ORIGINAL ARTICLE

Effect of enhanced ultraviolet germicidal irradiation in the heating ventilation and air conditioning system on ventilator-associated pneumonia in a neonatal intensive care unit

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Objective: The objective of this study was to test the hypothesis that enhanced ultraviolet germicidal irradiation (eUVGI) installed in our neonatal intensive care unit (NICU) heating ventilation and air conditioning system (HVAC) would decrease HVAC and NICU environment microbes, tracheal colonization and ventilator-associated pneumonia (VAP).

Study Design: The study was designed as a prospective interventional pre- and post-single-center study. University-affiliated Regional Perinatal Center NICU. Intubated patients in the NICU were evaluated for colonization, and a high-risk sub-population of infants <30 weeks gestation ventilated for ≥14 days was studied for VAP. eUVGI was installed in the NICU’s remote HVACs. The HVACs, NICU environment and intubated patients’ tracheas were cultured pre- and post-eUVGI for 12 months. The high-risk patients were studied for VAP (positive bacterial tracheal culture, increased ventilator support, worsening chest radiograph and ≥7 days of antibiotics).

Result: Pseudomonas, Klebsiella, Serratia, Acinetobacter, Staphylococcus aureus and Coagulase-negative Staphylococcus species were cultured from all sites. eUVGI significantly decreased HVAC organisms (baseline 500,000 CFU cm⁻²; P = 0.015) and NICU environmental microbes (P<0.0001). Tracheal microbial loads decreased 45% (P = 0.004), and fewer patients became colonized. VAP in the high-risk cohort fell from 74% (n = 51) to 39% (n = 18), P = 0.04. VAP episodes per patient decreased (Control: 1.2 to eUVGI: 0.4; P = 0.004), and antibiotic usage was 62% less (P = 0.013).

Conclusion: eUVGI decreased HVAC microbial colonization and was associated with reduced NICU environment and tracheal microbial colonization. Significant reductions in VAP and antibiotic use were also associated with eUVGI in this single-center study. Large randomized multicenter trials are needed.

Journal of Perinatology advance online publication, 24 March 2011; doi:10.1038/jp.2011.16

Keywords: nosocomial infection; antibiotics; prematurity; ventilator-associated pneumonia; neonatal intensive care unit

Introduction

Nosocomial infections constitute a major public health threat affecting many people worldwide, and are the direct cause of morbidity and death in large numbers of hospital patients.1 These nosocomial infections increase length of stay (LOS) and health-care costs,2 and the associated emergence of antibiotic-resistant microorganisms is viewed by the World Health Organization as a global threat.

Spread of nosocomial infections through contact and localized droplet transmission is long understood, and hand washing has been the primary focus of infection control groups. It is more recently recognized that many infectious diseases are transmitted through inhalation of airborne infectious particles, and that these particles can be disseminated through heating ventilation and air conditioning systems (HVACs).4 The Centers for Disease Control and Prevention recommend that high-efficiency particle air filters be applied downstream in hospital HVACs as a means to prevent spread of airborne microbes.5 However, all filter types can become leaky or contaminated, releasing significant quantities of pathogens into the indoor environment.6 In addition to surface
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Contamination secondary to contact spread, it may be that microbes housed in HVAC systems are contributing to the hospital bioload. Multiple studies have documented pathogenic bacteria, fungi, viruses and mold present in the air. Ventilator-associated pneumonia (VAP) is a nosocomial infection of the lung in intubated patients, including neonatal intensive care unit (NICU) patients. Rates have been reported as 16% of infants born at 25 to 29 weeks' gestational age, 1.4 to 3.5/1000 NICU ventilator days in all NICU patients and 12.5/1000 ventilator days in babies born at <1500 g. A comprehensive study carried out at the Washington University St Louis Children’s Hospital in infants <2000 g at birth demonstrated that VAP occurred in 28% of ventilated patients, the VAP rate was 6.5/1000 ventilator days for babies <28 weeks’ gestation at birth and VAP was an independent predictor of mortality (odds ratio 3.4). Tracheal colonization rates as high as 87% have been measured in intubated NICU patients.

