found that women who had had surgery were about 30 percent as likely to develop gestational diabetes, which can lead to pre-eclampsia, low blood sugar, birth defects and miscarriage. They were about 40 percent as likely to have overly large babies, whose challenges can include lung and blood problems. The outcomes were worse in some categories. Women who had surgery were twice as likely to have babies who were small for their gestational age, suggesting the need for better nutrition for pregnant women with surgically-reduced stomachs. And more of their babies were stillborn or died within a month after birth, although the number of such deaths in each group was very small and might have been due to chance, experts and the authors said. There was no significant difference in rates of premature births or babies with birth defects.

Pioneering Research Presented

Chiesi USA, dedicated to assisting with the advancement of neonatal research, hosted a complimentary seminar to provide a glimpse of the latest developments from the company's

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ISSN 1062-2454
Published seven times each year by

Goldstein and Associates, Inc.

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innovative neonatology R&D hub as well as philanthropy efforts serving the neonatology community. The seminar was held during NEO: The Conference for Neonatology. The event focused on Chiesi USA's commitment to advancing the field of neonatology through its portfolio of pioneering treatments, education initiatives and partnerships with leading organizations that share a mission to provide the highest level of care to the youngest of patients. Highlights included a panel discussion with a question and answer session with Rangasamy Ramanathan, M.D., NICU medical director at LAC+USC Medical Center, Linda Storari, Head of Neonatal Drug Development for Chiesi Group, and Josh Franklin, Vice President of Strategy and Business Development with Chiesi USA. Chiesi's industry-leading neonatology platform was established through the clinical success of CUROSURF (poractant alfa), which is an FDA approved surfactant indicated for the rescue treatment of Respiratory Distress Syndrome (RDS) in premature infants. CUROSURF reduces mortality and pneumothoraces associated with RDS. Transient adverse effects seen with the administration of CUROSURF include bradycardia, hypotension, endotracheal tube blockage, and oxygen desaturation.

Partnership Inked

Children's National Health System has inked yet another partnership with a regional hospital: It will offer neonatal services at the 183-bed Sentara Northern Virginia Medical Center in Woodbridge. Children's National neonatologists will provide the two most basic levels of nursery coverage at Sentara's neonatal intensive care unit and connect those services to the more serious level of care in the NICU at Children's main campus in Northwest D.C., officials said in a statement. The hospital has similar neonatal affiliations with Holy Cross Hospital in Silver Spring and Mary Washington Hospital in Fredericksburg, Va. The partnership is one of many for Children's, which also works with the emergency department at United Medical Center, a fetal echo clinic at Holy Cross and a pediatric cardiology program at MedStar Georgetown University Hospital.

Tetanus Not Going Away Globally

A new study has revealed that despite progress in maternal and neonatal health; globally, an estimated 58,000 neonates and an unknown number of mothers die every year from tetanus. The study observed that as of June, 2014, 24 countries were still struggling to eliminate the disease. According to the study, maintenance of elimination needs ongoing vaccination programs and improved public health infrastructure. It said maternal and neonatal tetanus is still a substantial but preventable cause of mortality in many developing countries. The study indicated that a case fatality from these diseases remains high and treatment is limited by scarcity of resources and effective drug treatments. The study observed that the "Maternal and Neonatal Tetanus Elimination Initiative", launched by World Health Organization (WHO) and its partners, had made substantial progress in eliminating maternal and neonatal tetanus. It said sustained emphasis on improvement of vaccination coverage, birth hygiene, and surveillance, with specific approaches in high-risk areas, had meant that the incidence of the disease continues to fall. The Lancet is the world's leading independent general medical journal. The journal's coverage is international in focus and extends to all aspects of human health. According to the WHO in many countries, deliveries take place in unhygienic circumstances, putting mothers and their newborn babies at risk for a variety of life-threatening infections. It said maternal and neonatal tetanus had been among the most common lethal consequences of unclean deliveries and umbilical cord care practices. WHO said when tetanus develops; mortality rates are extremely high, especially when appropriate medical care is not available. In 1988, WHO estimated that 787,000 newborns died of neonatal tetanus; thus, in the late 1980s, the estimated annual global neonatal tetanus mortality rate was approximately 6.7 neonatal tetanus deaths per 1000 live births - clearly a substantial public health problem. WHO estimates that in 2013, 49,000 newborns died from neonatal tetanus, a 94 per cent reduction from the situation in the late 1980s.

Genome Sequencing

Doctors expect soon to begin sequencing the genomes of healthy newborn babies as part of a government-funded research program that could have wide implications for genetic science. The research, to be conducted at major hospitals around the country, stems from a growing recognition that genome sequencing could someday be part of routine testing done on every baby. Such testing could provide doctors and parents a vast pool of data likely to reveal a wider range of potential medical risks than the traditional heel-prick test, in which a small sample of newborns' blood is taken to check for more than two dozen possible conditions. Genome sequencing of infants also someday could provide people with a genetic blueprint to carry through life. The data could be used years later to help develop personalized medical treatment, such as choosing the most effective asthma medication. Early identification of diseases can save a child's life or lead to interventions that change the course of the disorder. Whole genome sequencing or whole exome sequencing, which focuses on the 1% to 2% of the genome believed to be responsible for most genetic disorders,

can help identify mutations associated with some diseases. Some hospitals already perform sequencing on a small number of newborns who show signs of illness or developmental disorders. Those experiences so far suggest the procedure can help doctors identify the underlying problems.

Hospital Testing Google Glass

Boston's Brigham and Women's Hospital is testing out Google Glass technology to help parents of pre-term babies during a difficult time. The new program, Love at First Sight, utilizes Google Glass to help mothers of the smallest babies bond with the child even when they're separated by required medical equipment or protocols. The program took months to get regulatory approval after analyzing software providers and developing the product for neonatal care. It was adapted for hospital use by the start-up Pristine. The medical team wants to learn if video conferencing makes a difference to recovering moms. The hospital already has an 18-month long experience using iPads and FaceTime to allow conferencing. But the new Google Glass study, allowing interaction between mom, medical team and infant could be different. It should allow new mothers to see the infant and treatment from a first-person perspective. Software developed by Pristine allows all information to remain private.

Birth Defect Fears

Federal health authorities are reporting that nearly one-third of women of reproductive age had had an opioid painkiller prescription filled every year from 2008 to 2012. Experts said the practice carried considerable risks for birth defects. The Centers for Disease Control and Prevention analyzed health insurance claims data from Medicaid and private insurers for women ages 15 to 44 and found that an average of 39 percent of women on Medicaid filled an opioid prescription in a pharmacy each year from 2008 to 2012, compared with 28 percent of women with private insurance.Dr. Thomas R. Frieden, director of the C.D.C., described the numbers as "astonishing" and said they presented a substantial risk for birth defects. Women often do not know they are pregnant in the early weeks after conception, a critical time for organ formation, and could be "unknowingly exposing their unborn child" while taking the drugs.

Mothers' Sounds Studied

Researchers at Brigham and Women's Hospital in Boston studied 40 babies born eight to 15 weeks prematurely to demonstrate that the brain itself may rely on a mother's voice and heartbeat to grow. Like most severely premature babies, the infants were confined to incubators and spent limited time with their mothers. Using tiny speakers placed inside the incubators, half the babies were exposed to the sounds of their mothers' voices and heartbeats for three extra hours every day. The other half received no additional exposure to such sounds. After 30 days, babies in the first group had developed a significantly larger auditory cortex — the hearing center of the brain — than those in the second group. The findings, published in Proceedings of the National Academy of Sciences, could help guide doctors and parents caring for premature babies, who often suffer from developmental and cognitive disabilities.

Neonatal Care Booklet launched

A new publication designed to support parents during their baby's time in neonatal care has been launched. The booklet, which is now available at all neonatal units in Scotland, is intended to provide helpful information support and advice for carers, siblings and grandparents, and focuses on a parent's perspective. The South East and Tayside Managed Clinical Network for Neonatal Services established a parent group in 2012 to help develop the booklet based on their own experiences. Input also came from healthcare staff and the charity Bliss. The Scottish Government has now funded the publication and distribution to all neonatal units in Scotland.

Genetic Testing for Pregnant Women

Women expecting a baby or planning a pregnancy are being pitched a fast-growing array of tests to check if they are carriers for hundreds of mostly rare genetic diseases. Such genetic testing, called carrier screening, has long been targeted mainly at people of certain ethnic groups such as Ashkenazi Jews, who are at higher risk for some conditions such as Tay-Sachs disease. Now, companies that offer carrier screening are promoting the idea that testing everyone for many diseases is a more effective way to reduce the number of babies born with serious disorders, including cystic fibrosis, a life-limiting lung condition, and Canavan disease, a fatal neurological disorder. Scientists keep identifying new gene mutations, or variations, associated with specific diseases. Advances in DNA technology allow companies to quickly screen large numbers of people, using saliva or blood samples, to determine if parents could pass the genetic variations to their children. Counsyl offers tests that aim to detect heightened genetic risk for at least 98 different diseases, for between $599 and $999. Another company, Gene by Gene Ltd., of Houston, plans in the next few months to introduce First Look, a test billed as the most comprehensive on the market that can screen for more than 300 diseases. The company expects the price could be close to $1,500.

New Tech Personalizes Nutritional Needs

Several new technologies being used in the Cedars-Sinai Neonatal Intensive Care Unit, part of the Maxine Dunitz Children's Health Center, are helping our smallest babies with more rapid and healthier weight gain. Doctors have begun routinely using a device known as the Pea Pod to measure the body composition of the infants. The Pea Pod looks like a mini MRI machine. It is heated, and the baby is placed inside for approximately three minutes. Using an air displacement method, the machine senses change in pressure and can determine the percentage of body weight that is fat and the percentage that is lean body mass. With this information health care workers can then personalize the baby's nutritional supplements to help with appropriate weight gain. Charles Simmons, MD, chair of the Department of Pediatrics and director of the Division of Neonatology, says, "the Pea Pod is important in helping the NICU team facilitate a healthy weight gain in the smallest infants by calculating the amount of lean mass and body fat in the infant on a daily or weekly basis." At the same time, Cedars-Sinai is continuing a study of breast milk composition, using a device that analyzes the percentages of fat, protein and carbohydrates in breast milk. To date, health care workers have performed hundreds of analyses of breast milk.

Human Placenta Project Gets Funding

The National Institutes of Health has dedicated $41.5 million for an initiative to understand and monitor the development of the human placenta during pregnancy. The funding will support the development of new technologies to assess the health of the placenta as it grows and matures, with the ultimate goal of improving the health of mothers and children. The placenta is a temporary organ that ferries oxygen and nutrients from the

mother to her fetus while at the same time removing potentially toxic substances like carbon dioxide. It also produces hormones to help maintain pregnancy and perform the unique immunologic function of allowing the mother and fetus to coexist. Problems with the placenta may lead to negative pregnancy outcomes for mother or fetus, such as preeclampsia (a disorder of high blood pressure in pregnancy), gestational diabetes, preterm birth, and stillbirth. Placental problems have also been linked to a higher risk of heart disease later in life, for both mother and child. Until now, most studies of the placenta have been limited to ultrasound exams, blood tests, and the examination of placental tissue after delivery. The initiative seeks to spur new technologies or innovative applications of existing technologies, such as imaging tools or sensors, that would allow practitioners to safely track placental functioning during pregnancy. Such technologies might gauge how blood and oxygen flow through the placenta, how it attaches to the uterine wall, and how it conveys nutrients to the fetus. The latest funding announcement for the Human Placenta Project, its third and largest to date, also requires applicants to address the effects of environmental factors — such as air pollution, medications, and maternal diet — on the placenta during pregnancy.

Treatment for Neonatal Abstinence Syndrome

In the past decade, the number of Kentucky babies starting life with a drug dependency, or neonatal abstinence syndrome (NAS), has skyrocketed from 1.3 per 1,000 births to 19 per 1,000 births. Just like adults coming off drugs, babies whose mothers used opiate drugs during pregnancy, will suffer from a number of withdrawal symptoms, including tremors and irritability. The most common form of treatment for babies suffering from withdrawal is the opiate morphine, which can hinder brain development during a critical growth period in a baby's life. The treatment period for infants requires hospitalization and can last weeks or even months, resulting in high hospitalization costs. Dr Henrietta Bada, a neonatologist at Kentucky Children's Hospital, has conducted preliminary research supporting an alternative drug to morphine that will help babies recover from NAS faster and with fewer neurological effects. Bada recently published findings from a pilot study determining whether clonidine, a non-opiate, non-addictive drug commonly used to treat hypertension, would result in improved neurobehavioral performance in babies when compared with morphine, an opiate. The research, which was published in the February 2015 issue of the journal Pediatrics, presents encouraging evidence that clonidine was as effective as morphine.

Premature Babies With Asthma Outgrow It

Parents of premature babies worry about many things, including an increased risk of asthma. But a large Danish study has found that asthma, common in premature babies, disappears as the children grow older. By the time they are adults, their risk of asthma is no greater than that of babies born full term. Researchers combed birth and health data on 1.8 million people born from 1980 to 2009, checking for gestational age and neonatal respiratory problems. The study found that 27 percent of infants born earlier than 27 weeks required asthma medication during childhood, compared with 18 percent of those born at 28-31 weeks, 13 percent at 32-36 weeks, and 9 percent at full term. But after controlling for socioeconomic status, maternal asthma, multiple birth and other factors, they found that by adolescence, the association had weakened, and by adulthood 2.4 percent

of the former preemies required medication compared with 2.1 percent of those born full term, a clinically insignificant difference.

Invictus Adds to Board

Invictus Medical, the San Antonio, Texas-based medical device company dedicated to providing newborns with healthy early developmental milestones, has appointed Dennis P. Kane to its Board of Directors. Kane brings more than 35 years of medical technology experience to the Invictus board. Kane's background includes expertise in the global sales and marketing of devices, pharmaceuticals and diagnostics, including global and US sales and marketing at Phadia AB (formerly Pharmacia Diagnostics). Responsible for building and expanding operations related to disease management and pharmacoeconomics, he was with Upjohn, Inc. prior to its merger with Pharmacia.

Lysosomal Dysfunction Studied

Neonatal intestinal disorders that prevent infants from getting the nutrients they need may be caused by defects in the lysosomal system that occur before weaning, according to a new Northwestern Medicine study. Lysosomes are cellular recycling centers responsible for breaking down all kinds of biological material. The study links lysosomal dysfunction with intestinal disorders for the first time, pointing to a previously unknown target for research and future therapies to help infants unable to absorb milk nutrients and gain weight, a diagnosis called failure to thrive.

Company Adds to Portfolio

News reports have confirmed that Mallinckrodt Plc has agreed to acquire Ikaria Inc. for about $2.3 billion from a group of investors led by Madison Dearborn Partners to expand in neonatal intensive care treatments. The acquisition improves Mallinckrodt's hospital business by adding to its current base of diagnostic radiology and pain management in surgical specialties, the company said in a statement on Thursday. Ikaria's top product, INOmax, treats premature infants with breathing difficulties. Chief Executive Officer Mark Trudeau has been using acquisitions to broaden Mallinckrodt's collection of health-care assets since the company was spun off from Covidien Plc in 2013. Mallinckrodt acquired pain-drug maker Cadence Pharmaceuticals Inc. last year for about $1.3 billion, then followed up by buying Questcor Pharmaceuticals Inc. for $5.6 billion.

Distributor Found for Thermoregulation System

International Biomedical, a medical device company based out of Austin, Texas and specializing in neonatal products, will serve as the distributor of the Tecotherm Neo thermoregulation system for transport incubators in the United States. The Tecotherm Neo is compatible with International Biomedical's Airborne infant transport incubators. The Tecotherm provides programmed hypothermia protocol, is baby servo controlled and provides mobile therapeutic hypothermia treatment.

Company Launches Gauge

Medical device company Respiralogics has introduced the GiO 1 Digital Pressure Gauge for real-time display of airway pressure. GiO provides a simple method for bedside pressure measurement in an easy to read digital display. There are no complicated buttons or display screens with the GiO. Start with two buttons and unlock the possibilities. The GiO Digital Pressure Gauge can be used in a wide range of applications and clinical settings. GiO is backlit for ease of reading in minimal light environments. Battery operated; the GiO has an auto power off feature to extend the life of the lithium battery. GiO 1, with pressure measurement between 0 to 30 cmH2O, is specifically designed for both non-invasive and invasive infant/neonatal applications. Portable and compact, GiO can accomplish your pressure monitoring needs. GiO is packaged complete with hydrophobic filters, pressure line, elbow connectors and straight luer connector. For additional GiO information, contact customerservice@respiralogics.com.

Making the Connection: Interventions to Help Nurses Promote Positive Parenting in the NICU

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When parents find themselves in the Neonatal Intensive Care Unit (NICU), they may not have had the time to educate themselves on breastfeeding. Parents may not have made the decision on how to feed their baby. Neonatal nurses have an immediate and lasting effect on the lives of their tiniest patients and families. We enter the lives of parents without invitation, but out of necessity. The words, actions, and interventions we share with parents and families can be helpful and healing, promote bonding, strong parent-infant ties, and best practices. Among these practices are breastfeeding and providing breast milk. The neonatal environment offers significant challenges to the parents of premature infants. Technology, noise, lack of privacy, and stress all have the potential to negatively impact parental health, wellbeing, and attachment. Nurses have little preparation for such encounters; time and experience are often our only teachers. Looking to evidence can help us to prepare our families, promote bonding, and guide discussions to make the best choices. The decision of how to provide nutrition to babies is often thought of as a personal choice which should not be driven by nursing staff. Science proves however, that human milk is the best for all babies; nursing education needs to be presented to help parents understand its importance. As the architect for parental education, bonding, and attachment, nurses must be prepared to support, with evidence as our backer, choices regarding infant nutrition and human milk.

Parents need assistance

The Neonatal Intensive Care Unit (NICU) proves a formidable opponent for most parents. Expectations of a healthy pregnancy and uncomplicated delivery are destroyed when families are faced with just the opposite. The NICU is a stressful and emotional environment; one that is technical and discomforting. Parents may feel vulnerable, unable to bond and attach to their infant. The NICU can prevent positive parent interactions (Hopwood, 2010). Parental separation from the child, along with maternal stress, can reduce successful attachment (Smuker, Brisch, & Kohntop, 2005). Parental stress and anxiety in the NICU has been measured and individualized to show reduced bonding and attachment with very premature infants. Some parents will still show the effects of stress endured in the NICU countless years later (Wigert, Johannson, Berg, & Hellstro, 2006).

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Many studies correlate NICU stress to altered parenting behavior, health changes, and long term emotional issues (Busse, Stromgren, Thorngate, & Thomas, 2013). Studies have also shown a correlation between poor psychological functioning, negative interactions between parent and baby, depression, and altered sleeping patterns in parents of NICU babies (Flacking, Lehtonen, Thompson, Axelin, Ahlqvist, Moran, Ewald, & Dykes, 2012). Choices regarding treatment, including how to provide nutrition for their infant, may be difficult to make for parents under such stress. The parents of extremely premature babies have often times not had the opportunity to investigate and educate themselves on feeding choices. When thrown into the situation of a premature delivery and hospitalization, pumping human milk may seem too much.

Nurses aren't prepared

Neonatal nurses have an abundance of knowledge and skills; answering medical and technological questions while attending to the treatment and needs of such critical patients is never a problem. But promoting best practice with regards to the use and promotion of human milk can be another story. In cultures such as the United States where breastfeeding is not the predominate choice, many nurses feel uncomfortable endorsing and supporting breastfeeding and the provision of breast milk as the absolute best (Mohrbacher, 2010). The standard question "breast or bottle" no longer has a place as science has proven the many benefits of human milk as well as the detriments of formula! When mothers state that they wish to formula feed their premature infant, many neonatal staff feel uncomfortable providing education and may feel that they have no ability to influence mom's decision.

Nurses may feel that educating parents about the benefits of human milk and creating a stance for exclusively providing it is coercion. They may feel that the parent's right to choose is so strong, that any information which might contradict the parent's understanding is "forcing" or "opinion" and therefore wrong. Many studies show just the opposite. With the evidence now backing the benefits of exclusive human milk, many mothers express disappointment and disbelief that nurses, pediatricians, and neonatologists are not endorsing exclusivity as best practice. A study which included 21 mothers who changed their decision to formula feed after hearing about the benefits of breast milk for their premature infants is a perfect example of the influence of educated, caring staff. These mothers of very low birth weight infants quickly changed their opinion of formula after hearing that human milk was a critical component in the positive

outcome and progress of their baby. Moms reported feeling empowered and educated to make an informed choice while denying any feelings of pressure (Miracle, Meier, & Bennett, 2004). Prenatal consultations with information regarding the advantages of mom's milk have also been shown to increase initial provision as well as length of human milk feedings, including feedings after discharge (Friedman, Flidel-Rimon, Lavie, & Shinwell, 2004). Fully informing mothers of the health benefits of human milk is an ethical and professional expectation of neonatal staff; nurses are encouraged to be involved, not passive, in their endorsement of breastfeeding exclusivity (DiGirolamo, Grummer-Strawn, & Fein, 2003) (Miracle, & Fredland, 2007).

Evidence to guide practice

Parental feelings of bonding and attachment can be influenced and affected by interactions with nursing staff. Feelings of inclusion and participation positively affect mother's bonding (Wigert, et al, 2006). Forming bonds in the NICU may not be an instinctive or easy process, but can be encouraged and nurtured by knowledgeable and involved nurses. Communication between parents and staff is a vital first connection which must be made to facilitate a relationship and trust. This relationship can help to ease familial stress and support bonding. Communication is not only a sharing of verbal information, but also the non-verbal cues and behavior of staff that interact with every member of the family as well as the baby (Wigert, Blom & Bry, 2014). Parents have individual responses and needs; previous experiences, health considerations, and perceived support all impact the attachment process. Nurses must become proficient at assessing families according to their needs and individual responses (Hopwood, 2010). Working in an interdisciplinary manner with Social Services and Neonatologists, a team approach can be made to improve parent's comfort and reduce anxiety.

