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Vital signs analysis algorithm detects inflammatory response in premature infants with late onset sepsis and necrotizing enterocolitis

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Abstract

Background—Nonspecific clinical signs and suboptimal diagnostic tests limit accurate identification of late onset sepsis (LOS) and necrotizing enterocolitis (NEC) in premature infants, resulting in significant morbidity and antibiotic overuse. An infant's systemic inflammatory response may be identified earlier than clinical suspicion through analysis of multiple vital signs by a computerized algorithm (RALIS).

Aim—To evaluate the revised RALIS algorithm for detection of LOS and NEC in preterm infants.

Methods—In this nested case-control study, VS data (heart rate, respiratory rate, temperature, desaturations, bradycardias) were extracted from medical records of infants 23–32 weeks gestation. RALIS generated an output, with score 5 triggering an alert. Patient episodes were classified based on culture, radiograph, and antibiotic data into categories: LOS, expanded LOS, NEC, and controls. Paired t-tests, linear regression and cross-validation analyses were used to evaluate the relationship between RALIS alert and LOS/NEC.

Results—Among 155 infants with 161 episodes, there were 41 expanded LOS (+ blood, CSF, urine, respiratory culture), 31 LOS (+ blood, CSF, urine), 9 NEC, and 93 controls. RALIS alert

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The study was conducted at Prentice Women's Hospital of Northwestern Medicine in Chicago, IL, USA affiliated with Northwestern University Feinberg School of Medicine.

was 43.1 ± 79 hours before culture in LOS ($p=0.012$). There was a significant association between RALIS alert and LOS/NEC ($\beta=0.72$, $p<0.0001$). Sensitivity and specificity for LOS/NEC were 84% and 80%, (PPV=63%; NPV=93%). The regression model demonstrated an AUC of 89.9%.

Conclusions—For infants ≥ 32 weeks, RALIS detects systemic inflammatory responses in LOS and NEC in the first month of life. The algorithm can identify infection earlier than clinical suspicion, even for NEC with negative cultures. RALIS has high NPV to rule-out LOS and NEC, and may, after prospective validation, aid in antibiotic treatment decisions.

Keywords

neonatal sepsis; late onset sepsis; necrotizing enterocolitis; vital signs analysis; systemic inflammatory response; premature infant

Introduction

Sepsis remains a critical issue and a major cause of death among infants in the U.S.(1) and causes over 200,000 annual neonatal deaths worldwide(2). Approximately 21% of very low birth weight (VLBW) infants are affected by at least one episode of late onset sepsis (LOS; with positive blood culture after 72 hours of life)(3). There has been some improvement in the incidence of LOS due to infection control measures(4), but LOS continues to disproportionately affect preterm infants and cause significant morbidities including neurologic impairment, prolonged hospitalization, and death(5). Because prompt recognition of infection and initiation of antibiotic therapy decrease sepsis-related morbidity and mortality, clinicians have a low threshold to evaluate for LOS and empirically treat with broad spectrum antibiotics. However, the lack of reliable diagnostic capability for LOS remains an ongoing issue in the neonatal intensive care unit (NICU). Clinical signs of sepsis are mostly nonspecific and detected late, and current laboratory tools are of limited utility. Specifically, the current gold standard for diagnosis of LOS is blood culture, which has delayed results and limited sensitivity in the setting of small specimen volumes and recent antibiotic administration(6). Adjunct markers of infection such as white blood cell count indices(7), C-reactive protein(8), hypoglycemia, and thrombocytopenia are also nonspecific with poor positive predictive value (PPV)(9, 10). Furthermore, there is lack of consensus on the definition of neonatal sepsis amongst providers(11). As a result, antibiotic treatment is overprescribed in the NICU with 56% of VLBW infants treated with antibiotics while only 21% had culture-proven infection in one large study(3). The adverse effects of prolonged antibiotic exposure in preterm infants include antibiotic resistance, fungal infections, necrotizing enterocolitis (NEC), and death(12–14). The nonspecific clinical presentation of NEC overlaps with that of sepsis. The early localized bowel inflammation, bacterial penetration, and tissue destruction is often difficult to identify until signs of abdominal distension, bloody stool, bowel perforation, or clinical deterioration appear, often accompanied by sepsis(15). Thus, development of more reliable and specific methods to predict and exclude LOS and/or NEC in preterm infants is essential to improve neonatal outcomes.