The germicidal effect of UV light, through dimerization of DNA, has been described for a wide range of microorganisms, including bacteria, fungi and viruses. Ultraviolet germicidal irradiation (UVGI) is recognized as an intervention that reduces dissemination of airborne infections, and has been applied with some success to ceiling air in tuberculosis clinics and operative suites. We hypothesized that enhanced UVGI (eUVGI, or Pathogen Control System) would decrease pathogens in the HVAC, thereby decreasing pathogens in the NICU air and surfaces, and thus decrease tracheal colonization in intubated NICU patients. We further hypothesized that this would decrease VAP prevalent in our smaller premature intubated infants.

Methods
Study design and setting
This is a prospective pre- and post-intervention design study. Although our NICU is supplied by two HVACs, the intubated patients were all in one area supplied by one HVAC, so a randomized study was not possible. The study was conducted from 2001 to 2003 at The Women and Children’s Hospital of Buffalo NICU. Several practices remained unchanged throughout the study period including: (1) infection control protocols for hand washing (in room sinks with running water and bactericidal soap) and universal contact precautions; (2) the NICU surface cleaning schedule and materials (Cavicide, Metrex, Romulus, MI, USA); and (3) respiratory protocols for equipment cleaning, ventilator circuit changes and daily humidifier water changes. Endotracheal tubes and flow-inflating bags were not routinely changed. Tracheal aspirate collections involved a gloved, open suction technique with sterile saline instilled into the trachea and then suctioned into a sterile trap and sealed. Although the practice of earlier extubation did increase over the study period, this was mitigated in our analysis by the high-risk sub-population, including only babies who were ventilated for at least 14 days. The HVACs were equipped with 95% filters (ASHRAE 52 to 76 specification) that were last changed 1 year before the study, and were not changed during the study period. The HVAC components were not manually cleaned.

Patient population
Tracheal microbial colonization was measured in all NICU patients who had an endotracheal tube in place at baseline, 1, 2, 7 and 12 months after eUVGI. The University at Buffalo Children and Youth Institutional Review Board waived the need for informed consent. To determine the impact of eUVGI on nosocomial lung infection, we identified a sub-population with a high incidence of VAP. Patients born <30 weeks gestation, who required ventilator support for >14 consecutive days beginning in their first week of life, had a >70% incidence of VAP in our NICU, and so we defined this sub-population as ‘high risk’ for VAP. Patients were excluded from the high-risk sub-population analysis if they had congenital heart disease, complex congenital anomalies, other NICU stays, ventilator days >200 or died or were transferred while on ventilator support. There were 2, 0, 3 and 1 baby (babies) who met exclusion criteria, respectively, in each 6-month epoch.

Patient data were analyzed in 6-month epochs: 6 months before eUVGI installation in July 2001; the next 6 month transition period during which the HVACs’ microbial contamination was progressively eliminated; the following three 6-month intervals, ending June 2003. After July 2003, patients were moved to a newly built 64-bed NICU with eUVGI in place, and the study was closed.

Intervention
In July 2001, eUVGI, also called Pathogen Control System, was installed in the HVACs supplying the NICU (eUVGI schematic, Supplementary Information). The eUVGI system design included UV lamps (Sterile-Aire, Burbank, CA, USA) of optimal size and placement for specific microbial elimination. eUVGI had been placed in 12 buildings (to treat ‘sick building syndrome’ and improve HVAC efficiency) or hospitals. Also designated as Qualified Anti-Terrorism Technology by the Department of Homeland Security, eUVGI had been installed into the United Nations building. By 2009, eUVGI had been placed in 35 hospitals in 13 states.

Microbial studies
Cultures of HVACs, the environment and intubated patients’ tracheas were obtained before eUVGI installation and over the next 12 months. Six HVAC sites were tested including the cooling coils’ air effluent side, drain pan, and bulk water condensate. A total of 43 environmental sampling sites were designated: ambient air, outside air and NICU surfaces, including work stations, laundry...
hamper, diaper weigh stations, tap water, sink drain traps and ceiling diffusers for supply air.