Communication is so vital to making connections with staff who then guide attachment between baby and parents. In a study looking at parental feelings regarding their experiences with staff, relationships were described and categorized. Themes of staff/family relationships included: meeting a fellow human being, being included or excluded, and bearing unwanted responsibility. Parents felt that staff who took the time to notice when they needed to talk, reassured them, engaged them in conversation and chatting while calmly answering questions, met their needs and encouraged trust and security. Being included in baby's care helped parents to form a bond with the staff as well as the baby; encouraging them to stay at the bedside and in the unit longer, communicating and trusting more. Being excluded from cares and communication, being left out of unit rounding, and waiting for reports, caused a feeling of loneliness. These feelings isolated parents from the staff, the NICU, and ultimately their baby. Parents also noticed trends and differences in communication styles of staff and between disciplines. Urgent conversations with the doctor were associated with bad outcomes and news. Report results which took longer to occur or took place with a nurse were considered positive. Physician communication often required an "interpretation" by the nurse. These types of communication were felt by parents to require them to understand differences and take on a responsibility for understanding (Wigert, et al, 2014). Having to bear these responsibilities is a hardship on parents. Nurses who showed caring, warmth, genuine concern, empathy, and reassurance, were able to create supportive relationships with mothers and assist them with infant attachment according to several studies (Bialoskurski, Cox, & Wiggins, 2001) (Fenwick, Barclay, & Schmied, 2001). Nurses and staff are in a unique position to create effective, meaningful communication helping parents to process emotions and develop healthy bonds. Staff can assist with communication by individualizing messages for parents and really understanding needs to promote positive interactions.

The effects of touch and early social behavior in the NICU are directly related to positive bonding and attachment and have been reported on since the 1980's and 1990's. Eye contact, holding, and kissing, have all been correlated to deeper parental bonds (Hopwood, 2010). These very important promoters of attachment are often difficult to obtain in an environment full of technology, noises, and people. Coupled with the needs of critically sensitive patients, true moments for physical touch can be hard to come by. Babies are often thought to need quiet and decreased stimulation due to their unstable status. Current studies show the human touch to be desperately needed by both parent and child. Parent and child can build attachment through touch (Feldman, Elderman, Sirota, & Weller, 2002) (Weiss, Wilson, Hertenstein, & Campos, 2000). (Miles, Cowan, Goven, Stevenson, & Modi, 2005).

Kangaroo Mother Care (KMC) or Skin to Skin (STS) care is a highly studied and applied therapy which provides opportunities for mother and baby, a dyad in care, to coexist together. Physiologic effects of KMC are well studied and published. The maternal experience of KMC and the benefits for bonding with both parents, satisfaction and connection, and increased breastfeeding and provision of breastmilk are also noted. Reports of these results along with the continued practice of skin to skin care long after discharge are documented (Neu, 2004). A strong maternal sense of being needed and importance in their baby's life was a theme in one study which used 60 minutes of KMC three times a day. Moms were initially concerned and afraid to hold their tiny baby in this manner, but by the third encounter with support from staff, they felt competent and satisfied. Holding their baby during STS care gave a sense of connection, warmth, and joy to moms. By providing this care, moms felt that they were needed by nurses and provided a therapy that only mother could give (Johnson, 2007). Nurses can assist moms and dads with STS care by educating them about the benefits, and encouraging parents by telling them it is a good thing for their baby; one that only they can provide. Scheduling holding times and being available to parents is another positive action that nurses and NICU staff can provide. Creating a quiet, private spot with screens and by directing conversations and housekeeping to other areas in the absence of individual patient rooms, gives parents a comfortable environment to share with their baby (Johnson, 2007).

Neonatal nurses, neonatologists, and support staff should all familiarize themselves with the benefits of human milk and breastfeeding so that they can adequately educate and support parents when making decisions regarding breastfeeding and breastmilk. The American Academy of Pediatrics (AAP) states that the first postdelivery conversation between parents and physician should include the role of human milk in the infant's recovery as well as the urgent need to begin pumping (American Academy of Pediatrics, 2009). Mothers may not be aware of the benefits of human milk for their premature newborn and must be educated. Parents may know of the basic advantages of providing human milk, but the extended benefits of this milk which provides passive then active immunity, reduces

neonatal morbidities such as Retinopathy of Prematurity, Necrotizing Enterocolitis, and Chronic Lung Disease, and promotes neurodevelopmental outcomes may be completely new information (Manzoni, et al, 2013) (Meier, Engstrom, Patel, Jegier, & Bruns, 2010) (Sullivan, Schanler, Kim, et al, 2010) (Vohr, Poindexter, Dusik, McKinley, Higgins, Langer, & Poole, 2007).

Moms often base their decisions on the recommendations of nurses and health related benefits as presented by staff. It is the recommendation of AAP to stress protective properties of human milk and recommend that mothers provide breastmilk even if they do not wish to breastfeed (American Academy of Pediatrics, 2009). Messages from physicians and hospital staff are important factors in a mother's intent to breastfeed or provide breastmilk past the initial postpartum period. In one study, polling moms on the length of time they breastfed, their original intent to breastfeed, and the recommendation of both their doctors and staff members, correlations were made between concrete recommendations and length of breastfeeding. Nurse's neutral breastfeeding attitudes and messages resulted in a reduction in the provision of human milk (DiGirolamo, Grummer-Strawn, & Fein, 2003). The recommendation of physicians during prenatal consultations was associated with an increase in the provision of human milk (Friedman, Flidel-Rimon, Lavie, & Shinwell, 2004). Initial discussions of providing pumped milk to the premature infant should focus on the immediate need and positive outcomes. All discussions should be respectful, focusing on the need and the evidence; the same as providing support for any medical therapy or treatment (Rodriguez, Miracle, & Meier, 2005).

The AAP places responsibility for initiating and maintaining pumping on nursing staff. Pumping is to begin at a maximum within six hours of birth (American Academy of Pediatrics, 2009). This tight timeline is critical to initiating breast milk production and building the supply to keep up with the needs of a growing infant. The ability to provide the needed amounts of milk as the baby requires more is directly linked to pumping early and often. Using the Medela (McHenry, IL) Symphony® breast pump, Preemie+™ Pattern, which was created specifically for initiation of milk and Lactogenesis II in the pump dependent mom, is another very important factor which will help mom to produce higher quantities of milk (Meier, Engstrom, Janes, Jeiger, & Fabiola, 2012). The Preemie+™ Pattern was tested in pump dependent mothers who delivered at 34 weeks gestation or less and proved to bring the change from colostrum to plentiful milk more quickly (average 3-5 days) and also significantly more milk at 200-250 milliliters (mls) milk more per day (Meier, et al, 2012). In a very recent study at Children's Hospital of Pennsylvania (CHOP), pumping was initiated using the Preemie+™ technology in a cohort of term gestation congenital heart disease infants. These mothers were pump dependent as their infants, though term, were too weak and sick to feed at the breast. These moms pumped an average of five to six times a day with the Preemie+™ Pattern and yielded volumes that approached 500 mls per day by one week of pumping. This evidence now supports the use of this important initiation pattern in mothers of term infants (Torowicz, Seelhorst, Froh, & Spatz, 2015).The National Association of Neonatal Nurses (NANN) also supports evidence behind the initiation of milk and frequent pumping. Interventions from the NICU nurses are critical to ensure that mom is pumping enough milk during the first two weeks postdelivery to ensure adequate volumes of milk for baby at the time of delivery (NANN position statement #3046, 2009).

Neonatal nurses are influential in the facilitation of effective communication, family bonding, and the breastfeeding success of mothers. Nurses can create a positive environment in the NICU by blending knowledge and practice. Communication and promotion of human touch and skin to skin care can facilitate a warm and nurturing experience for parents and newborn. By understanding and actively educating moms on the science behind human milk, nurses can assist mothers in making evidence based decisions which affect the outcomes of their babies. No longer can nurses, advocates of health and reporters of research, hide behind neutrality. Nurses and health care providers hold an ethical responsibility to educate parents on the facts of human milk. The evidence and research supporting human milk as the ultimate best practice is plentiful and concrete. Nurses must present the evidence and facts to ensure best outcomes. In keeping with the pledge initiated by Florence Nightingale, nurses shall do all in their power to maintain and elevate the standard of the nursing profession. The standard of nursing practice is rooted in evidence which dictates behavior. NICU staff hold that responsibility, and as such, must do all in their power to promote excellence and share the science with the families who speak for our tiniest and most vulnerable patients.

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Analysis of the Influence of Processing on Human Milk's Macronutrient Concentrations

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Abstract

Human breast milk is the optimal source of nutrition for all infants but especially for those born prematurely. As stated by the American Academy of Pediatrics, breast milk, especially in neonatal intensive care units is the optimal food for infants. When a mother is not able to produce enough milk for her infant, the next best source of nutrition is donated human breast milk. However, human breast milk is not a sterile fluid and therefore can contain microorganisms that could be transferred to the infants who consume it. Because of this risk donor milk must be processed to ensure its safety, but with processing the milk's important nutritional components can be altered or removed entirely. The objective of this study was to assess the impact of the processing method on the macronutrients of human milk. During a 1-year period (2013-2014), more than 400 milk samples from individual donors were analyzed for fat, protein, lactose and energy density. Banked donor milk mean values (in weight/volume) were found to be 1.1% ± 0.04% for protein, 3.3% ± 0.03% for fat, 6.1% ± 0.6% for lactose, and mean total energy was 60 kcal/dL. Amino acids of pooled donor milk were also compared before and after processing. Our data shows that the processing only had a minor effect on the macronutrients of the donor milk. Additionally, the macronutrients were stable and remained constant over time.

Introduction

It is recommended by the American Academy of Pediatrics, and the World Health Organization (WHO) that mothers exclusively breastfeed from the time an infant is born to the first 6 months of life for all healthy women and infants, and to continue breastfeeding for up to 2 years and beyond (American Academy of Pediatrics, 2012; WHO United Nations Children's Fund (UNICEF), 2003). Breastfeeding exclusively for this long is not possible for all women. Only 27% of mothers of premature babies can produce enough milk for their infants (Ewaschuk et al., 2011). This leaves only two options to fulfil the required nutritional needs for the infant, one is formula and the other is donated human breast milk. Donor milk is preferable to formula because it contains the same important nutritional components including native human protein needed for the overall growth and development of the infants. Multiple studies have found that preterm infants have a lower risk of developing necrotizing enterocolitis (NEC) when consuming donor breast milk compared to consuming formula (Buescher, 1994; Lucas and Cole, 1990). In addition, Sullivan et al (2010) reported significantly lower rates of NEC for premature infants when exclusively fed with human milk that contained human milk-based fortifier compared with a mother's milk-based diet that includes bovine milk-based products.

Human milk banks across the world collect donated human breast milk to feed infants in need. Human breast milk is a highly nutritious liquid that is not only beneficial for infants but also provides an ideal growth medium for many microorganisms. Because of this, breast milk needs to be handled and stored properly otherwise microbial contaminates can be introduced. Microorganisms can be introduced to the donated milk from a variety of sources such as the mothers body, skin, breast-pump or milk containers (Landers and Updegrove, 2010; Lindemann et al., 2004). Therefore, the pasteurization or processing of donor milk is required to minimize the possibility of the transmission of infectious agents from the milk to the infants (Akinbi et al., 2010). Depending on the amount of time and temperature used, exposure to heat may alter or eliminate certain nutritional components which in turn could result in insufficient levels of major and minor nutrients needed to serve the specific nutritional needs of infants (Modi 2006; Tully et al., 2001). This is especially a concern for premature infants that have a heightened need for the many different important nutritional components found in human breast milk.

Given the current trend in increased use of donor breast milk as an alternative nutritional source to mothers own milk and the limited data regarding the effects of processing on the nutrient composition of the milk, we analyzed the nutritional components of pooled donor milk before and after processing. A range of 60 to 180 samples from individual donors were collected, pooled and processed per production lot. Each production lot consisted of 500 to 2,000 gallons of human milk. We compared four different production lots for nutritional components and three production lots for amino acid content before and after processing. The macronutrients were analyzed by 3rd party certified laboratories as well as our own lab at Medolac Laboratories. In addition, we compared the levels of major nutritional components found in our pooled donor's breast milk to the national populations pooled milk using previously reported levels in human milk.

Methods and Materials

Breast milk samples (50mL) were obtained from a partner

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milk bank, Mothers Milk CO-OP (Lake Oswego, Oregon). Milk was collected from approved donors who answered medical history questions and went through blood testing. The mothers were required to pass blood tests for specific diseases such as HIV1, 2, HTLV I, II, HBV, HCV, syphilis, Chagas and West Nile virus. The phlebotomy was performed by Labcorp and serology testing was done by the American Red Cross National Blood Testing Laboratories. Overall, donors were healthy before and after delivery and received negative results from the blood tests. Breast milk samples were stored in the donor's home freezer before being shipped overnight to our lab (Medolac Laboratories) in an insulated cooler. Informed consent was obtained from the participating mothers. For this study samples were collected during a one year period (2013-2014). The milk was collected from all states except for Alaska, Arkansas, California, Hawaii, Maryland and New York.

Microbiology Screen

All donor milk samples were screened for microorganisms including aerobic bacteria, *Staphylococcus aureus* and Enterobacteriaceae. Upon receiving, the milk samples were maintained at -20 °C. Donor milk samples were thawed in a water bath and diluted 200-fold with sterile water. A 1-mL aliquot of each diluted sample was plated on the 3M Petrifilm for testing (3M, St. Paul, MN). All plates were incubated according to the manufacturer's instructions. The colonies were counted and the results were expressed in colony-forming units per ml (CFU/ml). Milk samples within the suggested ranges were pooled and processed. The criteria includes: $<10^5$ CFU/ml for aerobic; $<10^4$ CFU/ml for Staphylococcus aureus and Enterobacteriaceae (UK National Institute for Health and Clinical Excellence). Once a donation had passed, the milk was pooled in a large tank that contained 60-180 different donations per production lot. A total of four production lots were analyzed for nutritional components as well as three production lots for amino acid content. The analysis was performed on samples before and after processing.

Nutrient Analysis

Major nutrients analysis

Approximately 20 ml of each sample was used to determine the nutrient content. A Calais Mid-range Infrared Milk Analyzer was used to analyze the milk and was previously calibrated for human milk measurements using the Kjeldahl method for protein, Chloramine-T for lactose and Mojonnier for fat. Energies derived from protein and lactose was determined by multiplying the number of grams of each component per 100 ml by a factor of 4 kcal/gram, and energies derived from fat was determined by multiplying the number of grams of fat component per 100 ml by a factor of 9 kcal/gram (Dewey et al., 1984).

Amino acid analysis

Amino acids were analyzed by AAA Service Laboratory (Damascus, Oregon) using a Hitachi ion-exchange high-pressure liquid chromatography amino acid analyzer with a post-column, ninhydrin derivatization instrument (Hitachi L8900, Hitachi High-Tech Trading Corp, Minato-ku, Japan).

Others

Samples were sent to IEH and Eurofins to be analyzed. Vitamin C was tested by AOAC 984.26 (IEH) or AOAC 967.22 method (Eurofins). Calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc were tested with modified EPA 6020 (IEH) or AOAC 965.17/AOAC 985.01 method (Eurofins). Iron and sodium were tested with the WRE 063 method. Phosphorus was tested with the AOAC 965.17 method. Cholesterol was tested using the WRE 053 method.

Statistical analysis

The effects of processing were analyzed through a paired t-test of matched samples collected before and after processing (Microsoft excel, Redmond, WA, USA).

Results and discussion

Nutrients were consistent among different production lots

Nutritional components of human breast milk are derived from three sources: synthesis in the lactocyte, diet, and maternal stores. It has been established that the composition of human milk varies significantly among different lactation periods, time of day, length of gestation, and dietary habits (Modi 2006; Sala-Vila et al., 2005; Yamawaki et al., 2005). To minimize variability, donated human breast milk of different production lots is routinely pooled together (Human Milk Banking Association of North America, 2005). The nutritional components were found to be consistent among the four different production lots tested (Table 1). The mean values for pooled milk samples was 1.1% for protein, 3.1% for fat, and 6.1% for lactose with the exception of production lot A, which had a higher reading for fat (4.2%) and a lower reading for lactose (4.5%). Production lot A also had a higher energy density (71 kcal/dL) which is attributed to the higher fat content. The other production lots had a mean energy density of around 60 kcal/dL (Table 1). The variation observed in production lot A could be due to the different analysis method used by IEH. Smilowitz et al (2014) reported that fat, protein and lactose in human milk was 3.2%, 1.0%, 6.2%, which is comparable to our observation. The current nutrient recommendation for preterm infants is 120 kcal with 3.5 to 4 g protein/day (American Academy of Pediatrics, 2009). Preterm infants are fed 150 ml/kg of breast milk, therefore the maximum protein intake would be 3.3 g/kg making fortifier a required supplement for preterm infants.

Vitamin C and other macronutrients in pooled donor milk samples were also analyzed. The macronutrients were consistent among samples except for potassium, which was about 10-fold higher in production lot B than what was observed in production lots C and D (Table 2). Though nutritional components in breast milk vary among mothers and among pumping sessions, there is consistency in nutritional concentrations when breast milk is combined into large pools.

Table 1: Major nutrient analysis of production lots before processing. Data is mean ± SD.

Samples	A_a	B_b	C_b	D_b
Protein %	1.2 ± 0.05	1.1 ± 0.04	1.1 ± 0.04	1.0 ± 0.04
Fat %	4.2 ± 0.04	3.3 ± 0.03	3.1 ± 0.03	2.7 ± 0.03
Lactose %	4.5 ± 0.5	6.4 ± 0.6	6.4 ± 0.6	5.9 ± 0.6
Energy kcal/dL	71	64	64	58

a Analyses were performed by IEH.
b Analyses were performed by Eurofins.

Nutrients were retained after processing

The medical community is interested in understanding the effect of processing on the nutritional composition of donated human breast milk, especially the possibility that nutrients could be altered or eliminated completely. However the major nutrients of pooled breast milk before and after processing were not

significantly different. The mean content for post-processing samples was 1.03% ± 0.03% for protein, 3.01% ± 0.26% for fat, and 6.20% ± 0.26% for lactose, compared to pre-processing samples of 1.06% ± 0.03% for protein, 3.07% ± 0.13% for fat, and 6.54% ± 0.15% for lactose (Table 3). Little to no difference was observed therefore our processing does not affect the major nutrients of human breast milk. On the contrary, a significant reduction in fat, energy content (Garcia-Lara et al., 2013), and protein (Akinbi et al., 2010; Koenig et al., 2005) of donor human breast milk was observed after Holder pasteurization.

Table 2: Macronutrient analysis of production lots before processing.

Samples	B	C	D
Vitamin C	<4.4 ppm	<4.4 ppm	<4.4 ppm
Calcium	0.03%	0.02%	0.02%
Copper	<1 ppm	<1 ppm	<1 ppm
Iron	<2 ppm	<2 ppm	<2 ppm
Magnesium	0.003%	0.003%	0.003%
Manganese	<0.5 ppm	<0.5 ppm	<0.5 ppm
Phosphorus	0.01%	0.01%	0.01%
Potassium	0.33%	0.04%	0.03%
Sodium	0.01%	0.01%	0.01%
Zinc	1 ppm	1 ppm	1 ppm
Cholesterol	0.01%	NA	0.01%
Saturated Fat	1.30%	NA	1.16%

Table 3: Nutritional comparison of three production lots between pre- and post-processing. Pre-processing samples are randomly picked from frozen samples, post-processing data are from Eurofins reports. Data is mean ± SD (n ≥ 3).

Samples	Fat %	Protein %	Lactose %	Energy kcal/dL
PRE	3.07 ± 0.13	1.06 ± 0.03	6.54 ± 0.15	57.67 ± 1.53
POST	3.01 ± 0.26	1.03 ± 0.03	6.20 ± 0.26	58.00 ± 0.00

The protein quality and quantity of human milk are an important factor for infant growth and development (Zhang et al., 2013) and the amino acid profile is recognized as an indicator of the overall protein quality (Raiten et al., 1998). Amino acids are required for protein synthesis facilitate the uptake of other nutrients and also enhance the infants' immune system against potential pathogenic bacteria, viruses and yeasts (Carratu, 2004; Ogechi and Irene, 2013). The complete characterization and quantification of proteins especially amino acids in human milk serves as an appropriate nutritional guide for understanding and defining an infant's protein and amino acid requirements. Therefore, amino acid levels in pooled samples of three production lots were measured before and after processing (Table 4). Minor increases was found in Alanine (0.24 ± 0.00 vs 0.27 ± 0.00; $p < 0.001$) and a decrease of 20% was found in Lysine, which is an important biological indicator of the nutritional value of milk (0.45 ± 0.01 vs 0.36 ± 0.01; $p < 0.001$). In a previous evaluation of amino acid stability during manipulation of human breast milk, Silverstre et al. (2006) observed a significant 30% decrease in lysine content after Holder Pasteurization. Overall, our processing only had a minor effect on the total amino acid levels in pooled donor breast milk (Table 4). Plasma glutamine levels fall during critical illness or following major surgery and glutamine deficiency may limit tissue recovery in these situations (Newsholme, 2001). The retention of glutamate from our processing enables infants who have severe gastrointestinal disease or who are recovering from major gastrointestinal surgery, which is commonly seen among preterm babies, to receive sufficient glutamine to meet demands.

A previous study has evaluated the effect of Holder pasteurization (62.5°C for 30 minutes) on free amino acid content in pooled donor breast milk (Valentine et al., 2010). There were significant increases in arginine and leucine, significant decreases in aspartate and no significant difference in lysine after pasteurization. To our knowledge, no data has been published on the effect of Holder pasteurization or the processing of human milk on total amino acid profile.

Table 4: Amino acid concentration of three production lots before and after processing. Data is mean ± SD (n = 3).

Amino acid (mg/ml)	PRE	POST
ALA (A)	**0.24 ± 0.00**	**0.27 ± 0.00**
ARG (R)	0.21 ± 0.00	0.20 ± 0.00
ASP (D)	0.68 ± 0.01	0.68 ± 0.02
GLU (E)	1.09 ± 0.03	1.32 ± 0.09
GLY (G)	0.16 ± 0.00	0.17 ± 0.01
HIS (H)	0.16 ± 0.00	0.15 ± 0.00
ILE (I)	0.38 ± 0.01	0.39 ± 0.01
LEU (L)	0.92 ± 0.03	0.99 ± 0.04
LYS (K)	**0.45 ± 0.01**	**0.36 ± 0.01**
MET (M)	0.10 ± 0.00	0.11 ± 0.00
PHE (F)	0.31 ± 0.01	0.29 ± 0.02
PRO (P)	0.60 ± 0.01	0.66 ± 0.01
SER (S)	0.32 ± 0.00	0.34 ± 0.00
THR (T)	0.32 ± 0.01	0.34 ± 0.00
TYR (Y)	0.25 ± 0.01	0.25 ± 0.01
VAL (V)	0.39 ± 0.01	0.41 ± 0.01

Significant differences between pre- and post-processing samples were labeled in bold. A p value ≤ 0.001 was considered significant.

Table 5: Nutritional comparisons between original processed samples and samples been stored at room temperature for 5 to 7 months. Data is mean ± SD (n = 4).