The advantage of automated vital signs monitoring as an objective tool to detect evolving sepsis is being increasingly recognized(16–18). Several studies have suggested that analysis

of vital signs patterns can help clinicians evaluate for impending infection or inflammatory response (e.g., sepsis, NEC) prior to obvious deterioration(19–22). For example, the HeRO monitoring system detects heart rate variability characteristics that change with LOS. The use of HeRO was associated with a reduction in 30 day mortality in VLBW infants(23, 24). The RALIS software was the first to incorporate multiple vital signs monitoring into a predictive algorithm to detect systemic inflammatory responses including LOS(25, 26). As we previously reported, RALIS detected sepsis 2.5 days prior to clinician suspicion of infection (defined as the time blood culture was obtained) with a sensitivity of 82% for LOS in infants < 28 weeks gestational age (GA)(20). However, the PPV and negative predictive value (NPV) were only 67% and 65% respectively. The RALIS algorithm was changed, primarily by incorporating more appropriate ranges for temperature and more accurate input of skin temperature rather than incubator values. The objective of the current study was to evaluate the performance of the revised RALIS algorithm to detect inflammatory responses from LOS and NEC in a cohort of preterm infants (23–32 weeks GA). Specifically, we compared time and frequency of RALIS alert to clinical suspicion of LOS among preterm infants with culture-confirmed LOS, NEC, and controls. Cross validation analysis was also performed to assess the robustness of the RALIS model.

Materials and methods

Patient population

Medical records of infants enrolled in a premature birth cohort study at Prentice Women's Hospital and who were admitted to the NICU were reviewed. Per standard study protocol, parental consent is obtained, patients are assigned a study code, and a de-identified database is stored on a secure server. Included infants were born at <33 weeks GA between 2008 and 2012 with complete VS data from birth to 28 days of life available in the electronic medical record. In this study of diagnostic utility, infants transferred out of the NICU, who died early within the first month of life, and those with congenital syndromes were excluded. Infants requiring oscillator or jet ventilation were also excluded because of inability to accurately interpret respiratory rates. Maternal and infant characteristics including demographics and clinical course were collected using the cohort database, Northwestern University Enterprise Data Warehouse system, and standardized medical record review. Extracted data included comprehensive microbiologic culture information, type and duration of antibiotics, and laboratory results. This study was approved by the Institutional Review Board of Northwestern University and Ann and Robert H. Lurie Children's Hospital of Chicago.

Vital signs and RALIS output

Vital signs (VS) data are entered into the electronic medical record (PowerChart, Cerner, MO) by experienced nurses per routine NICU protocol. Nurses read the VS from the standard continuous cardiorespiratory monitors (IntelliVue Neonatal, Philips, MA), verify values clinically, and input values into the Powerchart record. All patients had vital signs documented at least every 3 hours for 28 days of hospital admission. The VS data was extracted retrospectively, coded in Excel, and uploaded into the RALIS program in order to generate continuous RALIS output.

RALIS is a mathematical algorithm developed for monitoring of preterm infants to detect inflammatory response, such as in LOS. As previously described, the algorithm was originally developed from a derivation cohort of 200 infants at Bikur Holim Hospital, Jerusalem, Israel(26). The software generates a RALIS score based on significant VS changes from the individual patient's baseline, which is initially calibrated from the first 72 hours of life and evolves over the monitoring period. Additionally there are GA and birth weight specific VS ranges incorporated into the algorithm. VS data incorporated into RALIS include: heart rate, respiratory rate, temperature, desaturation events, and bradycardia events(20). The heart rate, respiratory rate, and temperature values are numerical, while the low SpO₂ (oxygen saturation) and bradycardic inputs are binary, indicating the presence or absence of events during the preceding 2–3 hour interval. Nurses record the lowest oxygen saturation and heart rate during an episode and the duration (seconds) in the electronic medical record. For RALIS coding, a desaturation event was defined as an oxygen saturation of <80% for >10 seconds. A bradycardia event was defined as a heart rate <100 beats per minute for >10 seconds. Weight is entered once every 24 hours. Each VS has a weighted contribution to the final algorithm output. There is a real-time current monitoring period, which the program uses to calculate significant aberrations from the baseline period, both of which are changing over time. The final RALIS score is reported on a 0–10 scale, with 5 as the threshold for an acute inflammatory response based on an initial derivation cohort in Israel. A RALIS score ≥ 5 for 6 consecutive hours generates an alert to the clinical team. We limited our analysis to LOS and NEC episodes within 3–28 days of life for consistency and comparison with previous studies(20, 26). A RALIS alert was considered associated with a LOS or NEC episode if it occurred within 7 days before or after infection was suspected. The absence of any alert within 7 days before or after culture was considered false negative result for a LOS or NEC episode. A false positive alert in control infants was any signal throughout the 28-day monitoring period.