HVAC and environment samples were collected using methods standardized for inanimate surface and air contamination, and were analyzed by an independent laboratory (Pure Earth Environmental Laboratory, Pennsauken, NJ, USA). NICU mixed air and outdoor air samples were collected in duplicate over 2 min by an Andersen N-6 Impactor (Graseby/Andersen, Atlanta, GA, USA) using malt extract agar and Sabouraud dextrose agar media (Hardy Diagnostics, Santa Maria, CA, USA) for environmental fungi and blood agar plate media for environmental bacteria. Surface-wipe samples were obtained using a BBL culturette (Becton Dickinson, Franklin Lakes, NJ, USA) with a sterile rayon-tipped swab that was moistened with a modified Stuart’s transport medium before sampling 1 square inch of surface area. The completed swab specimen was placed back into its original container, sealed, placed immediately into a clean cooler and shipped next day via air to the laboratory for identification and quantification of fungus, mold and bacteria to the species level. Upon arrival at the laboratory, each surface wipe was immersed in a sterile test tube containing 10 ml of sterile distilled water. The test tube sample was kept at room temperature for 10 min and then placed in a rotary shaker (3.81 throw, 220 r.p.m.) for 1 min. The resulting suspension or dilution was then inoculated (0.1 ml aliquots) on a 2% malt extract agar (for saprotrophic fungal growth) and a trypticase soy agar (for environmental bacteria growth). The results provided estimates of the total number of viable propagules per ml of suspension. The samples were immediately incubated at 25 ± 1 °C, along with laboratory controls.

Tracheal aspirates from routine suctioning of intubated patients by bedside nurses at predetermined sampling times were analyzed by the hospital laboratory (Kaleida Labs, Amherst, NY, USA) using standard hospital microbiology laboratory techniques with plated culture media for clinical bacterial and fungal isolation. Positive cultures were reported as rare, few, moderate or as heavy growth for each organism.

The diagnosis of VAP was derived from the Centers for Disease Control and Prevention/NNSI age-specific definition of nosocomial pneumonia, and required all of the following: a tracheal aspirate culture positive for pathogens, increased ventilator support requirements, new and persistent infiltrates on chest radiographs and a 7-day course of antibiotics. Decisions on patients’ treatment with antibiotics, ventilator support and LOS were made by a qualified neonatologist (who was unaware that VAP was being ascertained) based on assessment of clinical, laboratory, radiographic and culture results.

**Outcome measures**

To quantify NICU tracheal colonization, we defined an airway microbial load index (MLI), whereby each pathogen per patient sample was quantified on a scale of 1 to 4 for rare, few, moderate or heavy growth, as reported by the hospital microbiology laboratory, and totaled. Patients whose tracheal aspirates showed no growth were assigned a zero. MLI scores for all patients were averaged as a measure of overall NICU patient microbial load for a given sampling time point. The length of time of intubation at the time that the samples were obtained did not correlate with colonization as measured by tracheal MLI (mean days of ventilation for each sampling time point was 22.19 days at time 0 days, 31.46 days at 1 month of eUVGI, 28.29 days at 2 months, 59.75 days at 7 months and 27.6 days at 12 months of eUVGI).

VAP episodes, types of organisms, number of antibiotic courses, antibiotic days, ventilator days and LOS were compared between the pre- and post-eUVGI time periods. There was a trend for the age at first VAP episode to increase over time (P = 0.08 by one-way ANOVA).

**Statistical analysis**

The relationship between post eUVGI installation time and the tracheal and HVAC microbial load was assessed with linear regression, and a log transform was applied to the MLI variable. A mixed model was used, which fit the environmental samples, as a function of a random time and random location effect.

To statistically assess observed differences in time, a likelihood ratio test was used. Logistic regression was used to assess differences in VAP; secondary end points were analyzed using Poisson regression. Because gestational age independently predicted LOS and ventilator days (P < 0.001), models were adjusted for gestational age. If overall differences were significant (P < 0.05), eUVGI groups were compared with the control group in pairwise manner, with a Bonferroni adjustment. The Cochran–Armitage test for trend and the Pearson correlation coefficient were used where appropriate.

Simple with- and without-eUVGI comparisons were analyzed by the Student’s paired t-test (SAS statistical software; Cary, NC, USA). All statistical analyses were performed by a biostatistician (GEW).