Samples	Protein %	Fat %	Lactose %	Energy kcal/dL
Processed samples	1.04 ± 0.00	3.08 ± 0.00	6.19 ± 0.00	58.00 ± 0.82
5-7 months later	1.06 ± 0.00	3.03 ± 0.00	6.51 ± 0.00	57.75 ± 2.87

Nutrients were stable over time

There is a concern about the milks stability over time. Nutritional composition of four different production lots was analyzed after production and after being stored at room temperature for 5 to 7 months. Protein and fat were both stable over time and a slight increase in lactose was observed (Table 5). Nutritional composition of the processed pooled donor milk is not affected by storage at room temperature and is ready for use when needed. To our knowledge, our data demonstrate that our product is the only room temperature stable donated human breast milk in the world. Traditionally, donor breast milk is stored frozen and must be defrosted to use, which allows more time for microorganisms to grow and results in a delay before feeding. Room temperature stable milk is more convenient for the NICU since no defrosting is needed.

Conclusion

Human breast milk has the optimal levels of many different nutrients for neonates although addition of protein fortification is needed for preterm babies weighing less than 1,500 g, as recommended by the American Academy of Pediatrics. Mother's own milk remains the optimum feeding choice, followed by donor breast milk and as a last resort, infant formula (Akinbi et al., 2010). Breast milk from individual mothers differs in composition with each pumping and among different mothers, but the nutritional components of multiple lots of pooled breast milk showed consistency over time. Although concerns have been expressed that processing human breast milk could potentially affect or alter nutritional components of the breast milk, our results show that little to no change occurs after processing. In addition, the concentrations of amino acids in breast milk is consistent before and after processing, with one exception, lysine, which was decreased by 20%.

Though mother's own milk is the best nutritional source for nutrition for infants, there is consensus in the medical community that donor human breast milk is a better alternative than formula. Our pasteurization and processing method does not affect the nutritional composition of the milk. Donor breast milk provides the necessary nutritional food that is needed for infants, although protein and manual fortification is required for very low birth weight preterm infants.

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Clinical Data Needs in the Neonatal Intensive Care Unit Electronic Medical Record

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Abstract

Background: The amount of clinical information that providers encounter daily creates an environment for information overload and medical error. To create a more efficient EMR human-computer interface, we aimed to understand clinical information needs among NICU providers.

Methods: A web-based survey to evaluate 98 data items was created and distributed to NICU providers. Participants were asked to rate the importance of each data item in helping them make routine clinical decisions in the NICU. Results: There were 23 responses (92% – response rate) with participants distributed among four clinical roles. The top 5 items with the highest mean score were daily weight, pH, pCO2, FiO2, and blood culture results. When compared by clinical role groupings, supervisory physicians gave individual data item ratings at the extremes of the scale when compared to providers more responsible for the daily clinical care of NICU patients.

Conclusion: NICU providers demonstrate a need for large amounts of EMR data to help guide clinical decision making with differences found when comparing by clinical role. When creating an EMR interface in the NICU there may be a need to offer options for varying degrees of viewable data densities depending on clinical role.

Background

The combination of continuous monitoring and the ability of the electronic medical record (EMR) to store large amounts of data creates a potential for information overload in the intensive care (ICU) setting [1]. The potential dangers underlying this information overload relate to the inability of practitioners to discern pertinent from irrelevant information [2] and the accumulation of errors of cognition and performance associated with data corruption [3,4].

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A possible way to combat the risks of information overload may center on the development and implementation of advanced health information technologies (HITs). The Institute of Medicine and the United States Department of Health and Human Services have both advocated for the enhanced creation and use of efficient EMRs [5]. Recently, the HIT for Economic and Clinical Health (HITECH) Act was enacted which has allocated federal funds to aid in this endeavor [6].

Previous EMR implementation experiences in academic institutions have been met with conflicting outcomes. Although most institutions have demonstrated improved outcomes, increased productivity, and fewer errors [3,7-10] associated with EMR use, there is still guarded optimism on how best to design and integrate EMRs into clinical practice [11].

The development of a novel EMR human-computer interface, Ambient Warning and Response Evaluation (AWARE), at our institution resulted in improved performance and decreased errors of cognition in the adult ICU setting when compared to the standard EMR system [3]. The creation of this specific interface was based on expert panel input and data utilization models designed to assess the specific information needs of the unit [12,13]. This design methodology is in contrast to the vendor-generated platforms most commonly used in hospital EMRs [14]. The critically ill pediatric population is a unique group with specific medical information needs [15,16]. Additionally, there are special considerations that need to be taken into account when creating EMR interfaces for use specifically in the neonatal intensive care unit (NICU) [17]. As a result there have been standards suggested to help guide the creation of pediatric and neonatal specific EMRs [18,19]. The creation of these guidelines underscore the importance of creating efficient EMR human-computer interfaces, such as AWARE, to help decrease medical errors and improve clinical care.

In order to create an end-user designed patient-centered EMR interface we endeavored to better understand the exact clinical information needs of NICU practitioners. In the present study we address this gap in knowledge by a survey to determine what data NICU providers find useful in clinical decision making for inclusion into future EMR human-computer interfaces.

Methods

Study design

A web-based survey was conducted at Mayo Clinic, Rochester, MN, an academic tertiary health care center, equipped with a

comprehensive EMR. The Mayo Clinic NICU has 26 level III beds and admits approximately 350 infants per year. The survey was conducted among NICU providers of varying clinical roles. The study was approved by the Institutional Review Board (IRB) at Mayo Clinic. The study was deemed exempt from consent requirements by the IRB (13–003930).

Study subjects

Twenty-five subjects for the survey were selected from 4 clinical role designations; attending physicians (AP), neonatal fellows (NF), neonatal nurse practitioners (NP), and pediatric residents (PR). The AP group consisted of staff neonatologists and 1 neonatal hospitalist. NPs with significant clinical duty commitments (>50% of shifts occurring in the level III setting) were invited to participate. The PR group consisted of senior pediatric residents with NICU experience within the last year.

Data collection

Instruments

An expert panel, consisting of 2 APs and 1 NF, reviewed our current EMR and identified 98 unique data items that are available to clinical users. Using a 7-point Likert scale [ranging from not needed (0) to absolutely necessary (6)] subjects were asked to rate each of the 98 data items according to their opinion as to its importance in helping make routine clinical decisions in the NICU. Of note, only a0(not needed)or 6 (absolutely necessary) score was given a categorical description. Scores between these extremes (1–5) were exclusively numerical in nature.

Procedures

The survey utilized the Research Electronic Data Capture (REDCap) web-based application [20] and was distributed to the study participants via an embedded e-mail link to the survey. Two e-mail reminders were generated to enhance survey participation. The identity of participants and survey results were kept confidential from all subjects and investigators.

Data analysis

Survey responses were collected and tabulated by the REDCap tool. The mean score (MS) was calculated for each data item with items then ranked in descending order of MS for generation of the median value and interquartile ranges. The percentage of participants ranking each individual data item as not needed or absolutely necessary was also determined. The MS for each data item was also stratified according to the respondent's clinical role. For these analyses APs and NFs were grouped together (AP/NF) as they mainly perform supervisory roles, with NPs and PRs being grouped (NP/PR) as they often are charged with carrying out most pre-rounding, rounding, and post-rounding duties.

Statistics

All descriptive statistics and comparison of means (Wilcoxon's rank sum test) were performed in JMP (v 9.0.1, SAS Institute, Cary, NC); $p < .05$ was considered statistically significant. TABLEAU software (Seattle, WA) was used for data visualization. Study format, design, and statistical analyses were done in accordance with similar published studies performed at our institution and under the guidance of statistical support [12,21].

Results

Twenty-five survey requests were distributed with 23 responses obtained, giving a response rate of 92%. All 8 APs and 2 NFs completed the survey with 4 NPs and 9 PRs participating in the study.

Figure 1 shows each of the 98 data items listed by descending MS. The top 5 data items with the highest MS were daily weight, pH, pCO2, FiO2, and blood culture results. The median MS was 4.5 (maximum 6) with 71% of the data items falling within the top 2 quartiles when distributed by proportional quarters. The lowest 5 rated data items were RBC distribution width (RDW), QTc value, mean corpuscular volume (MCV), total number of transfusions, and hematocrit. Also displayed in Figure 1 is the percentage of respondents that rated each item as absolutely necessary or not needed. Daily weight received the highest percentage of responders (73%) rating it as an absolutely necessary data item. Sixteen of the 98 items (16%) received an absolutely necessary rating by more than 50% of respondents. The 3 items with the lowest MS also received the highest percentage of respondents rating each data item as not needed. Only 1 item (QTc value) was rated by more than 20% of respondents as not needed. Stratifying individual data item MSs by clinical role groupings (AP/NF versus NP/PR) resulted in an alteration of the order of highest ranking data items (Table 1). Seven data items (daily weight, pH, pCO2, FiO2, blood culture results, ventilator mode, and chest X-ray) were among the top 10 rated items in both groupings. Similarly, 6 data items (total urine output, hematocrit, blood type, MCV, RDW, and QTc value) were among the 10 lowest rated items in both groups.

Figure 2 shows the results of the distribution of MSs for every data item stratified by clinical role groupings. The AP/NF group rated data items significantly higher than the NP/PR group with the overall means of all data item MSs being 4.5 and 4.3 respectively ($p = 0.01$).Figure 2 also illustrates the same data analyses displayed in a proportion of densities chart (no statistical analyses performed). This chart demonstrates that the AP/NF group generated more data item MSs at the extremes of the scale with a larger proportion of both higher and lower rated data items compared to NP/PRs, who produced a larger proportion of moderately rated data items.

Discussion

We conducted a survey among NICU providers to assess the clinical information needs in an effort to create a more effective EMR human-computer interface. To our knowledge this is the first study attempting to assess these specific needs in a systematic way. Our survey had a high response rate with participants distributed among 4 distinct clinical role designations. The data demonstrates that when making clinical decisions NICU providers rely on a significant proportion of the large amounts of objective data provided to them by the EMR. This is evidenced by the fact that nearly three-fourths of the data items were ranked within the top 2 proportional quartiles with only 3 items contained in the bottom quartile. In addition, there was a large difference at the extremes of the rankings with many more respondents ranking items as absolutely necessary as compared to not needed. This finding of high EMR-derived data needs is consistent with the finding that NICU providers prefer objective data compared to verbal communication or clinical notes as their primary clinical information source [22].

The distribution of scores aligns with what one would expect clinically. Of the 10 highest rated data items, 7 were related to a respiratory parameter, one often associated with ventilatory

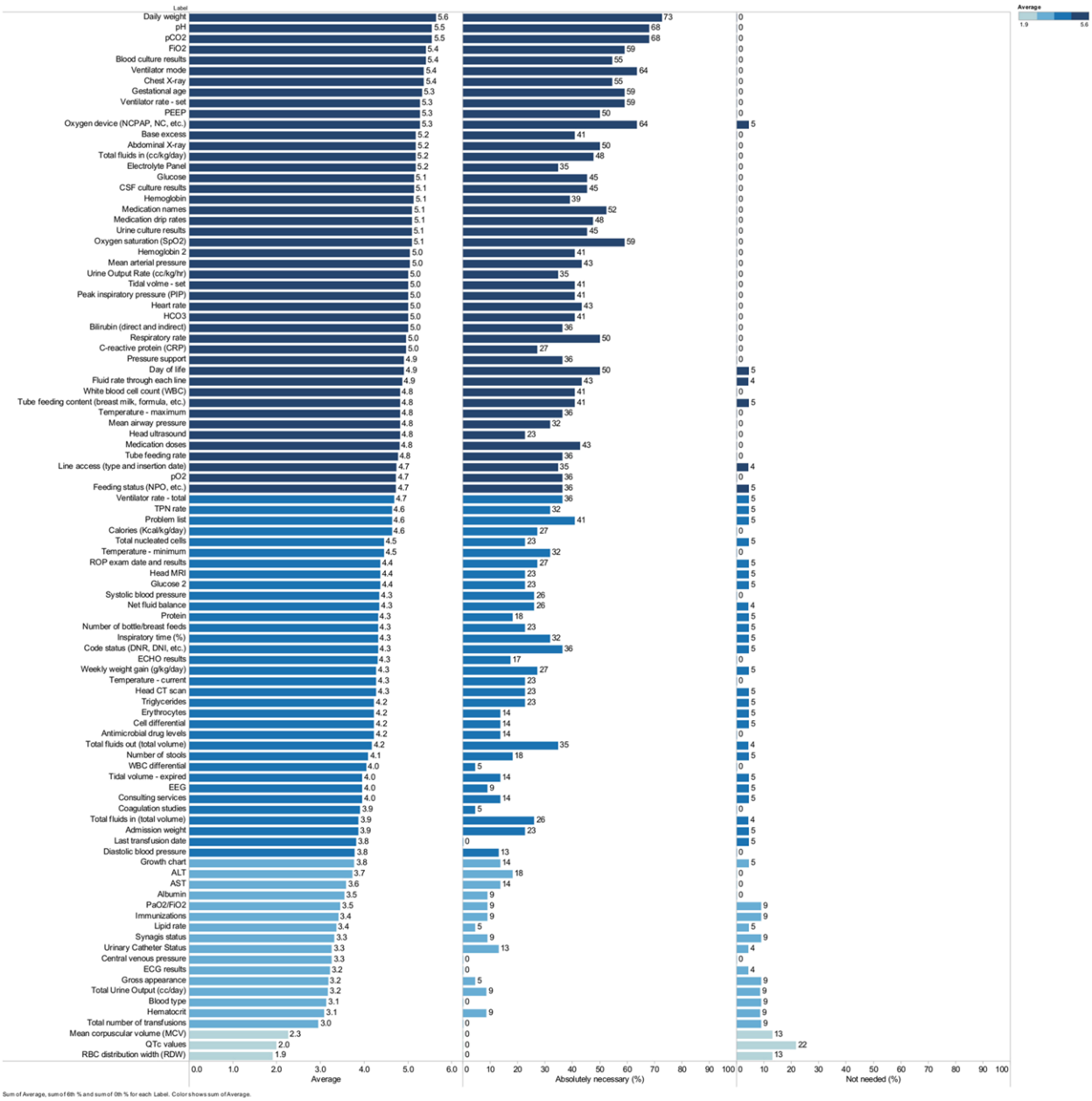

Figure 1. Data item ratings. Data items in descending order of the mean score (average) as rated by NICU providers to the perceived importance in guiding clinical care. Proportional quartiles are demonstrated by shading of the graph bars. Also shown are the percentages of respondents that rated each data item as absolutely necessary and not needed.

management. It is well known that a significant number of major morbidities among NICU patients are respiratory in nature with a significant amount of daily management related to this system [23,24]. As well, the highest rated item, daily weight, is vital for daily medication and fluid calculations and is marked as one the most fundamental data items required in the development of pediatric HITs [18,19].

In addition to our findings when respondents were grouped as a whole, there were important findings when data item ratings were stratified by clinical role. By grouping in this manner (AP/NF and NP/PR) we attempted to better understand the clinical information needs of users with similar clinical responsibilities. The results of these comparisons have clinical practicality and can offer insights into the appropriate development of HITs best suited to address differing clinical needs. NP/PRs are often responsible for most pre-rounding and rounding duties and are the primary care provider of NICU patients throughout a day [25-27]. As a result, providers in this role have a need for access to large amounts of data items, without much discrimination of importance, in order to collect all the necessary information for dissemination in multidisciplinary patient rounds and in the clinical note.

In contrast, supervisory physicians (AP/NFs) often give a disproportionate significance to a smaller set of data items that aid them in making the most critical decisions and care plans with certain data items being lightly regarded or even ignored.

These descriptions of differing roles are supported by our study as AP/NF data item ratings generated bimodal peaks at the extremes of the scale with a more consistent and evenly distributed rating pattern produced by NP/PRs. These findings may suggest that appropriate EMR interfaces for the NICU setting should include different viewing options that are catered to the primary clinical role of the user. Interfaces for

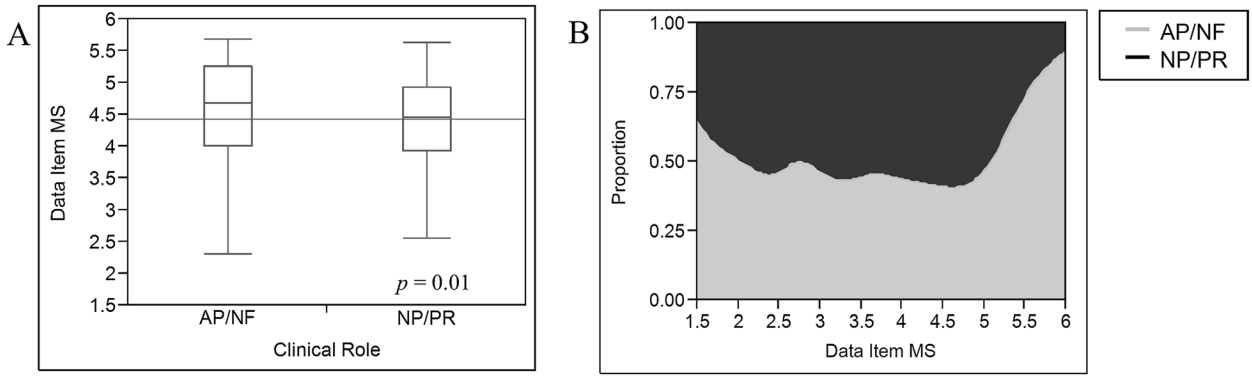

Figure 2. Variance by clinical role. (A) Variance in the individual data item mean scores stratified by clinical role (AP – attending physician; NF – neonatal fellow; NP – nurse practitioner; PR – pediatric resident). Data are displayed in box-whisker graphs (median, interquartile range, total range) with the lighter horizontal line showing the grand mean. (B) Proportion of density chart illustrating the distribution of data item mean scores by clinical role (no statistical analyses performed).

supervisory physicians may include less data items that highlight selected, critically relevant data whilethose forNPs andPRs maybesimilartocurrent EMR interfaces with most patient data items readily available and viewable. Further studies are needed to verify these findings and better elucidate what specific data items should be included in a proposed limited data option that can be utilized by supervisory physicians and others (ie consulting services), where large amounts of data can be safely omitted without patient care compromise.

Our study has some limitations which include the relatively small number of participants and a single institution survey. Our survey included 23 participants mainly due to the relatively low volume and staffing needs of our NICU. However, we were able to include every AP and NF with a large proportion of NP and PR participation. Despite the low absolute numbers, we feel these survey results accurately reflect the opinions of the care providers and can be used to help guide interface development. In addition, it is important to recognize that these results may reflect a local practice and should be generalized to other settings with these limitations in mind. Regardless, in the development of patient-centered clinical tools, the clinician'sperspective from a single hospital play a more important role than population based observations.

Another limitation of this study involves asking respondents to rate the importance of data items in helping make "routine clinical decision". We are aware that there are often times when clinical needs reach beyond the scope of what would be considered "routine" and require a different data requirement. It would be difficult to speculate the change in ratings, if any, if the word "routine" were removed from the survey. Regardless, we wanted the results of the survey to portray the ideas of providers in the most common and frequent clinical scenarios and designed the survey as such. We feel that options for viewing different levels of data density, as discussed earlier, would be a way to overcome this obstacle and provide adequate levels of data catered to each clinical situation.

This is the first step in a process of creating and adapting an EMR human-computer interface that involves a design allowing for end-users to influence its final product.

A methodical process such as this is important in creating an environment where the implementation of new technology is well accepted and tailored to the needs of the users [28]. In addition, the design of our study creates an opportunity for replication in other centers in an effort to compare and contrast the information needs among providers in various NICU settings.

Table 1 Top ten data items by clinical role

AP/NF		NP/PR	
Data item	**MS**	**Data item**	**MS**
Daily weight	5.7	Daily weight	5.6
Oxygen device (i.e. NCPAP)	*5.7*	pH	5.5
pCO2	5.7	pCO2	5.4
FiO2	5.7	FiO2	5.2
Blood culture results	5.7	Blood culture results	5.2
Ventilator rate - set	*5.7*	*Gestational age*	*5.2*
Ventilator mode	5.7	Ventilator mode	5.1
Chest X-ray	5.7	Chest X-ray	5.1
Medication names	*5.7*	*Total fluids in (cc/kg/day)*	*5.1*
pH	5.6	*PEEP*	*5.0*

A listing of the top 10 data items with the largest mean score (MS) stratified by clinical role groupings. Data items that differ between the groups are *italicized.*

Conclusion

Our study illustrates the myriad of data items available to NICU caregivers for use in clinical decision making and demonstrates that providers at our institution feel that a majority of those data items are important with significant differences found when comparing by clinical role. This creates the need to develop patient-centered EMR human-computer interfaces and other HITs that present vast amounts of data [1] in a way that is easily synthesized [17] and offers options for varying degrees of viewable data densities depending on clinical role. In this way one can better create EMR interfaces that are relevant to the clinical expectations of providers while at the same time achieve a goal of reducing information overload and lowering the risk for medical errors [3,13].

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Characterization of the Clonal Profile of MRSA Isolated in Neonatal and Pediatric Intensive Care Units of a University Hospital

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Abstract

Background: Methicillin-resistant Staphylococcus aureus (MRSA) are important pathogens in neonatal and pediatric intensive care units, which can cause severe infections in hospitalized children. Detection of the mecA gene and classification of the staphylococcal cassette chromosome mec (SCCmec) permit the characterization of MRSA strains isolated from infections caused by these microorganisms. In contrast, pulsed-field gel electrophoresis (PFGE) is used to type MRSA clones. This method is commonly used to analyze the epidemiology of bacteria causing nosocomial infections. The objective of this study was to detect and characterize MRSA isolated from clinical specimens of children hospitalized in the neonatal and pediatric intensive care units of the University Hospital of the Botucatu Medical School.

Methods: A total of 119 S. aureus strains were isolated from clinical specimens and the mecA gene was detected by PCR. SCCmec was detected by multiplex PCR and the clonal profile was analyzed by PFGE. Results: The mecA gene was detected in 17.6% (21/119) of the isolates; 42.9% (9/21) of MRSA were characterized as SCCmec type III and 57.1% (12/21) as type IV. Analysis of the clonal profile of these strains revealed three distinct clones, with SCCmec type III being related to the Brazilian endemic clone and type IV to clones JCSC4469 and USA800.

Conclusions: Replacement of clonal groups occurred in the neonatal and pediatric units over the period studied, a fact highlighting the importance of improving hygiene practices and control measures of nosocomial infections in these units.