LOS definitions

The primary outcome—LOS was defined as an episode (physician-initiated sepsis evaluation) occurring at >72 hours of life which resulted in a positive culture and was treated with an antibiotic treatment course. A positive culture was defined as any blood, cerebrospinal fluid (CSF), or urine culture with bacteria or yeast. We also collected data on episodes with a positive endotracheal tube culture (ETT) and chest radiograph infiltrate treated with antibiotics: which were included in the *expanded* LOS classification. Antibiotic treatment was receipt of a ≥ 7 day of antibiotic course. Neonatal complications such as NEC(27) and bronchopulmonary dysplasia (BPD)(28) were identified according to parent study protocol and widely used benchmarks. Specifically, an episode of NEC was defined as clinical and radiographic evidence of NEC (pneumatosis intestinalis) treated with antibiotics and bowel rest. Based on NICHD definitions for premature infants, episodes of culture-proven sepsis occurring <72 hours of life were considered early onset and excluded from analysis. Patients with no positive culture and no antibiotic course throughout their hospitalization served as controls. Clinical sepsis patient episodes with negative culture, but that received antibiotic treatment were classified as culture-negative LOS. A positive culture without antibiotic treatment in an infant who did clinically well was considered a false-positive culture episode.

Statistics and Modeling

For sample size determination, we considered LOS the primary outcome and the hours between RALIS alert and culture as the primary predictor. Based on our previous study, we used a mean time difference of 59 (± 67) hours in the LOS group and 22 (± 64) in the false-positive culture group (as a proxy for no LOS)(20). Thus, our cohort was adequate with greater than the estimated sample size of 24 LOS cases to achieve >80% power (alpha 0.05). Statistical analysis was performed by using SAS® 9.4 (Cary, NC) and Stata®/IC 13.1 (College Station, TX).

Demographic and clinical characteristics are reported as means with standard deviations and frequencies with percentages. Differences in characteristics among patients were assessed using independent samples t-tests, chi-square, or Fisher's exact tests. The association of RALIS with sepsis episodes was evaluated by comparing the hours between the time of RALIS alert and the time of clinical suspicion of sepsis, defined as the time a specimen was sent for culture. Time differences between RALIS and clinical suspicion were compared using the Wilcoxon signed-rank test. Linear regression was used to evaluate the relationship between the timing of RALIS alert and culture. We calculated sensitivity, specificity, PPV and NPV of RALIS for 1) expanded LOS (including blood, CSF, urine, and ETT positive cultures), 2) LOS (including only blood, CSF, and urine positive cultures), 3) NEC, and 4) LOS (blood, CSF, urine) plus NEC. All episodes were treated independently. Two-sided p-values <0.05 were considered statistically significant.

We implemented an *exploratory* cross-validation analysis using a modified hold out method to assess the validity of RALIS alert to predict LOS using logistic regression modeling. Patients with true positive (expanded LOS) and true negative status for infection (controls) were randomly assigned to the train dataset (75%) or the test dataset (25%). The train dataset was used to fit a multiple logistic regression model using a manual stepwise selection procedure with a model entry criterion of $p=0.10$. Patients in the test dataset were not included in model development. To assess the predictive value of this model, we evaluated the area under the curve (AUC) of the receiver operating characteristic (ROC) curve in the train and test datasets. The test dataset was used to cross-validate the model by comparing the R^2 of the predicted probabilities of infection and model-estimated coefficients for RALIS alert to those from the train dataset. A relative percent difference of 10% between the train and test model-estimated coefficients were chosen *a priori* as the validation threshold.

Results

Demographics

The 155 patients had 161 episodes including: 41 cases of expanded LOS, 14 culture-negative sepsis, 13 false-positive cultures, and 93 controls. Of the LOS positive cultures: 22 were blood, 8 urine, 10 ETT, and 1 blood+CSF. Organisms were gram negative rods ($n=10$), *S. aureus* ($n=8$), coagulase negative Staphylococcus species (CONS, $n=13$), group B Streptococcus ($n=1$), Enterococcus species ($n=6$), Candida ($n=1$), and 2 polymicrobial episodes with a gram negative rod and *E. faecalis* or *S. aureus*. There were 9 cases of NEC

within the first 28 days of life (2 concurrent with blood culture-proven LOS episodes and 7 with culture-negative LOS episodes). Thus, the total episodes of LOS and/or NEC was 38. The 13 false-positive cultures were 8 blood cultures with CONS, 1 blood culture with *S. aureus*, and 4 urine cultures (CONS, n=2 or polymicrobial, n=2).