**Results**

**Microbial studies**

Multiple bacteria, fungi and mold were isolated at various times from the HVACs, NICU environment and intubated patients’ tracheas to describe the microbial environment throughout the environmental study period (Microorganism Table, Supplementary Information). At baseline, the HVAC components, cooling coils and drain pan condensate were visibly contaminated (Figure 1) and cultured positive for moderate-to-heavy growth of fungi and Gram-negative rods, and lighter growth of Gram-positive cocci and Bacillus species. NICU surfaces and air showed a similar population of microbes. Sink traps were heavily colonized with Gram-negative rods; tap water was negative. Pseudomonas aeruginosa, Serratia marcescens and Klebsiella pneumoniae were the most common isolates in tracheal aspirates. eUVGI caused...
a 3-log reduction in the HVACs’ microbial load within 3 days (Figure 2). By approximately 6 weeks the HVACs had no visible contamination (Figure 1) and by 6 months HVAC cultures were negative (Figure 3). Similarly, NICU surface cultures approached zero during eUVGI ($P < 0.0001$, Figure 2). Baseline tracheal secretions had a heavy bioload (MLI: 4.2; $n = 21$), which decreased with eUVGI as HVAC colonization decreased (Figure 3). The percent of patients who had no or little tracheal colonization (MLI $\leq 1$) increased from 14% pre-eUVGI to 44% post-eUVGI (Table 1).

Ventilator-associated pneumonia

The clinical environment and patient population did not significantly differ among pre- and post-eUVGI groups (Table 1). Patient ventilator days, LOS, central-line days, gestational age and birth weight were similar for all groups. The percent of patients $< 30$ weeks gestation who met the criterion of $\geq 14$ ventilator days decreased over time (57 to 35%; $P = 0.01$, Table 1).

Approximately 74% of the high-risk sub-population had VAP before eUVGI was installed. After eUVGI, VAP decreased to 55% after 6 months and to 44% at 18 months ($P = 0.04$, Table 1). In addition, both the number of VAP episodes and number of antibiotics per high-risk patient decreased significantly (Figure 4). VAP was 88% polymicrobial with pathogen species similar to those identified in the HVACs, NICU environment and routine tracheal aspirates. Among the four 6-month cohorts of patients, Gram-negative bacteria were identified in 72 to 100% patients with VAP, and 0 to 62% grew Gram-positive bacteria. Gram-negative rods including *P. aeruginosa*, *Escherichia coli*, *S. marcescens*, *K. pneumoniae*, and *Stenotrophomonas*, *Acinetobacter* and *Enterobacter* species were identified three times as often as Gram-positive cocci (*Staphylococcus aureus*, *Coagulase-negative Staphylococcus* and *Enterococcus* species), and this proportion did not change with eUVGI. *P. aeruginosa* was the most common species isolated with and without eUVGI (57% pre-eUVGI; 43% at 18 months after eUVGI). Fungi, including *Candida albicans*, *Candida parapsilosis* and *Malassezia furfur*, were isolated in 17 and 14% of VAP cultures, respectively. During eUVGI, antibiotic use was reduced (Table 1). As expected, gestational age correlated negatively with LOS ($r = -0.34$) and ventilator days ($r = -0.51$);
Infections in the NICU. Gram-negative bacilli known to be associated with serious nosocomial load of the NICU environment. Many of the bacteria eliminated were cooling coils and components of the HVAC and decreases the microbial This study demonstrates that eUVGI eradicates microbes in the central

Discussion

This study demonstrates that eUVGI eradicates microbes in the central cooling coils and components of the HVAC and decreases the microbial load of the NICU environment. Many of the bacteria eliminated were Gram-negative bacilli known to be associated with serious nosocomial infections in the NICU. eUVGI was associated with a reduction in colonization of patient airways and VAP. These results suggest that it is possible that airborne pathogens may contribute significantly to surface contamination and patient colonization. The delayed improvement in tracheal colonization and VAP following reduction of bacteria in the HVGs and environment possibly reflects the persistent, although progressively diluting reservoirs in patients who were admitted before or early in the eUVGI period and who remained hospitalized in close proximity to patients admitted later.