Background

The genus Staphylococcus is a member of the family Staphylococcaceae, which comprises 49 species and 26 subspecies [1,2]. Staphylococcus aureus is the most important species of this genus and the causative agent of a range of infections, such as furuncles, cellulitis, impetigo, and wound infections. Some of the most severe infections caused by S. aureus include bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, meningitis, and abscesses in muscles, genitourinary tract, central nervous system and various intra-abdominal organs [3,4].

Studies have shown that 60 to 85% of staphylococci isolated from clinical samples are resistant to methicillin [5]. Methicillin-resistant Staphylococcus aureus strains (MRSA) are important pathogens in neonatal (NICU) and pediatric intensive care units (PICU), which can cause severe infections in hospitalized children who are generally exposed to several risk factors, such as prematurity, invasive procedures, mechanical ventilation, and drains [6].

Oxacillin is the drug of choice for susceptibility testing and treatment of infections caused by Staphylococcus. Intrinsic resistance of S. aureus to oxacillin is mediated by the production of a supplemental penicillin-binding protein (PBP 2a), which is encoded by the mecA gene [7]. This gene is found on a specific mobile genetic element identified as the staphylococcal cassette chromosome mec (SCCmec), which consists of the mecA gene complex, ccr gene complex, and region J. The mec complex comprises the mecA gene and its regulatory genes mecIand mecRI. The ccr gene complex is responsible for the integration and excision of SCCmec in the chromosome. In contrast, region J is not essential for the cassette chromosome, but can carry genes that encode resistance to non-beta-lactam antibiotics and heavy metals [8]. Eleven SCCmec types have been described so far [9]. These types are defined based on the combination of the type of ccr gene complex and class of the mec gene complex. Subtypes are defined based on polymorphisms in region J of the same combination of mec and ccr complexes [8].

SCCmec types I, II and III are classically found in nosocomial MRSA strains, whereas the other types are found in community-associated MRSA [10]. SCCmec type III encodes the largest number of resistance genes and strains harboring this type are important pathogens in hospitals where they cause severe infections [11]. In contrast, type IV is characterized by a smaller size and lower metabolic cost, a fact selectively favoring this element for transfer between staphylococci [12]. Community-associated MRSA have been reported to cause severe infections in NICU and PICU patients who never have been hospitalized [6]. According to these authors, the most frequent complications caused by these microorganisms are pneumonia and skin and

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Table 1 Detection of MRSA and SCC*mec* type according to hospital ward and clinical specimens

	Neonatal intensive care unit				Pediatric intensive care unit			
	N	% MRSA	SCC*mec* type		N	% MRSA	SCC*mec* type	
			Type III	Type IV			Type III	Type IV
Blood culture (N = 80)	39	17.5	2	5	41	14.6	1	5
Secretion (N = 22)	22	18.2	3	1	0	0	0	0
Fluid[a] (N = 2)	0	0	0	0	2	50.0	0	1
Foreign body[b] (N = 15)	15	20.0	3	0	0	0	0	0

N: number of strains.
[a]Peritoneal and pleural fluid.
[b]catheter and cannula.

soft tissue infections and strains carrying SCCmec type IV are the most common [6].

The increasing occurrence of MRSA in hospitals makes the typing of these microorganisms important in order to determine whether the strains involved in nosocomial infections or in possible foci of transmission are related to a specific clone, ie, whether they have a common origin [13]. Pulsed-field gel electrophoresis (PFGE) is commonly used to analyze the epidemiology of bacteria causing nosocomial infections. This method permits to clearly discriminate strains and to demonstrate the genetic relationship between isolates with high reproducibility [13]. The objective of the present study was to detect and characterize MRSA isolated from clinical specimens of children hospitalized in the NICU and PICU of the University Hospital of the Botucatu Medical School (HC-FMB).

Methods

Strains

A total of 119 S. aureus strains isolated from clinical specimens of children hospitalized in the NICU and PICU of HC-FMB between 1991 and 2009 were studied. Thirty-nine of the 76 neonatal strains were isolated from blood cultures, 22 from secretions, 12 from catheters, and three from cannulae. In the pediatric ward, 41 of the 43 strains were isolated from blood cultures, one from pleural fluid, and one from peritoneal fluid. The strains were isolated as described by Koneman et al. [14] on blood agar plates (Blood Agar Base, Himedia, Mumbai, India) and suspected colonies were submitted to Gram staining. After confirmation of morphology and specific staining, the isolates were identified using catalase and coagulase tests.

DNA extraction

Total nucleic acid was extracted from S. aureus isolates cultured on blood agar (Blood Agar Base, Himedia, Mumbai, India), inoculated individually into brain-heart infusion broth (Oxoid Ltd., Basingstoke, Hampshire, England), and incubated for 24 h at 37°C. Extraction was performed using the Illustra kit (Illustra, GE Healthcare, Pittsburg, PA, USA), which consisted of initial digestion of bacterial cells with lysozyme (Amresco, Solon, Ohio, USA) (10 mg/mL) and proteinase K (GE Healthcare, Pittsburg, PA, USA) (20 mg/mL). Five hundred µL of the extraction solution (Illustra, GE Healthcare, Pittsburg, PA, USA) was added and the mixture was centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 5,000 x g for 1 min. The supernatant was transferred to a column and centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 5,000 × g for 1 min. The collected fluid was discarded and 500 µLextractionsolution(Illustra,GE Healthcare, Pittsburg, PA, USA) was added again to the column. After centrifugation and discarding of the collected fluid, 500 µL washing solution (Illustra, GE Healthcare, Pittsburg, PA, USA) was added and the column was centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 20,817 × g for 3 min. The column was transferred to a 1.5-mL tube and 200 µL Milli-Q water heated to 70°C was used for elution. The samples were centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 5,000 × g for1minand the columnwas discarded. The extracted DNA was stored in a refrigerator (Brastemp BRD45, Whirlpool S.A., São Paulo, Brazil) at 4°C.

Detection of the mecA gene

The mecA gene was investigated in the S. aureus isolates for detection of oxacillin resistance. The primers and parameters described by Murakami et al. [15] were used for amplification: primers mecA1 (AAA ATC GAT GGT AAA GGT TGG) and mecA2 (AGT TCT GCA GTA CCG GAT TTG) that amplify a fragment of 533 bp. International reference strains were included as positive (S. aureus ATCC 33591) and negative (S. aureus ATCC 25923) controls in all reactions.

Determination of the SCCmec type

The SCCmec type was determined in the MRSA isolates by multiplex PCR. The primers and parameters described by Milheiriço et al. [16] were used for amplification.

Pulsed-field gel electrophoresis

The clonal profile of the Staphylococcus spp. isolates was determined using the modified protocol of McDougal et al. [17]. The strains were inoculated into brain-heart infusion broth (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated for 24 h at 37°C. The isolates were centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg) in microtubes at 15,294 × g for 1 min, the supernatant was discarded, 300 µLTE solution (10 mM Tris, 1 mM EDTA, pH 8.0) was added, and the strains were kept in a water bath for 10 min at 37°C. The cells were lysed by the addition of 5 µL lysostaphin (from Staphylococcus lyophilized powder, Sigma-Aldrich) and vortexed (Phoenix AP-56), and 300 µL of 1.8% low-melt agarose (Agarose-Low Melt, USB Corporation, Ohio, USA) was added at 37°C. Plugs were prepared from the strains and the agarose (Agarose-Low Melt, USB Corporation, Ohio, USA) was allowed to solidify. The plugs were transferred to a 24well plate containing 2 mL EC solution (6 mM Tris-HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2%

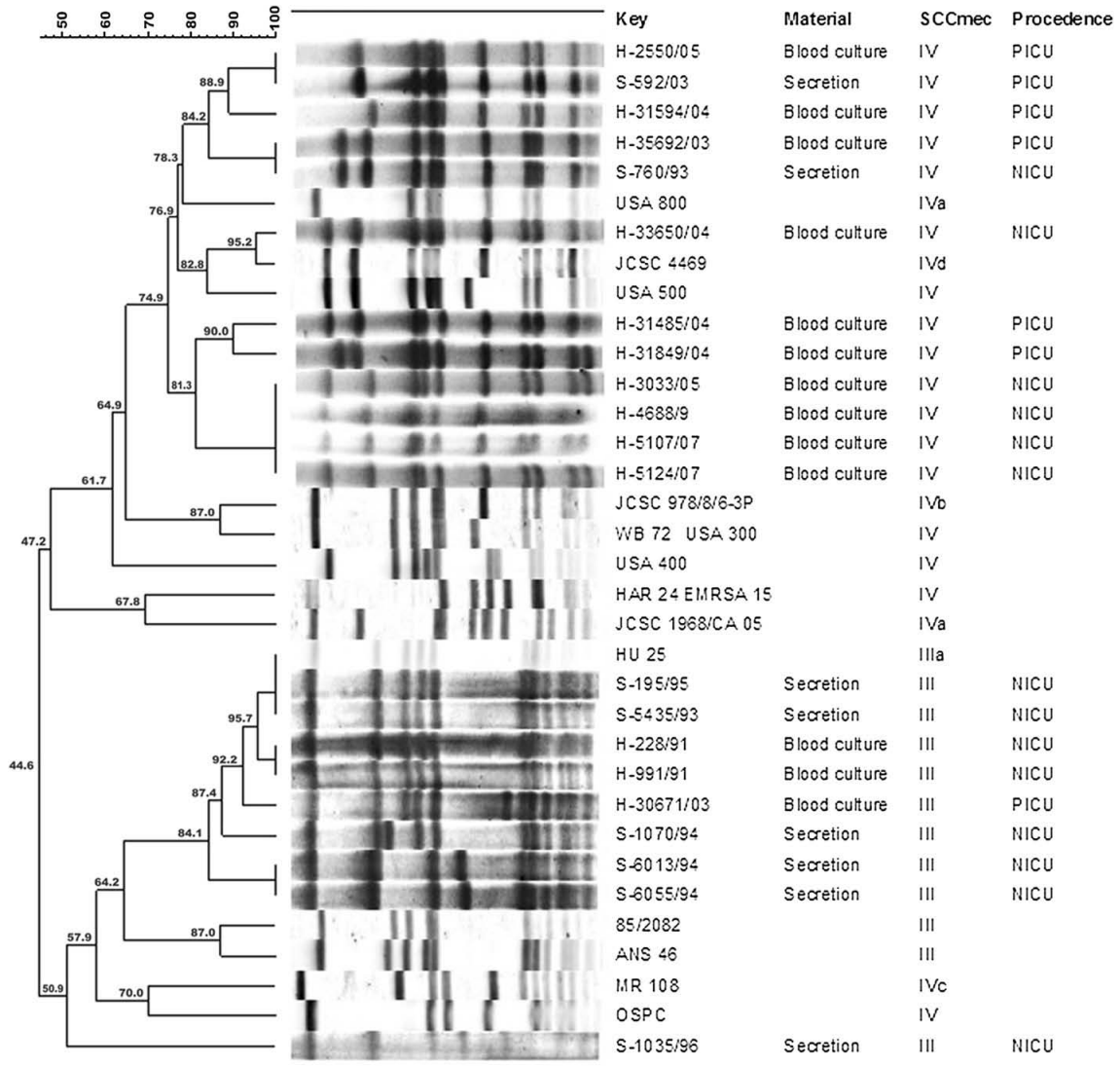

Figure 1. Determination of the clonal profile of MRSA carrying SCCmec type III and type IV isolated from clinical specimens of children hospitalized in the neonatal (NICU) and pediatric (PICU) intensive care units of HC-FMB.

sodium deoxycholate, 0.5% sodium lauroyl sarcosinate) and incubated for 4 h at 37°C. The EC solution (6 mM Tris-HCl, 1M NaCl,100 mM EDTA, 0.5%Brij-58,0.2% sodium deoxycholate, 0.5% sodium lauroyl sarcosinate) was removed and the plugs were washed four times in 2 mL TE solution (10 mM Tris, 1 mM EDTA, pH 8.0) for 30 min at 21°C.

One-third of the plug and 2 µL SmaI (Fast Digest SmaI, Thermo Scientific, Lithuania, EU) were used for the restriction of genomic DNA. For restriction, buffer without the enzyme (45 µL Milli-Q water and 5 µLof the enzyme buffer) was added to a 96-well plate and the plate was stored in a refrigerator (Brastemp BRD45, Whirlpool S.A., São Paulo, Brazil) for 30 min at 4°C. The buffer without enzyme was removed and buffer containing the enzyme (43 µL Milli-Q water, 5 µL enzyme buffer, and 2 µL of the enzyme) was added. The plate was incubated in an oven (Eletrolab 101 M/3, São Paulo, Brazil) for 6 min at 37°C. Electrophoresis was carried out in a CHEF-DR III System (BioRad Laboratories, Hercules, California USA) using 1% agarose gel (Pulsed-Field Certified Agarose, BioRad Laboratories, USA) prepared in 0.5 M TBE (0.1 M Tris, 0.08 M boric acid, 1 mM EDTA) under the following conditions: pulse times of 5 to 40 s for 21 h on a linear ramp; 6 V/cm; angle of 120°; 14°C; 0.5 M TBE as running buffer. The Lambda Ladder PFG Marker (New England BioLabs, Hitchin, United Kingdom) was used as a molecular marker. The gels were stained with GelRed (400 mL distilled, water and 30 µL GelRed) (10,000X in water, Biotium, Hayward, CA) for 1 h and photographed under UV transillumination.

The BioNumerics software, version 6.1 (Applied Maths, Belgium), was used for analysis of similarity, calculation of the Dice correlation coefficient, and construction of the dendrogram by the UPGMA method (unweighted pair group method using arithmetic averages). Band position tolerance and optimization were set at 1.25 and 0.5%, respectively. A similarity coefficient of 80% was chosen for the definition of clusters.

International clones kindly provided by Dr. Antonio Carlos Campos Pignatari, Laboratório Especial de Microbiologia Clínica, Disciplina de Infectologia, Universidade Federal de São Paulo/Escola Paulista de Medicina, and by Dr. Agnes Marie Sá Figueiredo, Universidade Federal do Rio de Janeiro,

Instituto de Microbiologia Prof. Paulo de Góes, Brazil, were used as controls: USA800 (SCCmec IVa), JCSC 1968/CA05 (SCCmec IVa), JCSC 978/8/6-3P (SCCmec IVb), MR108 (SCCmec IVc), JCSC 4469 (SCCmec IVd), WB72/USA300 (SCCmec IV), USA400 (SCCmec IV), USA500 (SCCmec IV), 0SPC (SCCmec IV), HAR24/EMRSA 15 (SCCmec IV), HU25 (SCCmec IIIa), 85/2082 (SCCmec III), and ANS 46 (SCCmec III).

Results

The mecA gene was detected in 17.6% (21/119) of the S. aureus isolates studied. MRSA were detected in 18.4% (14/76) of the S. aureus strains isolated from the NICU, including seven strains isolated from blood cultures, four from secretions, and three from catheters. Seven 16.3% (7/43) strains from the PICU carried the mecA gene, including six strains isolated from blood cultures and one strain isolated from pleural fluid (Table 1).

Characterization of the staphylococcal cassette chromosome mec

The 21 mecA gene-positive S. aureus isolates were submitted to multiplex PCR for characterization of the SCCmec type. Nine of the 21 strains (42.9%) were classified as type III and 12 (57.1%) as type IV. Eight of the nine MRSA type III strains were isolated from clinical specimens of children hospitalized in the NICU and one in the PICU. Six of the type IV strains were isolated in the NICU and six in the PICU (Table 1).

Evolution of oxacillin resistance in S. aureus strains isolated from patients seen at HC-FMB

Analysis of the period from 1991 to 2009 showed the early presence of SCCmec type IV in a strain isolated in 1993. Although the sample size of this study was too small to detect a significant difference, the results showed a decrease in the prevalence of SCCmec type IIIand arecentincreaseinSCCmec type IV-carrying isolates.

Analysis of the clonal profile of MRSA

Analysis of the clonal profile of the MRSA strains isolated in this study revealed four distinct clones. MRSA harboring SCCmec type III were divided into two groups, one related to the Brazilian endemic clone (HU25). The strains carrying SCCmec type IV were also divided into two groups, one related to a clone found in the United States (USA800) and the other related to a clone found in Japan (JCSC4469) (Figure 1).

Discussion

Oxacillin-resistant Staphylococcus aureus are important pathogens involved in infections that affect children hospitalized in intensive care units in many countries. Although the frequency of oxacillin resistance is high among S. aureus strains, particularly in large hospitals and universities, the frequency of isolation of MRSA in the NICU and PICU of HC-FMB was 17.6% (21/119) over a period of 18 years; 18.4% (14/76) of these isolates were detected in the NICU and 16.3% (7/43) in the PICU. Similar results have been reported in a study conducted in the United Kingdom, in which S. aureus strains isolated in the NICU and PICU of a hospital over a period of 10 years (1993 to 2003) were analyzed. The frequency of isolation of MRSA related to bacteremia was 15.1% (5/33) [18]. In a study conducted in New Zealand, the frequency of isolation of MRSA was 12.0% (7/58) in a PICU over a period of 11 years (1993 to 2004) [19]. In contrast, different results were found in the NICU of a hospital in the United States where 47.4% (8/17) of MRSA were detected among S. aureus over a period of 10 years (2000 to 2009) [20].

In the two wards, the S. aureus strains isolated from blood cultures exhibited a similar percentage of oxacillin resistance [NICU: 17.5% (7/39), PICU: 14.6% (6/41)]. In the NICU, MRSA were also isolated from other clinical specimens such as secretions [18.2% (4/22)] and catheters and cannula [20.0% (3/15)]. In the PICU, only one strain isolated from pleural fluid was resistant to oxacillin.

Among the MRSA detected in this study, 57.1% (12/21) were characterized as SCCmec type IV; of these, 83.3% (10/12) were isolated from blood cultures. In the study of Healy et al. [21], 75% (6/8) of MRSA strains isolated in the NICU were typed as SCCmec type IV. SCCmec type IV is the most frequent type found in the community and is also becoming predominant among healthcareassociated MRSA infections [8,22,23]. The smaller size of the cassette chromosome when compared to types I, II and III probably increases its mobility and transfer capacity between Staphylococcus, suggesting that clones carrying this SCCmec element may spread more easily and that diseases caused by these strains tend to increase [24,25]. According to Dolapo et al. [20], the incidence of MRSA infections in NICUs is still unacceptably high. This fact may be related to the acquisition of community-associated MRSA strains, which have evolved in the community and penetrated the NICU through parents or care providers.

SCCmec type III was identified in 42.9% (9/21) of MRSA and predominated among strains isolated in the 1990s. Only one strain was detected after 2000. SCCmec type III is commonly found in Brazilian hospitals and is highly resistant to various antimicrobial agents used to treated S. aureus infections, including resistance to beta-lactams, macrolides, aminoglycosides and trimethoprim–sulfamethoxazole [26]. In the study of Perez & D'Azevedo [27], nine MRSA were susceptible only to vancomycin, linezolid and teicoplanin. Eight of these strains carried SCCmec type III.

In the present study, SCCmec typing permitted to confirm the isolation of two types of MRSA in the NICU and PICU of HC-FMB over a period of 18 years. One important finding was the isolation of MRSA carrying SCCmec type IV in 1993 from the secretion sample of a newborn. SCCmec type IV was only typed again 10 years later in the pleural fluid sample of a child hospitalized in the pediatric unit. From that time on, this SCCmec was the predominant type among all MRSA isolated in the two units. According to Milheiriço et al. [16], the SCCmec element is an important marker for the determination of MRSA clones. In addition to being a valuable tool for the study of MRSA epidemiology, SCCmec characterization permits to investigate the evolution of MRSA clones in culture collections.

With respect to the epidemiology and evolution of MRSA clones, PFGE permitted a better analysis of the data obtained in this study. The MRSA isolates carrying SCCmec type III were divided into two groups, one of them related to the Brazilian endemic clone (HU25). According to Vivone et al. [28], this clone is responsible for most infections caused by MRSA. The MRSA isolates carrying SCCmec type IV could also be divided into two groups, one related to clone JCSC4469 and the other related to clone USA800. The strain mentioned above, which was isolated in 1993 and carried SCCmec type IV, was related to clone USA800. This group comprised strains isolated between 1993 and 2005. Trindade et al. [29] found a variety of MRSA that were related to the Brazilian endemic clone. In the present study,

strains related to the Brazilian endemic clone predominated until 2003, whereas strains related to clones JCSC4469 and USA800 were found after this period. A Brazilian study conducted in a university hospital that analyzed clonal groups over a period of 8 years found that the clones identified were replaced over time, without any predominance in a specific hospital area [30]. According to the authors, replacement of clonal groups over time might be explained by microevolution of the pathogen or by competition to adapt to the hospital environment. Furthermore, the report of the presence of the pediatric clone in central Brazil suggests that this clone is settling in Brazilian hospitals and spreading in the community, increasing the likelihood of expanding its reservoir [31].

Conclusions

The clonal MRSA groups found in the NICU and PICU of HC-FMB highlight the importance of improving hygiene practices and control measures of nosocomial infections in these units since hospitalized children are generally more vulnerable because of exposure to several risk factors. Furthermore, the clonal groups that predominated over the past years carry SCCmec type IV, an element that does not impose any metabolic cost on the host and that may spread in the absence of antibiotic selective pressure. This fact may result in the emergence of this type as a new pathogen in the world. Although the sample size of this study was too small to draw any definite conclusions, according to the literature, communityassociated MRSA are steadily increasing and may replace or be the more dominant population in clinical settings.

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Polymicrobial Bloodstream Infections in the Neonatal Intensive Care Unit are Associated with Increased Mortality: A Case-Control Study

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Abstract

Background: Polymicrobial infections in adults and children are associated with increase in mortality, duration of intensive care and healthcare costs. Very few studies have characterized polymicrobial bloodstream infections in the neonatal unit. Considerable variation has been reported in incidence of polymicrobial infections and associated clinical outcomes. We characterized the risk factors and outcomes of polymicrobial bloodstream infections in our neonatal units in a tertiary hospital in North America.

Methods: In a retrospective case control study design, we identified infants in the neonatal intensive care unit with positive blood cultures at Texas Children's Hospital, over a 16-year period from January 1, 1997 to December 31, 2012. Clinical data from online databases were available from January 2009 to December 2012. For each polymicrobial bloodstream infection (case), we matched three infants with monomicrobial bloodstream infection (control) by gestational age and birth weight.