As can be seen in Table 1, the control group had a significantly higher gestational age (30 (1.7)) weeks compared to the expanded LOS group (26.7 (2.3) weeks), the LOS group (27.2 (2.3) weeks), and the false-positive culture group (27.4 (1.1) weeks). The expanded LOS and LOS categorizations (with and without inclusion of ETT cultures n=41 and 31 respectively) had no notable differences in demographics. Controls also had a higher birthweight and lower incidence of BPD than other groups. Compared to controls, chorioamnionitis was more prevalent in the expanded LOS and LOS groups. Gender, multiple gestation, and prolonged rupture of membranes were not significantly different amongst groups.

RALIS alert

RALIS had an associated alert in 25 of 31 LOS episodes (81%). Of the 6 LOS episodes without an associated alert, 4 were CONS bacteremia. Among the 48 total expanded LOS and/or NEC cases, 40 episodes had an associated RALIS alert at ± 7 days (our standard definition). Of these, 32 episodes had an alert ± 4 days, and 27 had an alert prior to or on the day of clinical suspicion for infection. In the control patients, 74 of 93 had no RALIS alert (80%), and 19 (20%) had a false-positive alert (Table 2). The false-positive RALIS alerts in controls were distributed across GA with 11 occurring in 23–28 week GA infants and 8 occurring in 28–32 week GA range. Thirteen of the 19 infants with false-positive signal had 1 alert, and 6 had more than one alert. Two control infants had persistent elevated signal throughout most of the monitoring period.

The sensitivity of RALIS alert for LOS (± 7 day window) was 81% and specificity was 80%, with a PPV of 57% and NPV of 93%. For expanded LOS, the sensitivity was 84%, with a PPV of 63% and NPV of 93%. Amongst the 9 cases of NEC, 7 of 7 culture-negative cases had an associated RALIS alert (100%). Two episodes of NEC occurred with a concurrent blood-culture proven LOS episode, of which 1 had an associated alert. The sensitivity of RALIS alert for LOS and/or NEC (n=38) compared to controls (n=93) was 84%, with specificity of 80%, PPV of 63%, and NPV of 93%. The NPV predictive value of RALIS alert for LOS and/or NEC with ± 4 day window was 86%. Eleven of 14 culture-negative sepsis episodes had an associated RALIS alert (79%), 7 of which were NEC cases. Of the 7 culture-negative sepsis episodes *without* NEC, 4 had positive associated RALIS signal. Of the 13 false-positive culture episodes, there was a RALIS signal associated with a sent blood culture in 8 cases.

For the LOS group, the mean hours between RALIS alert and culture sent was -43.1 ± 79.4 hours [mean $\pm$ SD] ($p=0.012$, 95% CI: -75.8 to -10.3 hours). Linear regression analysis showed a significant association between RALIS alert time and culture time (β -coefficient = 0.72 , $p<0.0001$). The same statistical analysis was conducted for LOS and/or NEC as the outcome. Again, mean time of alert was earlier, -33.0 ± 79.3 hours before culture ($p=0.025$, 95% CI: -61.5 to -4.4 hours). There was a significant linear association between RALIS alert time and LOS/NEC (β -coefficient = 0.72 , $p<0.0001$) (Figure 1).

RALIS detection and individual VS changes

Figure 2 displays the contribution of each VS to the RALIS output score in a control and an infant with LOS. The control infant is a female born at 27 weeks GA and 925 grams who had no episode of LOS or NEC (Figure 2a). She received antibiotics for 48 hours at birth to rule-out early onset infection. This infant had desaturations during the monitoring period, but there was no RALIS score above the alert threshold. Figure 2b illustrates a female born at 26 weeks GA and 860 grams who developed LOS. There was a positive RALIS alert the day before a blood culture was sent due to clinical suspicion of sepsis. Antibiotics were not initiated until positive culture was resulted for *Staphylococcus warneri* and patient was diagnosed with NEC, for which she received a 14 day course of vancomycin and cefotaxime. To further demonstrate VS parameters in LOS and the contribution to a RALIS alert, we detailed the breakdown of individual vital sign changes in a case of LOS with VS data at baseline, preceding RALIS alert, and just prior to clinical suspicion of infection (Table 3). This 26 5/7 week GA female infant was born at 1140 grams, and infection was clinically suspected on day 11 of life (253 hours: 32 minutes of life). Cultures were sent at that time which later grew *Enterococcus faecalis*. In this infant, normal RALIS output throughout NICU hospitalization was noted until an alert on day of life 9 (197 hours: 47 minutes of life). There was no notable difference in mean temperature, but both heart and respiratory rates were elevated with increased periods of bradycardia prior to RALIS signal. In the timeframe between RALIS signal and clinical suspicion by a provider, the heart and respiratory rates further increased and significantly more desaturation episodes occurred, which likely led to the work-up and initiation of antibiotics. Of note, in this case RALIS alert occurred 55 hours prior to clinical suspicion of LOS.