Although contact is well-accepted as a means of transmission of nosocomial infection, concepts of airborne transmission of hospital infection are evolving. Early studies showed that all airborne and dry-surface microbes undergo desiccation; however, often overlooked were the genetic repair and secondary rehydration with ambient humidity that virtually ensure spread of disease by the aerobiological pathway. Many microbes have the capability of remaining airborne and viable, or settling and re-suspending for extended periods in the indoor environment, and may exist as single cells or spores, aggregates or as biological material carried by non-biological particles. In addition to reports of airborne transmission of infection in medical patients, surgical wound infections have been correlated with air bacteria levels. In a preliminary report of eUVGI placed in HVGs that supply air in operating rooms, eUVGI decreased the number of positive bacterial cultures in the HVAC system as well as in the air sampled at the level of the operating table.

Pneumonia is the second most frequent nosocomial infection for all patient populations. Although a VAP prevalence of 20% has been described for NICU infants who were intubated for >14 days, our rate of 1.7 episodes per high-risk patient is similar to our rate of 1.5 episodes per high-risk patient. The VAP rate of 74% is closer to this ARDS population, whereas pediatric patients with VAP contract 1.9 episodes per patient. Similar to our rate of 1.7 episodes per high-risk NICU patient. Comparable to adult and pediatric populations, VAP was largely polymicrobial in this cohort, and Gram-negative organisms were isolated more often than Gram-positive organisms. P. aeruginosa was the most common isolate, and all species in our population were virtually identical to those in other populations.

Table 1: Demographic profile of NICU and high-risk cohort and VAP results

<table>
<thead>
<tr>
<th>All NICU patients</th>
<th>Pre-eUVGI 1/01–6/01</th>
<th>Post-eUVGI 1/02–6/02</th>
<th>Post-eUVGI 7/02–12/02</th>
<th>Post-eUVGI 1/03–6/03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admissions, n</td>
<td>310</td>
<td>345</td>
<td>368</td>
<td>316</td>
</tr>
<tr>
<td>Average daily census, n</td>
<td>42.4</td>
<td>46.4</td>
<td>46.6</td>
<td>39</td>
</tr>
<tr>
<td>% Inborn</td>
<td>66</td>
<td>59</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>% Patients with tracheal MLI ≤1</td>
<td>14</td>
<td>30</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>No. of babies admitted &lt;30 weeks</td>
<td>54</td>
<td>57</td>
<td>73</td>
<td>51</td>
</tr>
<tr>
<td>No. of babies &lt;30 weeks and ventilated for ≥14 days</td>
<td>31 (57)</td>
<td>25 (44)</td>
<td>24 (33)</td>
<td>18 (35)</td>
</tr>
<tr>
<td>(%) of all babies &lt;30 weeks High-risk cohort (mean (s.d.))</td>
<td>66 59 69 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>26.4 (1.9)</td>
<td>25.7 (1.5)</td>
<td>26.2 (1.6)</td>
<td>26.0 (1.6)</td>
</tr>
<tr>
<td>Birth weight, (g)</td>
<td>901 (175)</td>
<td>816 (140)</td>
<td>853 (105)</td>
<td>845 (188)</td>
</tr>
<tr>
<td>Total parenteral nutrition/central-line days</td>
<td>37 (12)</td>
<td>41 (9)</td>
<td>45 (13)</td>
<td>51 (17)</td>
</tr>
<tr>
<td>Length of stay, days</td>
<td>98 (57)</td>
<td>92 (26)</td>
<td>89 (24)</td>
<td>105 (40)</td>
</tr>
<tr>
<td>Ventilator days</td>
<td>50 (33)</td>
<td>44 (23)</td>
<td>44 (27)</td>
<td>48 (28)</td>
</tr>
<tr>
<td>No. of VAP episodes per high-risk patient</td>
<td>1.2</td>
<td>0.7</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>% with at least one VAP</td>
<td>74</td>
<td>76</td>
<td>54</td>
<td>39</td>
</tr>
<tr>
<td>No. of VAP episodes per high-risk patient with any VAP</td>
<td>1.7</td>
<td>1.3</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>No. of antibiotics per high-risk patient</td>
<td>2.6 (2.7)</td>
<td>1.7 (1.7)</td>
<td>1.9 (2.4)</td>
<td>1.0 (1.5)</td>
</tr>
<tr>
<td>Antibiotic days</td>
<td>20.9 (24.2)</td>
<td>17.3 (20.8)</td>
<td>18.8 (25.5)</td>
<td>9.5 (14.7)</td>
</tr>
</tbody>
</table>

Abbreviations: eUVGI, enhanced ultraviolet germicidal irradiation; MLI, microbial load index; NICU, neonatal intensive care unit; VAP, ventilator-associated pneumonia.