Results: We identified 2007 episodes of bloodstream infections during the 16 year study period and 280 (14%) of these were polymicrobial. Coagulase-negative Staphylococcus, Enterococcus, Klebsiella and Candida were the most common microbial genera isolated from polymicrobial infections. Polymicrobial bloodstream infections were associated with more than 3-fold increase in mortality and an increase in duration of infection. Surgical intervention was a significant risk factor for polymicrobial infection.

Conclusion: The frequency and increased mortality emphasizes the clinical significance of polymicrobial bloodstream infections in the neonatal intensive care unit. Clinical awareness and focused research on neonatal polymicrobial infections is urgently needed.

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Background

Polymicrobial infections increase mortality more than 2-fold in adults and children, increase length of hospital stay and healthcare costs [1,2]. Risk factors for polymicrobial infections in children and adults include the presence of a central venous catheter, administration of parenteral nutrition, gastrointestinal pathology, especially short gut syndrome, use of broad-spectrum antibiotics and immunosuppression [1,3,4].

Neonatal polymicrobial infections are less well characterized compared to those in children or adults. Increased survival of extremely premature infants at the edge of viability, dependence on catheters and parenteral nutrition (PN), and antibiotic therapy may predispose to polymicrobial infections in neonates. The frequency of neonatal polymicrobial bloodstream infections reported in clinical studies varies from 4 to 24% of all bloodstream infections [5-10]. A standard definition for neonatal polymicrobial infections is lacking and the incidence varies from study to study partly due to variability in definition, neonatal population and practices [11]. Few studies have focused on polymicrobial infections in neonates, especially on risk factors and clinical outcomes in the western world [10-12]. In a neonatal review, the mortality due to polymicrobial infections was 3-fold greater than that of monomicrobial infections (70% vs. 23%) [10]. Organisms that are commonly implicated in neonatal polymicrobial bloodstream infections are coagulase-negative staphylococcus (CONS), Candida spp., Staphylococcus aureus and Enterococcus spp. [1,9,13-16]. A common risk factor appears to be multi-species biofilm infections originating from indwelling medical devices, notably indwelling vascular catheters or endotracheal tubes [1,17].

Polymicrobial bloodstream infections may be defined as multiple organisms isolated during an infectious episode including those from a single blood specimen [1] or more restrictively as isolation of more than one organism from a single blood specimen only [11,12,17]. Different definitions may partly explain the varying incidence of polymicrobial bloodstream infections reported. Bizzarro et al. (Yale, 1989-2006) and Faix et al. (Ann Arbor, 19711986) have reported the only two studies on neonatal polymicrobial infections from North America, [10,11]. Changing epidemiology of neonatal infections dictates a need to update and understand the epidemiology of poly-microbial infections in the hospitalized infant [11]. Lack of data on polymicrobial infections from our neonatal unit and concern of significant increase in mortality, morbidity and healthcare costs due to polymicrobial infections prompted us to investigate its

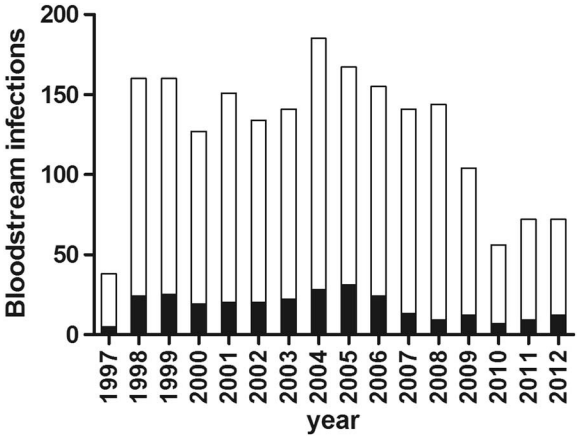

Figure 1. Frequency of polymicrobial bacteremia episodes. The frequencies of neonatal bacteremia episodes are plotted against the years from 1997 to 2012. The dark shaded portion of the bars indicate polymicrobial component of the infectious episodes, which ranged from 6.3 to 18.6% and on an average of about 14% during the 16-year study period. There were significant decrease in the number of infections and polymicrobial infections between the time epochs, 1998-2009 and 2010-2012 ($p < 0.01$).

frequency, risk factors and clinical outcomes at Texas Children's Hospital (TCH).

Methods

We hypothesized that polymicrobial infections comprise > 5% of bloodstream infections in infants residing in the neonatal intensive care units, have identifiable risk factors and are associated with higher mortality and morbidity than monomicrobial infections. We tested our hypothesis by performing a single center, retrospective, matched case-control study during a 16 year study period. Our study protocol was approved by the Institutional Review Board at Baylor College of Medicine, Houston. We followed the STROBE guidelines in reporting this study [18].

Identification of cases and controls

We identified blood culture positive infants from the clinical microbiology database, who were admitted to the neonatal intensive care units (NICUs) III and II at TCH from January 1, 1997 to December 31, 2012. Infants more than 28 days of age in the NICU at the time of the infection and those admitted from home to the NICU with positive blood cultures were also included. The data in the clinical microbiology database is entered by the microbiologist and all positive and negative cultures including those regarded as contaminants are recorded. The tertiary NICU at TCH has approximately 1500 admissions per year including inborn and outborn transferred neonates (ranged from 1338 to 1547 during the years 2009 to 2012). Very low birth weight (VLBW, birth weight < 1500 g) infant admissions ranged from 222 to 291 infants during the years 2009 to 2012.

Definitions

Polymicrobial bloodstream infection was defined as: i) isolation of more than one organism from a single blood culture specimen and ii) isolation of more than one organism in different blood culture specimens during the same bloodstream infectious episode. We defined 'bloodstream infection episodes' as time-periods associated with positive blood cultures [1] and the infection episode was considered resolved when at least two subsequent blood cultures performed every 24 hrs were negative. Usually when an organism is isolated from a blood culture specimen, blood cultures are repeated every 24 hr till two blood culture specimens are negative for organisms. Our neonatal units used only aerobic blood cultures and it is not routine to perform anaerobic blood cultures for the evaluation of neonatal sepsis. Single specimen polymicrobial infections were defined as more than one organism isolated from the same blood culture specimen. We also collected data regarding duration of the infection episode from culture positivity to culture negativity. For each polymicrobial bloodstream infection (case), we selected three gestational age matched neonates with comparable birth weights with monomicrobial bloodstream infection (control) during the time period 2009 to 2012. The matching was performed by an investigator who was blinded to neonatal risk factors and outcomes. An infant who had both polymicrobial and monomicrobial infections was included in the polymicrobial infection category for evaluation of neonatal outcomes because of the possibility that even one exposure to polymicrobial infection may increase mortality or morbidity. If an infant had multiple episodes of polymicrobial infections, the first polymicrobial infection episode was analysed. Coagulase negative staphylococcal (CONS) infections, skin flora (eg Micrococcus spp., Gamma Streptococci) and organisms infrequent in neonates (eg Bacillus spp.) were considered real infections when grown from two clinical specimens and a single culture of the above organisms were deemed contaminant and not included in the analyses.

Clinical data collection

Neonatal clinical data including demographics, risk factors and outcomes were identified by cross referencing our institution's neonatal clinical database (from Vermont Oxford Network (VON) database), for a 4 year period from January 1, 2009 to December 31, 2012. We collected the following clinical data for the identified cases and controls: demographic data (gestational age, birth weight, sex, age at the start of the infection episode and whether inborn or outborn), parenteral nutrition (PN) administration and its duration, presence of intravenous catheters at the time of the infectious episode (percutaneously inserted central catheters and broviac catheters but excluded umbilical catheters). We excluded umbilical venous catheters because our neonatal policy is to replace the umbilical venous catheters in the first few days of life with percutaneously inserted central catheters (PICC) and hence most umbilical lines lasted only a few days. Also, most of the bloodstream infections occurred at a time when umbilical catheters were no longer in place. We also collected data on important clinical outcomes (mortality, length of hospital stay, bronchopulmonary dysplasia, patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC, ≥ stage 2byBell's classification), intraventricular hemorrhage (IVH), periventricular leucomalacia (PVL) and retinopathy of prematurity (ROP), intermittent positive pressure ventilation (IPPV) at 36 wks corrected gestational age (GA), continuous positive airway pressure (CPAP) at 36 wks corrected GA, high frequency oscillatory ventilation (HFOV), inhaled nitric oxide (INO) therapy, extracorporeal membrane oxygenation (ECMO), surgeries, direct hyperbilirubinemia (direct bilirubin > 2 mg/dl or > 15% of total bilirubin) congenital heart disease and congenital malformations. All outcomes were defined as per VON database definitions.

Statistical analyses

Data were analyzed using STATA 11, Stata Corporation, Dallas, USA. Infants with polymicrobial bloodstream infections were compared with three gestational age and birth weight matched infants with monomicrobial bloodstream infections. Continuous

Table 1 Microbiology of monomicrobial and polymicrobial bloodstream infections

	Monomicrobial infections data as n (%)	Polymicrobial infections data as n (%)
CONS	16 (15.69)	12 (16.22)
Staphylococcus aureus	16 (15.69)	6 (8.11)
Streptococcus agalactiae	6 (5.88)	2 (2.70)
Candida albicans, C. parapsilosis, C. glabrata	7 (6.86)	7 (9.46)
Escherichia coli	18 (17.65)	5 (6.76)
Enterococcus faecalis, E. faecium	7 (6.86)	11 (14.86)
Enterobacter cloacae, E. aerogenes	8 (7.84)	5 (6.76)
Klebsiella pneumoniae, K. oxytoca	6 (5.88)	11 (14.86)
Pseudomonas aeruginosa	4 (3.92)	4 (5.41)
Bacillus spp.	4 (3.92)	1 (1.35)
Serratia marcescens	3 (2.94)	3 (4.05)
Others	7 (6.86)	7 (9.46)
Total	102 (100)	72 (100)

In monomicrobial bloodstream infections, 102 organisms were isolated from 102 infectious episodes. In polymicrobial bloodstream infections, 74 organisms were isolated from 34 infectious episodes. 'Others' include *Streptococcus salivarius*, *Citrobacter freundii*, *Clostridium tertium* for monomicrobial infections and for polymicrobial infections; they were Lactobacillus, *Proteus mirabilis*, *Streptococcus sanguinus*, Micrococcus spp., *Acinetobacter baumanii* complex and Gamma Streptococcus. All the organisms in the 'Others' group was cultured at least twice from 2 different samples. Candida species, Enterococcus species and Klebsiella species were isolated at higher frequencies in polymicrobial infections than monomicrobial infections. The most common combination of polymicrobial organisms were CONS and Candida (n = 6) and CONS and *Enterococcus fecalis* (n = 3).

data were analyzed for statistical significance by the Student's t test and categorical data were analyzed by chi squared analyses. A p value of < 0.05 was considered significant. A logistic regression analysis was performed for binary outcomes and odds ratios with 95% confidence intervals were estimated. The outcome of mortality was analyzed adjusting for 'any surgery' in the logistic regression model. Polymicrobial infections were used as the outcome of the logistic regression analysis for risk factors and as a covariate when assessing for neonatal outcomes. A subgroup analysis of VLBW infants was performed for mortality and risk factors in the logistic regression model.

Results

We identified 2007 bacteremia episodes during a period of 16 years in patients admitted to the NICUs at TCH; 280 episodes (14%) were polymicrobial (Figure 1). The percentage of annual polymicrobial bacteremia varied during the study period ranging from 6.3 to 18.6%. Polymicrobial bacteremia identified in single blood specimens constituted on an average of 56% (range 40 to 75%) during the 16 year study period.

Microbes isolated from monomicrobial (controls) and polymicrobial infections (cases) were similar but varied in frequency (Table 1). Microbes isolated at greater frequencies in polymicrobial infections were Candida spp. (>3%), Enterococcus faecalis (>2-fold) and Klebsiella pneumoniae (>2-fold). The most common combinations of polymicrobial organisms were CONS and Candida spp. (n = 6) and CONS and Enterococcus faecalis (n = 3). Gram positive organisms were isolated more frequently than gram negative organisms and the proportion of gram positive to gram negative organisms were similar in both monomicrobial (gram positive 51% and gram negative 42%) and polymicrobial infections (gram positive 48% and gram negative 40%).

We compared clinical data pertaining to demographics, risk factors and clinical outcomes between infants with polymicrobial bacteremia and gestational age and birth-weight matched infants with monomicrobial bacteremia (data from January 2009 to December 2012) (Table 2). We obtained neonatal data for 34 infants with polymicrobial bacteremia and 102 matched infants with monomicrobial bacteremia during that period. The demographics were similar between infants with polymicrobial compared to infants with monomicrobial bacteremia. As expected, the gestational age (30.5 vs. 30.4 wk, p = 0.90) and birth weights (1592 vs.1535 g, p = 0.77) were adequately matched between controls and cases respectively. Male sex and age at infection did not differ significantly between cases and controls.

In monomicrobial bloodstream infections, 102 organisms were isolated from 102 infectious episodes. In polymicrobial bloodstream infections, 74 organisms were isolated from 34 infectious episodes. 'Others' include Streptococcus salivarius, Citrobacter freundii, Clostridium tertium for monomicrobial infections and for polymicrobial infections; they were Lactobacillus, Proteus mirabilis, Streptococcus sanguinus, Micrococcus spp., Acinetobacter baumanii complex and Gamma Streptococcus. All the organisms in the 'Others' group was cultured at least twice from 2 different samples. Candida species, Enterococcus species and Klebsiella species were isolated at higher frequencies in polymicrobial infections than monomicrobial infections. The most common combination of polymicrobial organisms were CONS and Candida (n = 6) and CONS and Enterococcus fecalis (n =3).

We observed a significant difference between cases and controls in terms of surgery other than for NEC (p = 0.04) and any surgery (including NEC surgery) (p = 0.05, borderline significance). We observed an increase in the presence of a central venous catheter (97 vs. 91%, p =0.20) and incidence of direct hyperbilirubinemia (50 vs. 32%, p = 0.06) both of which did not reach statistical significance. We did not observe any significant differences in the proportion of infants receiving PN, days on PN, the incidence of NEC, surgery for NEC, congenital heart disease or congenital malformations between cases and controls.

We noted a significant difference in associated mortality between controls and cases (47 vs. 20%), odds ratio adjusted for 'any surgery', 4.3 [95% CI, 1.8 to 10.2] (p = 0.001). A significant increase in the duration of infection (culture positivity) was noted between cases and controls (2.91 vs. 2.07 days, p = 0.02). No significant differences were observed in length of hospital stay, BPD, IPPV at 36 corrected wks, CPAP at 36 corrected wks, HFOV, ECMO, PDA, severe IVH (IVH > grade 2), PVL, severe IVH or PVL, ROP or severe ROP (ROP > stage 2) (p > 0.05). An increase in INO therapy in cases were noted (41 vs. 25%, p = 0.08) but was not statistically significant.

In a subgroup analysis of VLBW infants, polymicrobial infections compared to monomicrobial infections, had a significantly higher mortality (OR 6.4 [95% CI 1.3 to 30.3], longer duration of infection (OR 1.4 [95% CI 1.04 to 1.8] and higher incidence of congenital malformations (OR 17.7 [95% CI, 2.1 to 148.7).

Discussion

We performed a case-control study of neonatal polymicrobial infections in a tertiary hospital in North America, and identified polymicrobial bloodstream infections in nearly 14% of bloodstream infections. We also probed the electronic neonatal clinical database over a four year period from January 2009 to December 2012 for relevant clinical data including risk factors and outcomes. We observed that polymicrobial bloodstream infections were associated with an increased mortality (>3-fold) and increased duration of infection compared to monomicrobial bloodstream infections. Surgical intervention excluding NEC surgery was a significant risk factor for neonatal polymicrobial infections.

The annual frequency of polymicrobial bloodstream infections in our study was approximately 14% over a 16 year study period. The frequency of neonatal polymicrobial infections reported in literature is variable, ranging from 3.9 to 25% [10-12,17,19-21]. Contaminated multi-dose lipid emulsions were responsible for the high frequency (25%) of polymicrobial infections in one study [20]. In most studies that report neonatal infections, the isolation of multiple organisms is often not discussed. The higher frequency of polymicrobial infections in our study compared to other reports in literature may be due to two reasons. First is the varying definition of polymicrobial infections in the different studies. We defined polymicrobial bloodstream infections as multiple organisms isolated during an infectious episode including those from a single blood specimen similar to Sutter et al., as we believe that 95% CI-95% confidence intervals, Bwt-Birth weight, GA-gestational age, PN-parenteral nutrition, NEC-necrotizing enterocolitis, BPD-bronchopulmonary dysplasia, IPPV-Intermittent positive pressure ventilation, CPAP-continuous positive airway pressure, HFOV-High frequency oscillatory ventilation, INO-inhaled nitric oxide, ECMO-extracorporeal membrane oxygenator, PDA-patent ductus arteriosus, ROP-retinopathy of prematurity, IVH-intraventricular hemorrhage, PVL-periventricular leucomalacia, severe IVH-IVH > grade 2, severe ROP-ROP > stage 2. *p values < 0.05. this is a true reflection of a polymicrobial infection [1]. 10% frequency reported in North American by Bizzarro et Other studies defined polymicrobial infections as multiple al. [11]. Secondly, increased incidence of polymicrobial pathogens isolated from a single blood specimen, which bloodstream infections in our study may also relate to the may account for the lower incidence of polymicrobial in-large percentage of medically complex infants referred to fections [11,12,17].

Using the latter definition the annual our NICU including infants requiring surgical intervenfrequency of polymicrobial bloodstream infections in our tions, complex congenital heart disease, ECMO, abdomstudy would be approximately 7.8%, comparable to the inal wall defects, short gut syndrome and other congenital anomalies. This complex group of infants often requires indwelling vascular catheters for parenteral nutrition or medications for extended periods of time. The annual frequency of polymicrobial bacteremia in the neonatal intensive care unit in our study varied from 6.3 to 18.6% of all bacteremia during the 16-year study period. This annual variation may be in part due to expansion of the NICU, changes in the case-mix and referral patterns, nursing policy changes or implementation of catheter care bundles. There were significant decrease in the number of infections and polymicrobial infections between the time epochs, 1998-2009 and 2010-2012 (p < 0.01). We did not note any significant differences in the number of admissions of VLBW and ELBW infants. A dedicated vascular access team was formed in June 2009 followed by better implementation of catheter care bundles in June 2009, which along with increased compliance with hand hygiene and other infection control measures may have contributed to the decrease in infections.

In our study, cases and controls had similar demographics, a mean gestational age of approximately 30 weeks, birth weight of approximately 1500 g, similar male to female ratio and similar percentage being inborn. We focused on risk factors reported for polymicrobial infections in existing literature. The average age at the onset of the infections was 41 days in controls and 38 days in cases and hence all infections were in the late onset sepsis category and mostly occurred beyond the first 28 days of life. Almost all of the infections were late-onset infections and hence we did not collect data on risk factors for early onset sepsis such as maternal prolonged rupture of membranes, group B streptococcus colonization, urinary tract infection or chorioamnionitis. Subgroups of infections in neonates less than 28 days of age or those that were admitted from home were too small for meaningful comparisons. We noted a significant association of polymicrobial infections with surgery other than NEC and a trend towards association with direct jaundice. Cases had a higher incidence of central venous catheter compared to controls (97 vs. 91%, p = 0.20) but this association was not statistically significant.

In our case-control study, we observed more than 3-fold increase in associated mortality in neonates with polymicrobial bacteremia, but the retrospective and database oriented nature of our study precludes any conclusions of causality. Mortality due to polymicrobial infections has been reported to be at least 2-fold more than that of monomicrobial infections both in adults and children [1,2]. Faix et al. reported that mortality due to polymicrobial infections in neonates is increased almost 3-fold in his study of 15 cases of polymicrobial infections in 1971-86 [10]. More recent studies by Bizzarro et al. (study period 1989-2006), Tsai et al (study period 2004 to 2011) and Gupta et al. (1 year period in early 2000) did not report an increase in attributable mortality to polymicrobial infections in their studies [11,12,17]. The variability in mortality rates reported by different studies on polymicrobial infections may be multifactorial including differences in study design, variable definitions of polymicrobial infections, patient population, study periods, virulence of the organisms isolated or other unidentified factors [11]. The mechanisms for increased mortality in polymicrobial infections are not clear. Similar increases in mortality are observed in

Table 2 Infant demographics, risk factors and outcomes in cases and controls

	Monomicrobial infections (n = 102), Mean [95% CI] or n (%)	Polymicrobial infections (n = 34), Mean [95% CI] or n (%)	p value	Odds ratio (OR) [95% CI]
Demographics				
GA (wks)	30.5 [29.9, 31.1]	30.4 [29.4, 31.3]	0.90	
Bwt (g)	1592 [1493, 1692]	1535 [1365, 1706]	0.77	
Male sex, n (%)	56 (54)	17 (50)	0.65	
Age at infection (days)	40.7 [35.8, 45.5]	37.7 [30.3, 45.0]	0.75	
Inborn (%)	43 (41.2)	14 (41.2)	1.00	
Risk factors				
Catheter, n (%)	93 (91)	33 (97)	0.20	3.6 [0.4 to 29.1]
PN, n (%)	92 (90.2)	30 (88.2)	0.74	
PN duration (days)	49.3 [43.9, 54.8]	47.5 [40.0, 55.1]	0.86	
NEC (%)	24 (23.5)	7 (20.6)	0.72	0.8 [0.3 to 2.2]
NEC surgery (%)	18 (17.6)	6 (17.6)	1.00	1.0 [0.4 to 2.8]
Surgery other than NEC (%)	55 (53.9)	25 (73.5)	0.04*	2.4 [1.0 to 5.6]
Any surgery (%)	56 (54.9)	25 (73.5)	0.05*	2.3 [1.0 to 5.4]
Direct hyperbilirubinemia (%)	33 (32.4)	17 (50.0)	0.06	2.1 [1.0 to 4.6]
Congenital heart disease (%)	28 (27.5)	9 (26.5)	0.91	1.0 [0.4 to 2.3]
Congenital malformations (%)	31 (30.4)	15 (44.1)	0.14	1.8 [0.8 to 4.0]
Outcomes				
Mortality (%)	19.6	47.1	0.001 *	4.3 [1.8 to 10.2] Logistic model adjusted for surgery
Hospital stay (days)	101.7 [93.6, 109.8]	100.5 [88.1, 112.9]	0.99	
Infection duration (days)	2.1 [1.9, 2.2]	2.9 [2.5, 3.3]	0.02*	
BPD (%)	35 (34.3)	9 (26.5)	0.40	0.7 [0.3 to 1.6]
IPPV at 36wks (%)	24 (23.7)	12 (35.3)	0.19	1.8 [0.8 to 4.1]
CPAP at 36wks (%)	8 (7.8)	4 (11.8)	0.49	1.6 [0.4 to 5.6]
HFOV (%)	33 (32.4)	10 (29.4)	0.75	0.9 [0.4 to 2.0]
ECMO (%)	16 (15.7)	6 (17.6)	0.79	1.2 [0.4 to 3.2]
INO (%)	26 (25.5)	14 (41.2)	0.08	2.1 [0.9 to 4.6]
INO or HFOV or ECMO (%)	52 (50.9)	18 (52.9)	0.84	1.1 [0.5 to 2.4]
PDA (%)	13 (32.4)	33 (38.2)	0.53	1.3 [0.6 to 2.9]
ROP (%)	12 (11.8)	4 (11.8)	1.00	1.0 [0.3 to 3.3]
Severe ROP (%)	7 (6.9)	3 (8.8)	0.70	1.3 [0.3 to 5.4]
ROP surgery (%)	6 (5.9)	2 (5.9)	1.00	1.0 [0.2 to 5.2]
Severe IVH (%)	18 (17.6)	5 (14.7)	0.69	0.8 [0.3 to 2.4]
Severe IVH or PVL (%)	19 (19.6)	5 (14.7)	0.52	0.8 [0.3 to 2.2]

95% CI- 95% confidence intervals, Bwt-Birth weight, GA-gestational age, PN- parenteral nutrition, NEC- necrotizing enterocolitis, BPD-bronchopulmonary dysplasia, IPPV- Intermittent positive pressure ventilation, CPAP- continuous positive airway pressure, HFOV- High frequency oscillatory ventilation, INO- inhaled nitric oxide, ECMO- extracorporeal membrane oxygenator, PDA-patent ductus arteriosus, ROP-retinopathy of prematurity, IVH-intraventricular hemorrhage, PVL- periventricular leucomalacia, severe IVH- IVH > grade 2, severe ROP- ROP > stage 2. *p values < 0.05.

animal models of systemic and local polymicrobial infections [22-25]. We have observed increased catheter infection and systemic dissemination in a poly-microbial biofilm catheter infection model compared to monomicrobial infection [11]. The increased mortality may also arise from inherent host vulnerability that causes the infection in the first place (eg prematurity, short gut) or facilitation of one infection by the other (synergism) [26]. Polymicrobial interactions in polymicrobial environments such as a mixed-species biofilms on catheters may induce a host of synergistic mechanisms including quorum sensing, induction of virulence among others that may contribute to enhanced mortality or morbidity of the host [12].