Cross validation

The expanded LOS and control patients were randomly divided into the train dataset (n=100, LOS cases=29) and test dataset (n=34, LOS cases=12). Birthweight ($p=0.0035$) was included in the model, while GA, gender, multiple gestation, chorioamnionitis, and prolonged rupture of membranes were not significant as predictors of infection. The AUC of the final logistic regression model in the training dataset was 89.9% [95% CI: 82.5–97.2%] (Figure 3). The model-estimated coefficient for RALIS alert in the training dataset was 1.7250; the model-estimated coefficient in the test dataset was 1.8276. The R^2 of the model was 0.3811 in the training dataset and 0.4598 in the test dataset, with an absolute change (increase) in R^2 of 0.0790 and a 5.95% relative percent change in model-estimated coefficients.

Discussion

We have previously tested the RALIS multiple vital signs analysis algorithm in preterm infants for detection of inflammatory response in the setting of LOS. We expanded our work in this study to test an improved version of the RALIS software in a broader GA range and including NEC. Our major finding is that the revised RALIS algorithm functions well in detection of LOS and NEC inflammatory responses within the first four weeks of life, and has particular utility in the exclusion of LOS and NEC with a high NPV. Also, there was an associated alert in the vast majority of NEC episodes, even with negative blood cultures. On

average, a RALIS alert occurred significantly earlier than clinical suspicion of LOS or NEC. Though limited in sample size, in exploratory cross validation analysis, the model estimated coefficients for RALIS in the train and test models remained similar. This reinforces the model of RALIS alert with birthweight in predicting inflammatory responses of LOS and NEC in premature infants < 32 weeks.

The reliable detection and exclusion of LOS and NEC are ongoing clinical challenges in the NICU, given limited diagnostic tools. Thus, vital signs analysis can be a useful, additive approach. The HeRO system has demonstrated signals in LOS approximately 24 hours prior to clinical diagnosis(22). In a subsequent study, Fairchild and colleagues reported a cross-correlation between heart rate and oxygen saturation, demonstrating the potential for other vital signs to improve HRC index monitoring for detection of LOS(29). There is evidence that acute increases in apnea, bradycardia, and desaturation episodes are some of the most common signs of LOS in preterm infants in the NICU(30). Thus, incorporation of multiple vital signs, including episodes of bradycardia and desaturations, as indicators of the pathophysiologic response to acute systemic inflammatory response is logical and innovative.

RALIS is based on this multifaceted approach and has now been tested through iterations of revised algorithms to optimize its predictive value (both positive and negative) for clinical use in the NICU. RALIS overcomes the issue of significant inter-individual variation in VS by using GA and birthweight specific ranges in combination with acute changes from an individual infant's own baseline. All included vital signs contribute to the algorithm output, with respiratory rate and heart rate providing key contributions. Our current data demonstrate an 84% sensitivity of RALIS for inflammatory response in LOS and/or NEC with a high NPV of 93%. It is notable that of the cases of false-negative RALIS output (cases of LOS with no associated alert n=6), two-thirds were CONS species in the blood (n=4), an organism that typically does not elicit a robust systemic inflammatory response. It is also possible that these 4 episodes were reflective of culture contaminants and may not have required antibiotic treatment.