 LOS (r = 0.37) and ventilator days (r = 0.53) correlated positively with VAP. All correlations were significant (P<0.001).
Nosocomial infections generate a significant financial burden to hospitals. In a recent study of a combined PICU–NICU population in which 32% developed VAP, the hospital cost for patients with VAP was $308,534 compared with $252,652 in the non-VAP patient. Thus, we speculate that overall costs could be decreased if eUVGI decreases VAP; also, we did not study other nosocomial infections. Finally, given the evidence for aerosol transmission of influenza viruses and their inactivation by UV radiation, eUVGI installed in health-care facility HVACs may contribute to pandemic preparedness.

Figure 3 Enhanced ultraviolet germicidal irradiation (eUVGI) decreased the microbial load of the heating ventilation and air conditioning system (HVAC) (mean CFU/cm² × 1000) and patients’ tracheas MLI*. MLI—microbial load index—a score to quantify overall NICU density of tracheal colonization at a point in time, whereby each airway pathogen is quantified on a scale of 1 to 4 (rare, light, moderate and heavy growth as reported by the laboratory) and totaled for each patient. For example, a patient with light growth of three pathogens would have an MLI = 3, whereas a patient with heavy growth of three pathogens would have an MLI = 12; patients whose tracheal aspirates showed no growth were assigned a zero.

Figure 4 During enhanced ultraviolet germicidal irradiation (eUVGI), the proportion of high-risk patients (<30 weeks gestation and ≥14 days ventilator support) who developed ventilator-associated pneumonia (VAP), as well as the number of episodes of VAP and the number of antibiotics per patient in this population decreased. Pt, patient; Abx, antibiotics.
by offering an enhanced level of protection to health-care workers, similar to its use in buildings for protection from bio-terrorism agents. eUVGI may also diminish the incidence of bacterial super infection, frequent causes of mortality during flu pandemics.

Several issues limit interpretation of these data. A more rigorous randomized design was precluded by the NICU layout, and the necessary pre- and post-comparisons are subject to possible clinical care changes over time. We cannot rule out that VAP may have decreased over time because of unidentified clinical or environmental interventions. Actually, the decreased percent of patients <30 weeks with prolonged ventilation over time may reflect, in part, early extubation practices, and suggests that those neonates who remained intubated and thus met criteria may have constituted an even sicker population in the later post-eUVGI cohorts. The close timing of environmental and clinical responses with HVAC improvements suggests these may have been affected by eUVGI. DNA testing would have more definitively linked the HVAC and NICU environmental reservoirs with the patients’ organisms, but was beyond the scope of this study and could be considered in a more definitive randomized controlled trial.

Conclusion

In conclusion, eUVGI eradicated microbes in HVACs, and was associated with a decrease in NICU environmental pathogens and tracheal colonization. Significant reductions in VAP and antibiotic use in NICU high-risk patients were associated with eUVGI in this limited study. Large multicenter randomized trials are needed to further characterize the effects of eUVGI on the full spectrum of adult, pediatric and neonatal hospital populations.

Conflict of interest

Corinne Leach, MD, PhD, is the spouse of Vigilair Systems stockholder and former CEO (Timothy Leach), and she introduced the concept to our group.

Acknowledgments

This work was supported in part by a grant from the NYSTAR Center for Advanced Technology in Biomedical and Bioengineering, by the Department of Pediatrics, SUNY at Buffalo, and by an in-kind contribution of eUVGI technology and installation and environmental sample collection from Vigilair Systems. We acknowledge, Diane Dryja, Director of Kaleida Microbiology Laboratory for culture analysis, Andrea Mattingly, for data base analysis, Sharrie Lacata, for guidance on billing, Michele Pamer for computer generated design and the NICU nurses for their invaluable assistance in sample collection.

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Supplementary Information accompanies the paper on the Journal of Perinatology website (http://www.nature.com/jp)