We also noted an increase in the duration of infection in cases compared to controls, which may be due to increased host susceptibility or a synergistic effect of the polymicrobial infection. We did not observe any differences in length of hospital stay or other clinically relevant neonatal outcomes such as BPD, IPPV or CPAP at 36 corrected weeks, HFOV, ECMO, ROP, NEC or IVH or PVL. We observed an increase in the use of INO therapy in cases compared to controls, which was not statistically significant.

The organisms isolated in our study were mostly similar in both monomicrobial and polymicrobial bloodstream infections,

similar in antibiotic susceptibility patterns and comparable to those organisms from polymicrobial infections reported in literature. The most common organisms isolated in both monomicrobial and polymicrobial infections were CONS, Staphylococcus aureus, Escherichia coli, Enterococcus species, Klebsiella spp. and Candida spp. Infections due to Candida spp., Enterococcus species and Klebsiella spp. increased in frequency in cases compared to controls. The most frequent polymicrobial combinations were CONS with Candida spp. and other organisms with Enterococcus faecalis, similar to reported literature [1,14,27]. Studies from Asia, by Tsai et al. and Gupta et al. report a high incidence of Gram negative infections (approximately 60%) in the polymicrobial group [12,17]. In the North American study by Bizarro et al., polymicrobial infections were preponderantly caused by Gram positive organisms (77%) similar to our study [11]. Geographic variations in organisms isolated from neonatal polymicrobial infections and their antibiotic susceptibility patterns may partly explain the variation in mortality and morbidity across the world due to polymicrobial infections. In our study we did not discern any differences in the treatment regimens used to treat polymicrobial infections and mono-microbial infections that could explain the differences in mortality. Polymicrobial infections were treated adequately for all the organisms isolated and duration of therapy was consistent with our written neonatal guidelines.

Limitations of our study include being a retrospective, observational, case-control study. Bloodstream episodes were identified from the clinical microbiology database and hence the clinical data associated with the infection or their severity were not available. The clinical risk factors and outcomes were identified from the clinical database, which was available only for a 4 year period. Being a retrospective study, it is difficult to assign a causal relationship to the increased mortality associated with polymicrobial infections in our study. We did not assess long term developmental or growth outcomes as longitudinal assessment data were not available.

The human microbiome project and other microbiome studies emphasize the polymicrobial nature of organism communities in the human body [28,29]. The availability of molecular culture-independent methods for detection of sepsis may increase the identification of polymicrobial infections [30]. Guidelines or recommendations for therapeutic and preventive strategies against polymicrobial infections do not exist. Prolonged therapy with antibiotics targeting all organisms isolated in a polymicrobial infection and removing infected catheters remain the mainstay in therapy. Defining the epidemiology and clinical impact of polymicrobial infections may be the first step towards delineating optimal therapy and clinical outcomes. Preventive strategies should emphasize catheter care bundles that decrease central line associated bloodstream infections. Focused research is necessary to prevent and treat polymicrobial infections to improve clinical outcomes in the vulnerable neonate.

Conclusions

The main aim of the study was to investigate the frequency of polymicrobial infections in our tertiary neonatal intensive care unit, understand its impact on neonatal outcomes and promote research in the management of these infections. Polymicrobial bacteremia is common in neonates and comprises nearly 14% of all infectious episodes. The most common organisms in neonatal polymicrobial infections are CONS, S. aureus, Enterococcus species, E. coli, and Candida species. Surgical intervention is a significant risk factor for neonatal polymicrobial infections. We observed a more than 3-fold increase in mortality in infants with polymicrobial bacteremia and a significant increase in duration of the bacteremia. Research focused to prevent and to improve clinical outcomes in neonatal poly-microbial infections is urgently needed.

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Epidural Analgesia, Neonatal Care and Breastfeeding

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Abstract

The objective of our study is to evaluate the correlation between epidural analgesia during labor, start of breastfeeding and type of maternal-neonatal care. Two different assistance models were considered: Partial and Full Rooming-in. In this cohort study, 2480 healthy infants were enrolled, 1519 in the Partial Rooming-in group and 1321 in the Full Rooming-in group; 1223 were born to women subjected to epidural analgesia in labor. In case of Partial Rooming-in the rate of exclusive or prevailing breastfeeding is significant more frequent in newborns born to mothers who didn't receive analgesia. Instead, in case of Full Rooming-in the rate of exclusive or prevailing breastfeeding is almost the same and there's no correlation between the use or not of epidural analgesia. The good start of lactation and the success of breastfeeding seems to be guaranteed by the type of care offered to the couple mother-infant, that reverses any possible adverse effects of the use of epidural analgesia in labor.

Background

Breastfeeding provides optimal nutrition for infants and improves maternal health. Some studies attribute to breastmilk the reduction of the frequency and severity of neonatal infectious diseases and a protective function against sudden infant death syndrome, diabetes, lymphomas, allergies, and chronic digestive diseases. Lactation explicates several benefits for mothers because it seems to reduce the incidence of postpartum bleeding and the risk of ovarian and breast cancer, it favors the return to pre-pregnancy body weight and improves bone remineralization [1,2].

Therefore the American Academy of Pediatrics (AAP) recommends breastfeeding for almost the first six months of life [3].

In recent years there was an increase of epidural analgesia for pain management during labor. Several studies tried to find an association between epidural analgesia and breastfeeding. Most of these studies are retrospective, observational and nonrandomized, and the confounding variables make the results unreliable and sometimes conflicting [4-10].

Recently some authors focused their studies on type of assistance given to newborns, analyzing mother-child relationship during the first days of life, and they don't consider epidural analgesia a risk factor for breastfeed [2,11-16].

The objective of our study was to evaluate the correlation between epidural analgesia during labor, start of breastfeeding and type of maternal-neonatal care. Primary outcome was the type of feeding assessed from the 48th to the 72nd hour of life, we also evaluated the influence of labor analgesia on neonatal Apgar score.

Methods

In this cohort retrospective study conducted for a period of two years, 2840 healthy infants born at the 'A. Gemelli' General Hospital (Rome) with gestational age (GA) ≥37 weeks were eligible. They were delivered vaginally after uncomplicated pregnancies. Most of the mothers were middle-class and graduated. Exclusion criteria were Apgar score <7 at 1 or 5 min and high risk pregnancy. Two different assistance models were considered to encourage and support breastfeeding by all eligible mothers:

- Partial Rooming-in (PR-I): newborns were in mother's room from 10 am to 8 pm, with the occasional presence of the nurse. At night newborns were transferred and assisted in the nursery to offer the necessary care by the caregivers.
- Full Rooming-in (FR-I): newborns and mothers were together all day long, with continuous assistance of the nursing staff, to make the neonatal-care easier for the mother and to assess the appropriateness of breastfeeding. The access to full rooming-in was possible only if it was available an appropriate room, without planning in advance [17].

Nowadays epidural analgesia is implemented in refracted bolus or in continuous infusion combining local anesthetics (Bupivacaine or Ropivacaine) with opioids (Fentanyl or Sufentanil) [18].

In our hospital laboring women received epidural analgesia with an initial bolus 18–20 ml of Sufentanil 10 µg plus Ropivacaine 0.10%, until reaching analgesic block and repeated bolus of Ropivacaine 0.15-0,2% of 8–10 ml every two hours, to maintain the analgesic block. The initial bolus was given only if there were

AAZ: conception and design, overall responsibility. RR: analysis and interpretation. MC: data collection. AI: writing the article. FC: statistical analysis. CR: obtained funding. All authors read and approved the final manuscript.

painful and effective contractions, of appropriate intensity and frequency, and if the fetal head was at the level of the uterine cervix.

Type of feeding was assessed from the 48th to the 72nd hour of hospitalization of the newborns involved in the study. Type of feeding was distinguished, according to WHO-guidelines [19] in:

- Exclusive Breastfeeding (EB): infant received only breast milk from mother and no other liquids or solids, with the exception of drops or syrups containing vitamins, mineral supplements or medicines;
- Prevalent Breastfeeding (PB): Newborns were mostly breastfed, but they could also have received water or other water-based liquids, (sweetened and flavored water teas, infusions);
- Mixed Feeding (MF): Infants received formula as integration to maternal milk;
- Artificial Feeding (AF): Newborns did not receive breast milk but only formula.

All data were retrospectively collected and stored on a database. Continuous variables were presented as mean ± standard deviation (SD) and categorical variables as percentage. Comparisons with the variables were performed using Student's T-test in case of normal distribution. Significance was accepted at $p < 0.05$. Statistical analysis was performed using the software Graph Pad 4.

A logistic regression was performed to evaluate the risk "mixed or artificial feeding in infants between 48th and 72nd hour of life" with regard to the independent variables: mother's parity, assistance model (partial or full rooming-in) and use of epidural analgesia during labor. The findings are presented as odds ratio (OR) with 95% confidence intervals (CI), standard errors and p-values. The analyses were performed using the "Stata Statistical Software: Release 10" (StataCorp LP, College Station, Tx).

Results

2840 term newborns, by vaginal childbirth have been enrolled: 1617 were born to women not subject to epidural analgesia in labor (No Analgesia group); 1223 were born to mothers subjected to epidural analgesia in labor (Epidural Analgesia group). There were no differences between the groups studied with respect to maternal age, gestational age and parity (Table 1).

Table 1. Demographic characteristics

	No Analgesia group			Epidural Analgesia group			P value
Gestational Age (weeks)	mean ± ds	39.1 ± 1.01		mean ± ds	39.4 ± 3.59		Ns*
	range	37 – 41		range	37 – 41		
Maternal Age (aa)	mean ± ds	32.9 ± 6.3		mean ± ds	32.4 ± 5.5		Ns*
	range	20 – 45		range	18 – 44		
Parity		N°	%		N°	%	
	Primiparous	885	54.8	Primiparous	680	55.6	Ns*
	Multiparous	732	45.2	Multiparous	543	44.4	

* ns= not significant.

Apgar score did not show any difference between the two groups, nor at one minute or at five minutes of life.

The newborns studied received milk from the 48th hour to the 72nd hour of life as follows: 2024 (71,3%) newborns received exclusively or predominantly breastmilk, 801(28,2%) received breastmilk with integration of formula, 15 (0,6%) were fed exclusively with formula.

Considering the use or not of epidural analgesia, the rates of newborns exclusive or prevailing breastfed are higher in the first group (73,5% in "no Analgesia group" vs 68,3% in "Analgesia group", p = 0,002). Mixed feeding is less frequent than exclusive or prevailing breastfeeding but its prevalence is higher in the second group (25,9% in "no Analgesia group" vs 31,2% in "Analgesia group", p = 0,002) (Table 2).

Table 2. Type of feeding in the two studied groups.

Type of feeding	No Analgesia	Epidural analgesia	P value
AF	9 (0.6%)	6 (0.5%)	Ns*
MF	419 (25.9%)	382 (31.2%)	0.002
EB o PB	1189 (73.5%)	835 (68.3%)	0.002

* ns= not significant.

Partial Rooming-in was the assistance model for 1519 newborns (53,5%) and Full Rooming-in for 1321 (46,5%). Prevalence of breastfeeding was higher in Full than in Partial Rooming-in (92,2% vs 53,1) (Table 3).

Table 3. Type of feeding and assistance model.

Type of feeding	Partial Rooming-in	Full Rooming-in
AF	0.8%	0.2%
MF	46.1%	7.6%
EB o PB	53.1%	92.2%

Stratifying infants by model of assistance and differentiating between the two groups "No Analgesia" vs "Epidural Analgesia" we note that in case of Partial Rooming-in the rate of exclusive or prevailing breastfeeding is significant more frequent in newborns born to mothers who didn't receive analgesia (p <0,001; 59,2% vs 44,1%). Instead, in case of Full Rooming-in the rate of exclusive or prevailing breastfeeding is almost the same and there's no correlation between the use or not of epidural analgesia (p <0,001; 91,7% vs 92,8%) (Tables 4 and 5).

Table 4. Type of feeding in the two studied groups in partial rooming-in assistance.

Type of feeding	No analgesia	Epidural analgesia	P value
AF	8 (0.9%)	4 (0.7%)	Ns*
MF	361 (39.9%)	340 (55.3%)	< 0.001
EB o PB	535 (59.2%)	271 (44.1%)	< 0.001

* ns= not significant.

Table 5. Type of feeding in the two studied groups in full rooming-in assistance.

Type of feeding	No analgesia	Epidural analgesia	P value
AF	1 (0.1%)	2 (0.3%)	Ns*
MF	58 (8.1%)	42(6.9%)	Ns*
EB o PB	654 (91.7%)	564 (92.8%)	Ns*

* ns= not significant.

Multivariable analyses

In partial rooming-in assistance, newborn were more likely to have received mixed or artificial feeding between the 48th and the 72nd hour of life (OR 10.99, p < 0.0001, 95% CI 8.76-13.79). Mixed or artificial feeding between the 48th and the 72nd hour of

life is also associated, but to a lesser extent, with maternal use of epidural analgesia during labor (OR 1.61, p < 0.0001, 95% CI 1.34-1.94). Maternal parity, instead, is not associated with type of newborn feeding (OR 0.99, p > 0.05, 95% CI 0.78-1.08) (Table 6).

Table 6. Multivariable analyses to evaluate risk of "mixed or artificial feeding in infants between 48th and 72nd hour of life" with regard to independent variables considered.

Comparison	Odds ratio	95% confidence interval	P value
Partial rooming-in vs full rooming-in	10.99	8.76 – 13.79	0.0001
Epidural analgesia vs no analgesia	1.61	1.34 – 1.94	0.0001
Primiparous vs multiparous	0.99	0.78 – 1.08	Ns*

* ns= not significant.

Discussion and conclusion

To minimize the maternal discomfort and its effects for the fetus, in recent years the use of epidural analgesia during labor is increased. The objective of this study was to determine whether epidural analgesia interfere with the start of breastfeeding in the first days of life.

The effects that drugs used in epidural analgesia may cause to fetus can be direct, which are rare and especially due to overdose of the drugs used, and indirect, linked to the physiological and biochemical changes that they determine in the mother that may indirectly affect the fetus and newborn.

As evidenced by Halpern et al. the use of epidural opioid analgesia is associated with greater incidence of higher Apgar scores at 1 'and 5' and to a lesser need of neonatal resuscitation and use of Naloxone than with systemic opioid analgesia [2]. Some authors have focused on the influence of epidural analgesia on starting breastfeeding.

The results of many studies in recent years are largely conflicting. First of all, the problem was whether the reduction of pain with epidural analgesia could affect breastfeeding. Some studies suggested that this is associated with a delayed start of lactation and to an earlier suspension, others have found no association. Halpern and co-workers in their observational study reported that analgesia in labor is not associated with a reduction of breastfeeding in their study population at 6–8 weeks postpartum [2,20]. In their work, rather, it is highlighted the importance of the promotion of breastfeeding in hospital management, as reaffirmed in 2002 by Leighton [21].

In our hospital there is a constant effort to promote breastfeeding, supporting early mother-infant relationship, increasing the rooming-in and the breastfeeding on demand.

Even Albani and colleagues in 1999 reported the absence of adverse effects of epidural analgesia on breastfeeding, with the same rate of breastfeeding at 1, 4, 6 weeks post-partum in case of use or not of epidural analgesia [22].

In a study of 2010 Reynolds and co-workers concluded that while epidural analgesia in labor may be associated with some short-term side effects, its effects on the child, when compared with systemic analgesia, are better for Apgar scores, acid–base balance and breastfeeding [13]. Beilin and colleagues performed the only randomized controlled trial to evaluate the dose-dependent effect of epidural fentanyl on breastfeeding success [23]. They showed that epidural analgesia with fentanyl at high dosage (>150 µg) associated with local anesthetics, compared with epidural analgesia with lesser amounts of fentanyl, is more often associated with stopping breastfeeding before 6 weeks of life.

Wilson and colleagues, in their randomized controlled trial, confirmed that women who did not receive epidural analgesia did not show higher rates of breastfeeding compared with women receiving epidural analgesia. In fact, there is a lower rate of breastfeeding in women not undergoing epidural analgesia but pethidine systemically [20].

In our study we have observed that the use of epidural analgesia does not adversely affect the Apgar score at one or five minutes of life: there are values comparable between the groups. As regards breastfeeding, we considered the women who breastfed their children between 48th and 72nd hour of life. We noted that, on the general population, there is a prevalence statistically significant of breastfeeding in the group of women who didn't undergo epidural analgesia. This result, however, is strongly due to the assistance model used. It's important to analyze type of feeding in relation to the type of hospitalization model, total or partial Rooming-in. In the first case there was a prevalence of exclusively or predominantly breastfeeding in both groups. Instead, in case of partial Rooming-in, we see a prevalence of exclusively or predominantly breastfeeding in the group of mother who didn't undergo epidural analgesia and this was statistically significant.

Although multivariate analysis indicates the use of epidural analgesia as an independent factor which interferes on breastfeeding, the protective effect of the type of rooming in seems to be very high and higher than the risk determined by epidural analgesia itself, but these results should be confirmed on other surveys.

As stated by Wieczorek and Pandya in their recent studies [24,25], we believe that the difference in the type of feeding is not due to the influence of analgesia used but rather to the type of Rooming-in carried out, total or partial, and to the clinical monitoring necessary to neonatal well-being. It seems, essential to adopt a model of care that favors an early mother-infant relationship and ensure its continuation with total Rooming-in.

Therefore, in full rooming-in, with an early and continuous mother-infant contact, there are no differences in the type of breastfeeding among the group of infants born to mothers who underwent epidural analgesia and the group of newborns born to mothers not subjected to analgesia. Furthermore if we consider only the group of infants born to mother that underwent epidural analgesia there is a high prevalence of exclusive or prevailing breastfeeding in full rooming-in model assistance compared with partial rooming-in, with a frequency of 92.8% vs 44.1%.

We can conclude that the good start of lactation and the success of breastfeeding seems to be guaranteed by the type of care offered to the couple mother-infant, that reverses any possible adverse effects of the use of epidural analgesia in labor.

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In Vitro Growth of Plasmodium Falciparum in Neonatal Blood

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Abstract

Background: Children below the age of six months suffer less often from malaria than older children in sub-Saharan Africa. This observation is commonly attributed to the persistence of foetal haemoglobin (HbF), which is considered not to permit growth of Plasmodium falciparum and therefore providing protection against malaria. Since this concept has recently been challenged, this study evaluated the effect of HbF erythrocytes and maternal plasma on in vitro parasite growth of P. falciparum in Central African Gabon.

Methods: Umbilical cord blood and peripheral maternal blood were collected at delivery at the Albert Schweitzer Hospital in Gabon. Respective erythrocyte suspension and plasma were used in parallel for in vitro culture. In vitro growth rates were compared between cultures supplemented with either maternal or cord erythrocytes. Plasma of maternal blood and cord blood was evaluated. Parasite growth rates were assessed by the standard HRP2-assay evaluating the increase of HRP2 concentration in Plasmodium culture.

Results: Culture of P. falciparum using foetal erythrocytes led to comparable growth rates (mean growth rate = 4.2, 95% CI: 3.5 – 5.0) as cultures with maternal red blood cells (mean growth rate =4.2, 95% CI: 3.4 – 5.0) and those from non-malaria exposed individuals (mean growth rate = 4.6, 95% CI: 3.8 – 5.5). Standard in vitro culture of P. falciparum supplemented with either maternal or foetal plasma showed both significantly lower growth rates than a positive control using non-malaria exposed donor plasma.

Conclusions: These data challenge the concept of HbF serving as intrinsic inhibitor of P. falciparum growth in the first months of life. Erythrocytes containing HbF are equally permissive to P. falciparum growth in vitro. However, addition of maternal and cord plasma led to reduced in vitro growth which may translate to protection against clinical disease or show synergistic effects with HbF in vivo. Further studies are needed to elucidate the pathophysiology of innate and acquired protection against neonatal malaria.

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Background

Although children below five years of age are disproportionally severely affected by malaria morbidity and mortality, there is a lag time of about three months after birth, before first disease episodes occur [1-4]. This period coincides with the persistence of maternal IgG antibodies in the infant's circulation but other protective mechanisms may also contribute to the reduced susceptibility [4-7].

Previous studies postulate a role for persisting foetal haemoglobin during the first months of life in protection against malaria [8]. The transcription of HbF starts around the 10th week of development and ends shortly before birth. This causes a linear decline in the number of foetal red blood cells from up to 90% at time of birth to around 5% at 3 months of age [8]. It was hypothesized that parasite strains — encountering HbF only on relatively rare occasions from an evolutionary perspective — may therefore be selected by strong adaptation to adult haemoglobin (HbA), a process resulting in limited intraerythrocytic growth of Plasmodium falciparum in predominantly HbF containing neonatal blood [9,10]. This paradigm of protection during early infancy — first published in 1977 — was held up since then until recently, when Amaratunga et al. failed to detect growth delays in neonatal erythrocytes and thus profoundly challenged this concept [11].

To further investigate potential mechanisms of protection, this study evaluated the comparative in vitro growth rates of a standardized P. falciparum clone under controlled culture conditions using either maternal, cord, or non-malaria exposed donor erythrocytes.

In addition, the growth modulating effect of maternal, cord, and non-malaria exposed donor plasma on P. falciparum growth was evaluated.

Methods

Study region and patient population

The study took place at theCentrede Recherches Médicales de Lambaréné, Albert Schweitzer Hospital and Georges Rawiri Regional Hospital in Lambaréné, Gabon. Gabon is a Central

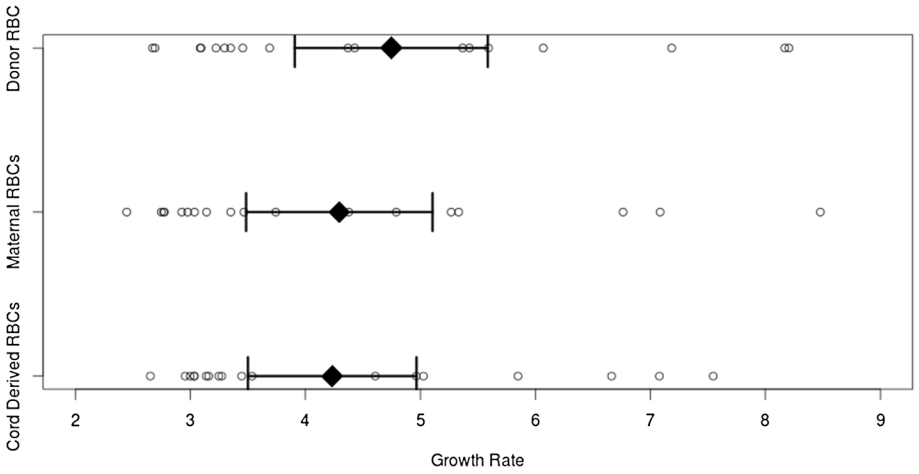

Figure 1. In vitro growth rates of Plasmodium falciparum when using cord, maternal or non-malaria exposed donor erythrocytes displayed by data points, means and 95% CIs.

African country characterized by a tropical climate and hyperendemic malaria transmission [12]. Participants were invited to join this study when attending the local maternity wards for delivery. Demographic data were obtained, however no validated information about intake of IPTp was available. All subjects were tested in routine antenatal care for HIV and only those with negative test result were invited to participate. The study protocol was approved by the institutional review board of the CERMEL and the ethical review committee of the Medical University of Vienna and all women provided written informed consent prior to blood sampling.

All samples were tested for sickle cell trait by haemoglobin electrophoresis and for plasmodial infection by thick smear at the time of delivery and samples were excluded in case of a positive result or when signs of haemolysis or clotting were apparent. At delivery 1.2 ml of blood was taken from the umbilical cord in an EDTA tube. Peripheral maternal venous blood was taken at the earliest convenience within three days. Non-malaria exposed donor blood was obtained from Caucasian male volunteers. Blood samples were collected in EDTA tubes and specimens were immediately centrifuged to separate red blood cells from plasma. Red blood cells were washed three times in complete parasite medium and stored at 4°C in 0.5 volume of saline adenine glucose-mannitol (150 mM NaCl, 1.25 mM adenine, 45 mM glucose, 30 mM mannitol) until further use. Plasma was immediately frozen at –80°C.

Parasite culture and growth assay

A laboratory-adapted clone of P. falciparum (3D7) repeatedly selected for presence of knob-phenotype was kept in continuous sorbitol synchronized culture throughout the course of the study. Parasites were maintained using a standard protocol in complete parasite medium (500 ml RPMI-1640, 10 mg/l gentamicin, 6 g/l (25 mM) HEPES, 292mg/l(2mM) L-glutamine, 50 mg/l (0.36mM) hypoxanthine, 5 g/l Albumax II) in a candle jar at 37°C. To test the effect of erythrocytes and plasma on plasmodial growth, micro-cultures of 200 µl each were established in 96-well plates (Corning Costar-3599) in duplicates. To investigate the effect of HbF on parasite growth, micro-cultures containing cord derived red blood cells and maternal red blood cells, respectively, as well as a positive control containing donor O + red blood cells, were set up in duplicates. Parasites derived from continuous culture in adult RBC were diluted with respective test RBC (>1 : 40) to obtain at 1.5% haematocrit, 0.05% parasitaemia using 5% pooled, heat inactivated serum from AB + donors for 72 hours before growth was stopped by freezing.

Plasma derived from mothers, newborns and a malaria-naïve adult was added to cultures in complete parasite medium to assess its effect on parasite growth at 1.5% haematocrit, 0.05% parasitaemia and 10% plasma supplementation. Frozen lysates of micro-cultures were used for histidine-rich protein II enzyme linked immunosorbent assay (HRP2 ELISA) for the quantification of parasite growth [13-15]. Commercial antibodies (MPFM-55A and MPFG-55P, Immunology Consultants Laboratories, Inc) and high binding plates (Corning Costar 3590) were used. Growth rates of P. falciparum were estimated by the assessment of HRP2 concentrations in cultures. Growth was defined as an increase in optical density in the HRP2 ELISA. An at least two fold increase in HRP2 concentration from prior to post incubation was set as threshold for further analysis. Statistical analysis was performed using "R" software. Differences between groups were assessed employing the Tukey's Honestly Significant Difference test. All reagents, unless stated otherwise were obtained from Sigma-Aldrich, St. Louis.

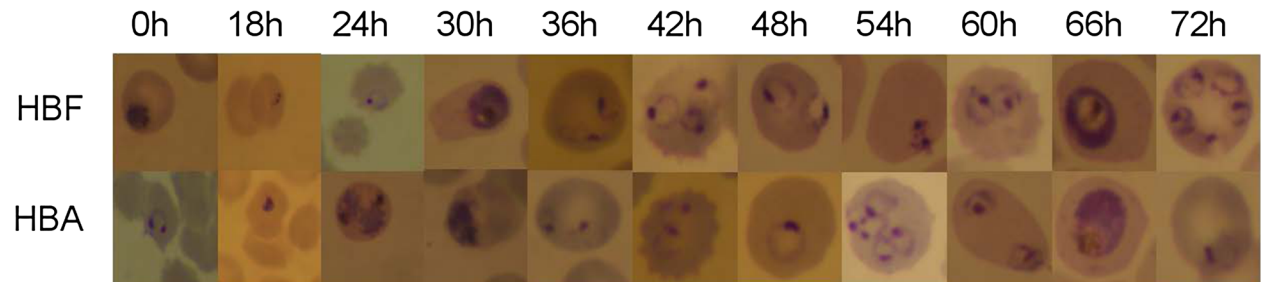

Figure 2. In-vitro development of Plasmodium falciparum over time in erythrocytes rich in foetal and adult haemoglobin respectively.

Table 1 Mean growth rates and lower and upper margin of 95% CIs in test given test erythrocytes of high and low growth strata as well as the cumulative data

All data	Lower margin	Mean	Upper margin
Cord derived RBC	3.50	4.23	4.97
Maternal RBC	3.38	4.19	5.00
Non malaria exposed donor RBC	3.79	4.63	5.47
Low growth			
Cord derived RBC	4.63	5.36	6.08
Maternal RBC	4.63	5.43	6.23
Non malaria exposed donor RBC	5.42	6.09	6.76
High growth			
Cord derived RBC	3.01	3.11	3.21
Maternal RBC	2.81	2.95	3.10
Non malaria exposed donor RBC	3.02	3.17	3.33

Results

Hundred and three pregnant women consented to participate in this study between July 2012 and June 2013, of which sixty-five were excluded from further testing (sickle cell trait: 12, clotting: 27, haemolysis after sampling: 13, Plasmodium infection: 7, presence of anti-Rhesus antibodies: 6). Thirty-eight participants finally met the criteria for analysis. Mother's age was ranging from 17 to 42 years with a mean of 25 years and a standard deviation of 7.1 years with a mean gravidity of 3.5 with a standard deviation of 2.4. Mean gestational age at delivery was 38.5 weeks of gestation with a standard deviation of 2.1 weeks and mean birth weight was 2,866 grams ranging from 1,900 to 4,400 grams. Among the recruited women, 18 paired erythrocyte samples and 22 plasma specimens were successfully used in parallel for further in vitro testing.

Maternal, neonatal, and non-malaria exposed donor erythrocytes

Parasite growth was quantified by HRP2 ELISA. 18 paired samples of maternal, cord and donor RBCs showed adequate growth and were used for comparative evaluation. Mean cumulative growth rates were 4.35 (95% confidence interval: 3.90 – 4.81) folds when compared with pre-incubation samples. No significant difference between cultures using non-malaria exposed donor erythrocytes (mean growth rate: 4.6, 95% CI: 3.8 – 5.5) or either maternal (4.2, 95% CI: 3.4 – 5.0) or cord derived erythrocytes (4.2, 95% CI: 3.5 – 5.0) was observed (Figure 1). Similarly, growth rates between maternal and cord derived erythrocytes supplemented cultures did not differ (p = 0.99). To test these findings under different culture conditions, cultures were classified in high growth (>4 fold increase) and low growth strata (2–4 fold increase in HRP2). When comparing growth rates within these strata, again no significant difference was observed (Table 1).

In addition to this quantitative analysis, a qualitative analysis of intra-erythrocytic growth of P. falciparum was performed microscopically. Parasites growing in umbilical cord erythrocytes displayed microscopically similar maturation characteristics and no other obvious morphological differences were observed (Figure 2).

Maternal, neonatal, and non-malaria exposed plasma

Growth rates of P. falciparum were compared under standard conditions by supplementing culture medium with either maternal or cord derived plasma and non-malaria exposed donor plasma serving as a positive control. Plasmodium falciparum growth was significantly higher in non-malaria exposed donor plasma containing culture medium than in either culture supplemented with test plasma (Figure 3). Whereas the mean increase in HRP2 concentration in culture supplemented with malaria-naïve plasma was 6.8 fold, growth rates in cultures supplemented with maternal and umbilical cord plasma averaged 2.1 fold (p <0.001). Umbilical cord plasma (mean growth rate: 2.1, 95% CI: 2.0 -2.3) and maternal plasma (mean growth rate: 2.1, 95% CI: 2.0 2.2) yielded close to identical results. (p =0.93)

Discussion

This study demonstrates that cord derived erythrocytes, harbouring a high concentration of HbF, are equally suited for P. falciparum invasion, maturation, and growth as adult erythrocytes containing predominantly HbA. Growth rates between maternal, neonatal and non-malaria exposed donor erythrocytes were very similar, and no morphological signs of growth inhibition were detected. These findings provide strong evidence that P. falciparum may similarly develop within HbF rich red blood cells and that HbF per se is no protective factor against malaria during the first months of life.

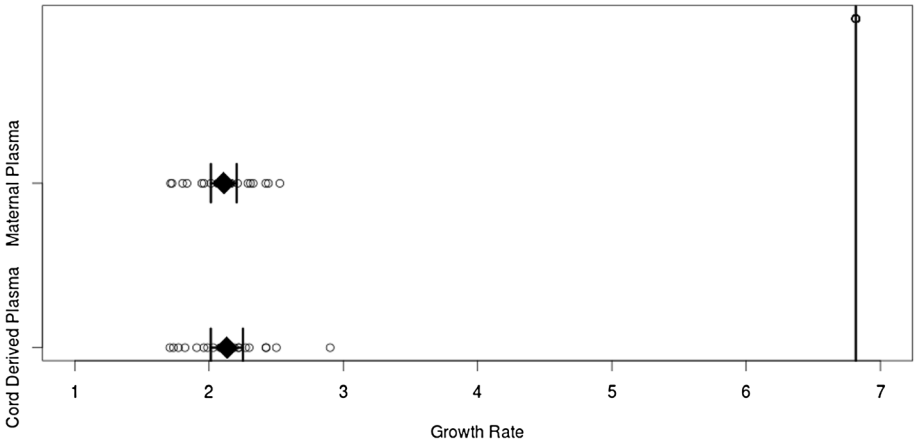

Figure 3. In vitro growth rates of Plasmodium falciparum when supplemented with either cord or maternal plasma displayed by data points, means and 95% CIs. Non-malaria exposed donor plasma used as a positive control yielded a growth rate of 6.8, indicated by the vertical bar.

These data are in line with results published by Amaratunga et al. [11] demonstrating normal invasion and development of P. falciparum in HbF containing red blood cells in a set of adult and six cord blood specimens. While this study focused on an overall assessment of parasite growth in a larger sample size, Amaratunga et al. could detect effects of neonatal haemoglobin, in particular in conjunction with immune IgGs, on the knob phenotype and PfEMP-1 mediated cytoadherence of parasitized red blood cells. Although erythrocytes rich in foetal haemoglobin seem to be permissive to infection with P. falciparum in vitro, reduced cytoadherence may, particularly in the presence of IgG, lead to lower parasite densities in vivo due to increased clearance of infected cells in the spleen.

To evaluate whether humoral immunity and transfer of maternal immunoglobulins or other plasmatic factors may play a protective role in newborns, cultures were supplemented with the respective plasma samples. Interestingly, a consistent growth-inhibition was observed in both maternal and neonatal plasma when compared to non-malaria exposed donor plasma. In vitro growth inhibition caused by a supplementation with immune plasma was reported previously in Thai individuals yielding comparable results to this study [16].

It is tempting to speculate that passive transfer of maternal antibodies to the newborn accounts for this growth inhibitory effect. However, it may not be excluded that other factors including cytokines, hormones, complement or indeed drugs with anti-malarial activity passing the placental barrier into the neonatal blood may have caused this inhibition of growth. One limitation of this study is the lack of valid information about IPTp intake of study participants — a factor potentially affecting plasma assays. In addition these data derive from experiments with one clone of P. falciparum and potential differences between wild isolates and laboratory adapted parasites may not be ruled out. Finally, behavioural factors certainly also contribute to young infants' protection from malaria, since neonates receivespecial careand attentionbytheir caregivers potentially leading to less exposure to infectious bites.

The amount of protection against malaria in early infancy might yet be caused by the interplay of several distinct factors, probably in conjunction with maternally derived antibodies. However, these data conclusively refute the hypothesis of HbF serving as an intrinsic inhibitor of P. falciparum growth in vitro, and indicate that maternal and newborn plasma show considerable inhibitory activity against in vitro growth of P. falciparum.

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The Characterization of Noise Levels in a Neonatal Intensive Care Unit and the Implications for Noise Management

Juan Carlos Fortes-Garrido, Andres Mauricio Velez-Pereira, Manuel Gázquez, Montserrat Hidalgo-Hidalgo and Juan Pedro Bolívar

Abstract

Background: The effects of noise are particularly harmful for the newborns, and therefore this study assesses and characterizes noise levels in a neonatal intensive care unit (NICU) in a medium-size hospital in the city of Huelva with the aim of optimizing the management and quality of care for newborns.

Methods: The equivalent continuous sound level was recorded as A-weighting curves using Type I sound level meters with levels measured during 100 milliseconds along to 15-day period in the both critical (in and out of incubators), and intermediate care units from a medium-size hospital. These devices were attached to a central beam 80 cm below the ceiling and into one of the incubators.

Results: The maximum noise levels measured for critical (C-in), C(out) and intermediate (I) were: 88.8 dBA, 97.2 dBA and 92.4 dBA, respectively, while for the equivalent noise levels for the total measuring period (15 d) were 57.0 dBA, 63.7 dBA, and 59.7 dBA, respectively. The Fourier frequency analysis has demonstrated several typical periods related to both work activities and family visit, which were: 7 days, 24 h, 12 h, and 3 h.

Conclusions: The statistical analysis revealed a clear correlation between the noise level, the kind of care room, and the time of the day. The results show that the values recommended by international bodies and agencies (AAP, WHO) are surpassed by a large margin, thus making it crucial that certain norms are followed in order to reduce the noise level in the NICU, by means of physical alterations to the layout, and raising awareness of health care personnel and visitors in order to encourage noise prevention in the daily care work and conversation. And finally, has been demonstrated that by applying the t-Student test the mean noise values in both wards are significantly different, which leads us to state that the noise level for the critical wards are higher than in the intermediate care ward.

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Background

Recent studies on the quality and risks associated with admission to a medical center have established a relation between the increase in morbidity and mortality, in addition to alterations in the quality of life following discharge from the health center which are usually linked to greater exposure to environmental pathogens, a lowering of the body's defenses and an increase in invasive techniques, among other reasons. Many authors have found a high correlation between noise levels in NICU and adverse effects arising from sleep disorders, underdevelopment of responses to stimuli, deterioration of the nervous system, etc. [1-6]. The noise that occurs in the NICU is linked to the monitoring and follow-up processes of the newborn's state of health, such as alarm systems, electro-medical equipment and incubators, and the general human ambient noise [7].

Several studies have measured the noise levels in NICU, finding very different values depending on the management of the work activities or customs of the place, with equivalent continuous levels in ranges from about 55 up to 83 dBA. These noise levels are high enough to stimulate the newborn's endocrinal and cardiovascular systems, resulting in significant alterations in sleep patterns [8-11]. Bushch-Vishniac et al. [12] determined that the average noise level for the John Hopkins Hospital has risen in the last 45 years by around 0.40 dBA a year, mainly due to the increase in audible alarm systems, the installation of air conditioning, control and surveillance systems and electro-mechanical therapeutics.

In terms of the legal framework, the World Health Organization (WHO) recommends that the newborn inside an incubator should not be exposed to noise levels higher than 35 dBA at night and 40 dBA during the day [13]. As proposed by the US Environmental Protection Agency and supported by the American Academy of Pediatrics (AAP) Committee of Environmental Health, noise levels within the NICU should be kept below 45 dB [14]. More specifically, the AAP recommends that hourly, a NICU's loudness equivalent (LAeq) should be below 50 dBA, the sound level that is exceeded 10% of the time (L10) should be at or below 55 dBA, and the maximum sound (Lmax) should be below 70 dBA [15]. In Spain, the Standards Committee of the Neonatology Society of the Spanish Pediatrics Association recommends a total background noise level in NICU

Figure 1. Location of sound level meter in the NICU critical care ward for newborns.

of no more than 55 dBA, and should not exceed 70 dBA [16]. However despite these recommendations, noise levels routinely oscillate between 65 dBA and 85 dBA, normally at low frequency [17].

In this study are correlated the temporal variations of the noise with its causes (Monitoring Systems, work activities, family visits, medical rounds, etc.). Spain is the second noisiest country in the world after Japan [18], according to the World Health Organisation (WHO), so this study is particularly important due to customs and the high noise level in the daily activities of the Spanish people.

With this in mind, the aim of our study is to identify, assess and make a space-time characterization of the noise levels in the NICU of a typical medium-sized hospital in Spain to find the main correlations with the noise sources, and, as consequence, to establish the right protection measures for noise reduction and elimination.

Methods

The study area is the NICU of the Juan Ramón Jiménez Hospital (HJRJ) in the city of Huelva (southwestern Spain). It is a general public hospital which also has specialist units. The NICU has 31 care units for newborns divided into three levels of medical attention: NICU-C has 9 incubators for newborns in a critical condition. NICU-I is an intermediate intensive care ward with 10 beds and the NICU-M has 12 beds for newborns requiring minimum intensive care.

The continues monitoring was developed for a period of 15 days and each individual noise measurement was made in time of 0.1 s in fast mode, in the NICU-C critical care ward (Figure 1) and the NICU-I intermediate care ward (Figure 2) since newborns in these two units are the most sensitive to noise. A sound level meter was placed in the NICU-C ward 80 cm below the ceiling and 153 cm from the wall. A second meter was placed in the NICU-I ward along the central beam 80 cm below the ceiling and 215 cm from the wall because it is the central point of the NICU, in order to achieve a representative point of the noise. A third meter was placed in the incubator. In line with regulation UNE-EN ISO 1996-1:2005, the equivalent continuous noise level was measured in A weighting and in time intervals of 1 s, and from these individual measurements the different noise index shown in this work were calculated; for example L10, L50, L90, Leq,1 h, etc, depending of the time interval of interest.

Two noise level meters used were the Type I Brüel & Kjäer model 2270 and 2250. The technical characteristics for two meters are: 4.2 HZ broadband linear frequency range with supplied microphone Type 4189, 16.6 – 140 dB A-weighted dynamic range with supplied microphone Type 4189. Outputs: Generator and Headphone. The third was the Rion NL-31, and the technical characteristics are: A weighting: 28-138 dB, C weighting: 33-138 dB, flat: 38-138 dB, Peak sound level: 141 dB, Ranges 100 dB dynamic, frequency 20-12,500 Hz (including microphone).

The sampling period lasted 15 days in order to ensure that the averages were representative and to offset the Hawthorne effect [19] of attention bias in which the study participants alter their behavior when they are aware they are being observed, and avoid interferences that can cause another unexpected variable to influence the study.

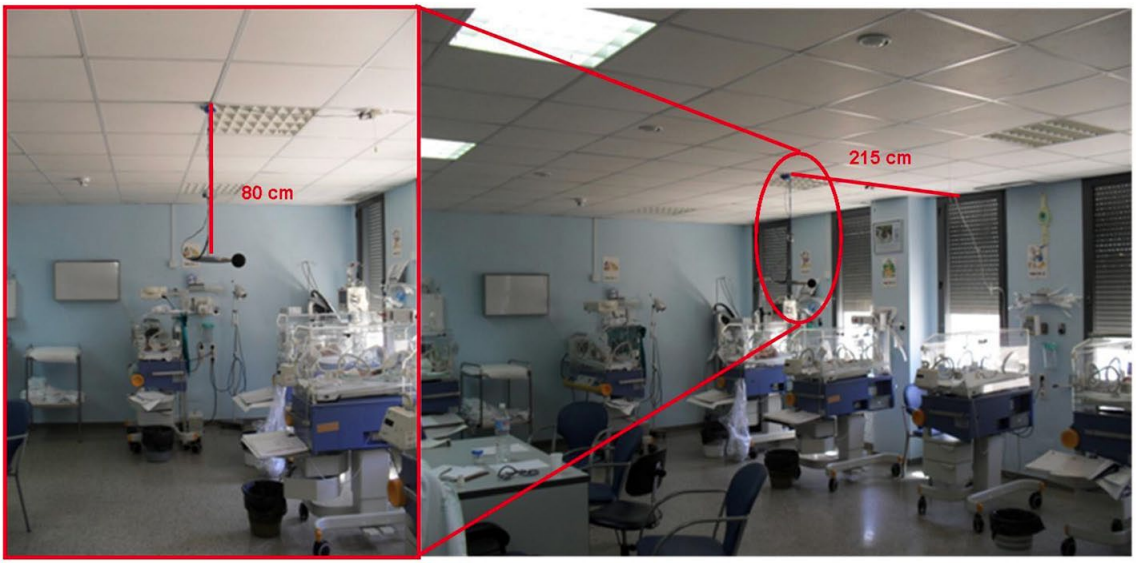

Figure 2. Location of sound level meter in the NICU intermediate care ward for newborns.

The collected data (individual data were for time intervals of 0.1 s) were recorder in the Secure Digital (SD) by Memory Card inside of the level meters and downloaded to PC and were treated and processed in the XLSTAT code developed by Addinsoft and SPSS 13.0,

Results

Figure 3 shows the LAeq,1 h for the 15-day sampling period in the two NICU wards studied. The values for the intermediate care ward are generally lower than those for the critical care ward. The hourly LAeq,1 h minimum and maximum values fluctuate in a small range of about 49-52 dBA for the minimum values (3 dBA), and 67-72 dBA (5 dBA) for the maximum. The values are constant during the fifteen days. The maximum LAeq, 1 h (about 72 dBA) occurs at around 14:00 on 29/06/2010 (end of visits with medical information to the families), in the NICU-C. The Figure also shows that the equivalent continuous hourly level exceeds 70 dBA in many hours (12 times), in clear breach of WHO recommendations. The LAeq, 1 h in the incubators are, clearly, more lower than out (54dBA-62dBA). This is because the incubator absorbs something the noise.

An analysis of the LAeq, 1 h in Figure 3 demonstrates that a possible Hawthorne effect is not applicable since the values recorded in the first three days were similar or slightly higher than those for the following days. In general, the LAeq, 1 h values for the afternoons are significantly higher than those for the mornings due to visiting hours.

Also in Figure 3, we see that 50% of the noise level recorded between 01:00 and 07:00 is less than 53 dBA in NICU-I and under 52 dBA in NICU-C. Noise levels increase early in the morning, reaching a peak when care of the newborns is at its busiest and when shift changes take place between 12:00 and 14:00, with noise levels falling during the night.

Figure 4 was designed to represent the average hourly rates of the percentiles of the equivalent continuous noise level in NICU-I and NICU-C (in and out), in order to establish whether there were peak-time events that could be associated to specific hours of the day. This Figure shows that the background noise level (quantified by L90) varies between 46 dBA and 51 dBA in NICU-C, and between 49 and 52 dBA in NICU-I, which is higher than that recommended by the WHO [13]. Likewise, the L99 (background noise) minimum noise level of about 50 dBA in NICU-I, 46 dBA in NICU-C (out) and 52 dBA in the incubator, was to be expected due to sounds coming from outside the hospital, such as passing traffic in the surrounding area. The noise inside the incubator is higher and constant due to electromechanical equipment.

The L10 levels (peak noises) are similar in both wards, between 55 dBA and 65 dBA in NICU-C and 53-67 dBA in NICU-I. Inside the incubator the L10 is around 54- 57 dBA. This acoustic rate remains stable during the day (8:00 to 22:00) with oscillations below 5 dBA, and the minimum recorded at around 05:00 when activity in these wards is at its lowest. Also noteworthy is that noise levels reach a maximum when care activity is at its busiest, between 08:00 and 15:00, which demonstrates that the wards are noisiest when nursing and monitoring are at their most intense.

We also observe that acoustic levels in NICU-C are generally more uniform than in NICU-I. In the incubator is more uniform without large swings. In relation with the climate noise, L10-L90, in NICU-C (in) the Figure 4 shows a constant range between 2 and 4 dBA, reflecting a constant climate noise with maximum to 7:00, 11:00, 13:00 and 14:00 with values around 4 dBA. On the other hand, the values of NICU-C (Out) show a wider range between 9-16 dBA, with maximum in the visits hours 13:00-14:00 h and 19:00 h, as we shall see in Figure 5. In relation to the NICU-I, we can observe that the range of climate noise is wider than in NICU-C (out), with values between 4-16 dBA, being highest in the shift changes. Lastly, we can observer that in all cases the minimum values were obtained during the night period (0:00-6:00 h).

Figure 5 presents the hourly integrated values of the equivalent continuous noise level for the 15-day period. There are certain similarities. Higher levels of noise happens with the staff sift changes (8:00, 15:00 and 22:00 hours). In the night there is a lower noise level due to lower activity in the care. The highest levels are in the feedings times.

Obviously there are more instances of intervention, monitoring

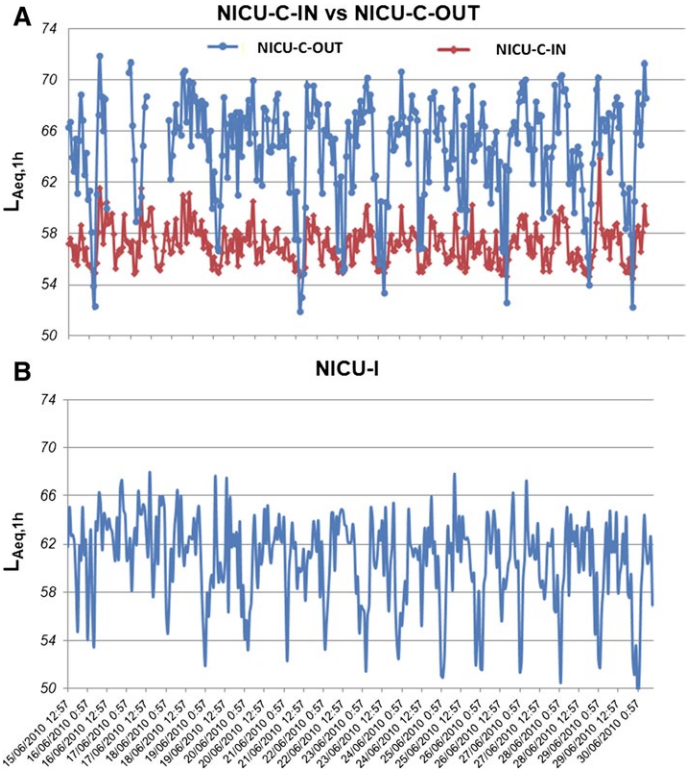

Figure 3. Comparative equivalent continuous noise levels in the NICU-I and NICU-C (OUT and IN) at HJRJ – (1 hour integration).

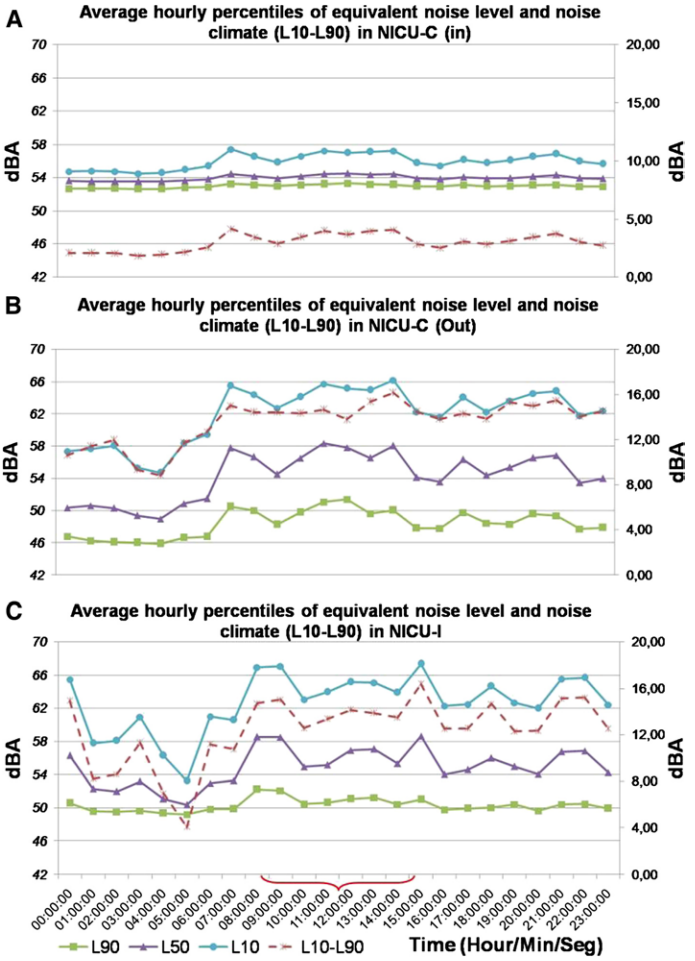

Figure 4. Average hourly percentiles of equivalent noise level in the NICU-C (in), NICU- C (Out) and NICU-I.

and follow-up of newborns in the NICU-C ward where the most critical care takes place, with a consequently greater noise level throughout the day. The NICU-C-out ward recorded LAeq,1 h of 69 dBA at 08:00, which is when the biggest number of health care personnel starts work. Hence, the high noise level is directly attributed to talking by the staff, again verified by the second peak which occurs at 15:00 when the majority leaves work. This Figure also shows the close relation between the activities of the health care personnel in the NICU and the noise level in each ward.

These activities are periodical which is verified by a temporal series analysis of the noise, which yielded the periods of greatest significance: 7 days, 24 hours, 12 hours and 3 hours. The first of these periods relates to the weekend effect in which work activity decreases markedly towards the end of this seven-day period. The 3-hour period is also noteworthy as the newborns are fed every three hours. In the NICU-C, the relation between feeding times and noise levels is weak since most newborns are fed by nasogastric drip. In terms of visiting times at 13:30 and 18:45, there is a rise in the average hourly noise level in the NICU-C while the average noise level in the NICU-I remains stable or falls.

The normality of the distribution of LAeq,10 min data was tested with the result that the noise in each ward did not distribute normally, as was to be expected since there are considerable internal correlations in the noise measured for each hour of the day (see Figure 6). By contrast, if we apply a normality test to LAeq, 10 min at a specific hour of the day for the 15 days of the sampling we find that these data have a high degree of normality. It does not have the normal distribution that would be obtained for the mean value and standard deviation measured experimentally (N = 326 values, μ = average = 64.65 dBA, Sx = Standard Deviation = 3.98 dBA, U = Standard Uncertainty of the Average = 0.22 dBA). It can be demonstrated that by applying the t-Student test the mean noise values in both wards are significantly different, as obtained from their intervals at 95% confidence (μC = 64.6 ± 0.4 dBA; μI = 60.6 ± 0.4 dBA), which leads us to state that the noise level in the critical care ward for newborns is 4.0 dBA higher than in the intermediate care ward.

By contrast, if we take a specific hourly period such as 00:00-01:00 in NICU-I, the resulting data set (N = 6 · 15 = 90 data) still has a normal distribution with 95% confidence. Likewise, if we apply the Shapiro-Wilk test we get W = 0.838 (p-value = 0.159), so the null hypothesis is acceptable at a significance level of 5% (alpha = 0.05) since the p-value obtained (0.159) is greater than the alpha considered (0.05). This result confirms our hypothesis that the noise level has a normal distribution if the sampling period is restricted to 1 hour, which is less than the shortest of the characteristic periods of the data series (3 hours or the newborns' feeding time).

Discussion

The results are consistent with those of other authors [20-28] in which the hours with the highest levels of noise are between 08:00 and 15:00, when care activity around the newborn in NICU is at its busiest. In terms of the maximum, minimum and average noise levels recorded, the studies in NICU that we have

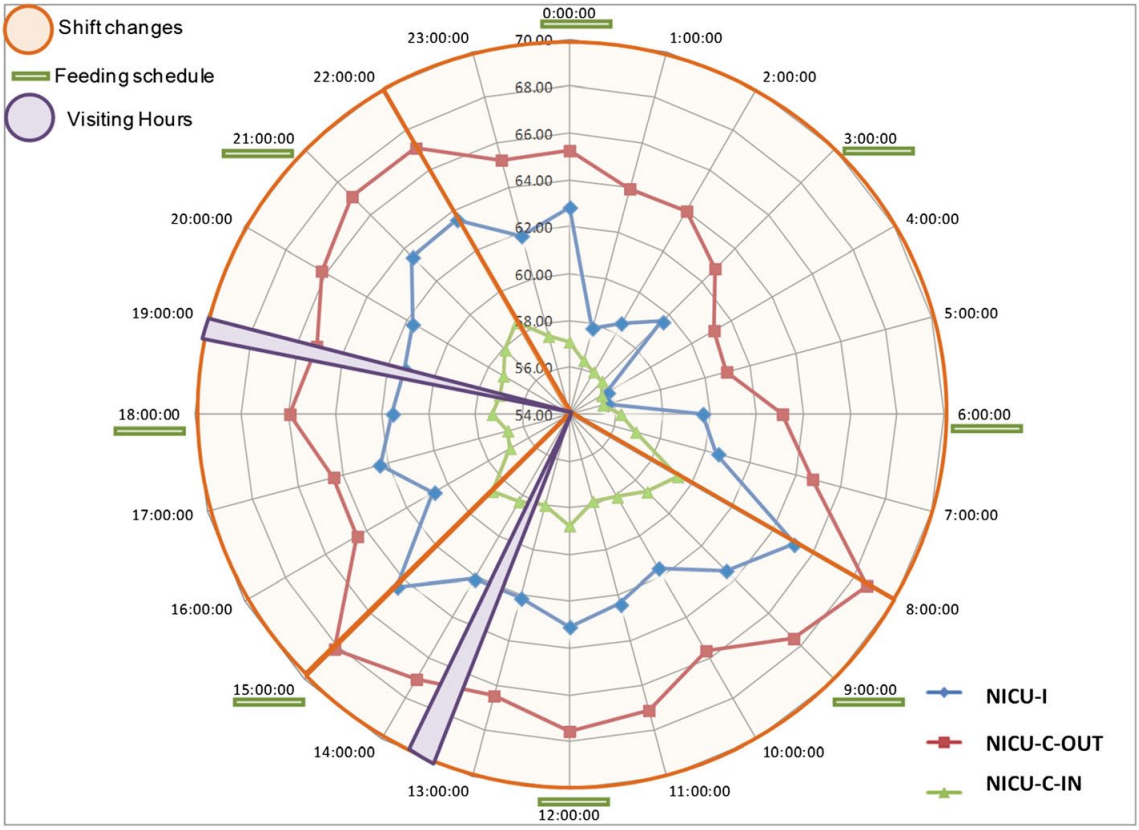

Figure 5. LAeq,1 h register (24 h) in the NICU at HJRJ. Circle graph.

analysed present a wide range of values oscillating between 36-80 dBA [21-25]. The results of our study show that the noise levels in NICU at HJRJ fall within a similar range. However, our study recorded maximum values that exceeded those of other researchers, at 80 dBA in LAeq,10 min averages. The equivalent sound level in other studies has a broader range (between 40 and 90 dBA) than the one recorded in our study (between 48.8 and 72.2 dBA), and with an integrated hourly average of LAeq,24 h noise of 64.5 dBA, which easily surpasses international standards and recommendations for newborns in NICU [13,14]: LAeq,1 h = 45 dBA, L10 (hourly) = 50 dBA and Lmáx = 65 dBA, which is a considerable problem that remains unresolved [29,30].

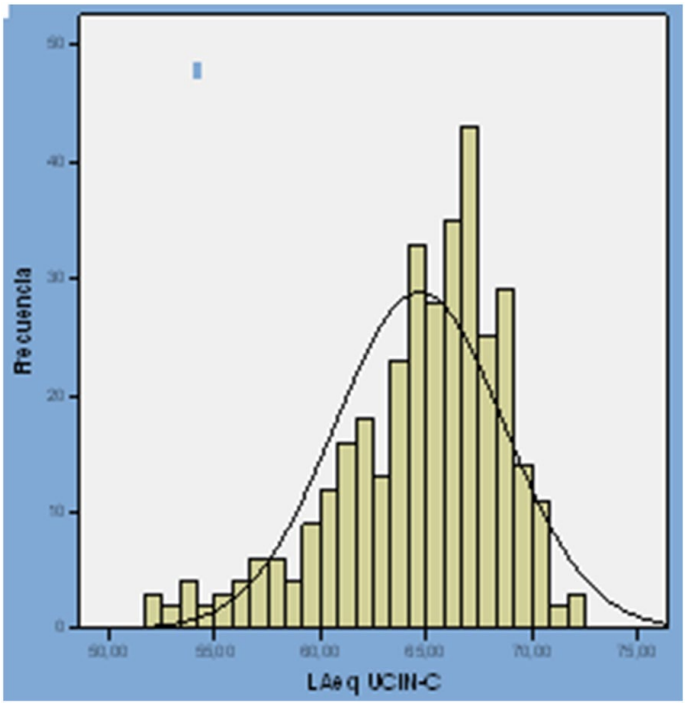

Figure 6. Experimental frequency distribution of the LAeq, 10 min.

The behavior of the hourly noise level in our study is consistent with that of Mackenzie [31], who established that the level of noise increased as the working day progressed and decreased in the afternoon. This is in accordance with Argote et al. [24] and Brandán et al. [22] who stated that noise levels rise at those times when the ward is cleaned in the mornings, and when relatives visit in the afternoons. Another characteristic that influences noise level relates to the health care work performed by personnel in shifts, with the night shift (22:00 – 08:00) showing the lowest levels and the morning and afternoon shifts registering the highest values, which is in line with various other studies [20-26].

Our study agrees with other authors [32-34] that noise levels exceed advisable. This may be due to a high number of patients, poor sound insulation, among other factors.

Conclusions

This study has demonstrated the high levels of noise pollution that newborns and health professionals are exposed to in NICU. Noise varies according to the shift, with levels at their highest during the morning and falling substantially at night. The critical care unit endures the highest noise levels. Measures to reduce these high noise levels in NICU include sound insulation (design, walls, closed doors with silent locking), drawing up an preventative maintenance equipment programme, placing the

newborn as far as possible from machines, which could also be removed from the ward (fridges, computers, medical case history trolleys, etc). It would be advisable to raise awareness among personnel of the noise they make during work, and achieve a gradual decrease in the noise emanating from alarms to acceptable levels. Also needed is a reduction in conversational noise among personnel and visitors to the wards, which could be achieved by hanging the appropriate warning signs in these areas. In addition, it would be needed to develop a guideline for noise mitigation, and educational preparation of the staff and patient's visitors with the implementation of protocols and to assess the progress.

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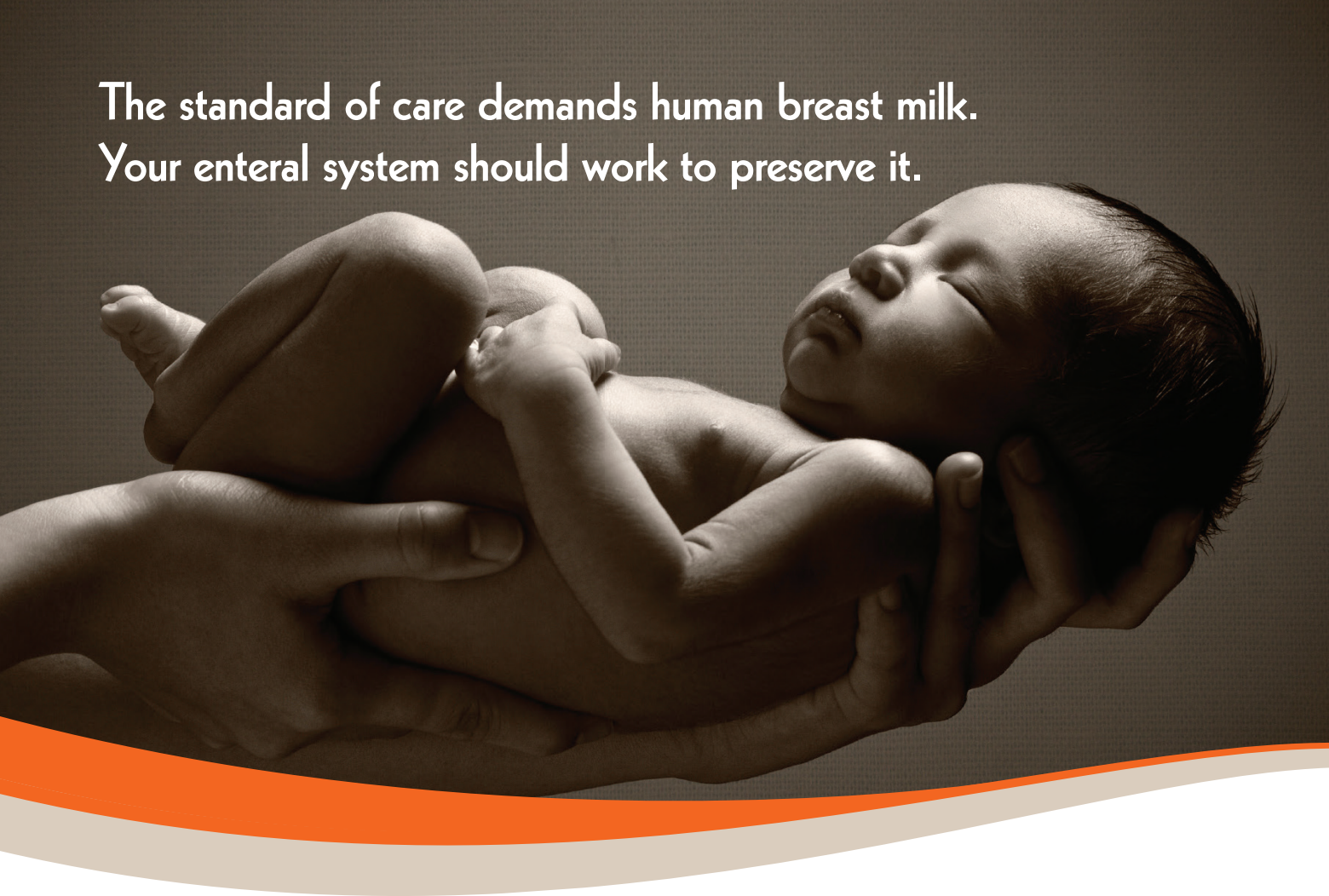

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