RALIS can detect LOS and NEC before a potential decompensation with mean alerts 33 hours prior to clinician suspicion. Although sensitive, the PPV remains moderate and thus the positive diagnostic value of RALIS to identify LOS and NEC is limited. However, perhaps more importantly, RALIS may reassure a clinician of the absence of LOS and NEC by displaying a normal score as evidenced by the high NPV. The PPV of a RALIS alert is similar to that reported in our previous study (67% vs 63%)(20), with a notably higher NPV (65% vs 93%). An associated RALIS alert was within 7 days of clinical suspicion of infection, with the majority prior to or on the day of culture having been sent. Using a more conservative 4 day window, the NPV remained high at 86%. Of note, alerts that occur *after* clinical suspicion/work-up of an infection are only helpful prior to culture results by reinforcing the clinical decision to evaluate and treat. After infection is confirmed or if a patient continues to be clinically ill, the RALIS results are only helpful in verifying the diagnosis and would not change outcome.

By RALIS providing objective evidence that systemic inflammatory signs are not present (NPV), clinicians may more confidently withhold or discontinue antibiotics, thus potentially reducing the burden of empiric antibiotic overuse in preterm infants. Early life antibiotic exposures not only cause short term complications during the NICU hospitalization, but there is growing literature about the far-reaching effects of antibiotics on risk of chronic conditions such as obesity and asthma later in life(31–33). Antibiotic use for LOS in the NICU remains highly variable and clinicians could benefit from additional evidence-based data to combine with existing and novel tools (34, 35). Of note, there were also false-positive alerts in control patients, limiting the PPV and that would trigger clinicians to evaluate the patient and likely send diagnostic tests for infection. The majority of control infants with false-positive alerts had 1 abnormal signal early in the monitoring period (with 48 hours of output reporting after initial baseline assessment) and normal-range output thereafter. In a few infants, there were multiple false signals or persistently elevated signal. Furthermore, a RALIS alert that occurs many days before positive culture and returns to normal threshold prior to treatment may not be helpful in informing the physician of impending LOS. Prospective study will be essential to understand the factors or conditions that lead to false positive alerts or persistent abnormal inflammatory signal in certain high-risk infants, if not infection. The balance between additional diagnostic laboratory tests and possible antibiotic therapy during a rule-out of sepsis versus discontinuation of antibiotics attributable to use of the RALIS algorithm also requires further prospective studies.

The only change in the study subjects was an expansion of gestational age range. All other exclusion criteria (oscillator ventilation, EOS, transfer or death in first month of life) remained the same between our previous and current study. The exclusion of these infants, who were potentially more severely ill, is a notable limitation to general applicability of this technology at this point. RALIS monitoring for critically ill infants requiring oscillator ventilation, with EOS, and with severe illness leading to early death within first month of life requires further study. Alternative approaches may need to be developed to incorporate respiratory rate and alternate normal ranges in these patients at high risk for negative outcomes.

Sample size constraints remain a limitation in this study, particularly for cross-validation analysis, as is the retrospective nature of the study in a single center. Retrospective review is limited by electronic medical documentation and antibiotic/laboratory data, and thus prospective study of RALIS monitoring can provide more granular information on the utility of this algorithm in real-time NICU care. Infants in the control group had significantly higher GA and birth weight than LOS and NEC groups. This is a source of potential bias, and thus future studies may require a frequency matching approach with these parameters. The strengths of this study include 1) detailed review of the patient's clinical data and precise definitions of culture-proven LOS (with clear delineation of culture source) and 2) removal of both culture-negative LOS and false-positive culture cases from regression analysis, because clinical definitions of LOS are biased by variable physician antibiotic prescribing practices. The inclusion of NEC adds to the confidence in RALIS for detection of systemic inflammatory response from an early evolving infection. In the future, the utility of RALIS for detection of inflammatory response in LOS and NEC prior to clinical suspicion and for its reliable negative signal requires confirmation and further study in a

larger, multi-center prospective trial. In addition, RALIS monitoring needs to be investigated for its utility after the first month of life and in combination with existing or novel laboratory diagnostic tools.

Conclusions

The revised RALIS algorithm of multiple VS analysis can detect acute subclinical changes indicative of systemic inflammatory response such as in LOS and NEC. This retrospective study provides evidence that the RALIS alert identifies preterm infants with developing infection in the first month of life, often earlier than suspected by a clinician and appears to do so even in cases of NEC with negative cultures. The high NPV suggests RALIS may be particularly useful in ruling out LOS and NEC, allowing discontinuation of unnecessary antibiotics in preterm infants who are at highest risk of adverse effects from antibiotic overuse. RALIS may aid in medical decision-making in combination with other clinical and laboratory assessment. Thus, the real time application of this tool is promising and warrants further evaluation in prospective trials.

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Figure 1. Linear Regression Plots of RALIS alert with Culture

a) The regression line for the bivariate association between culture hours of life (HOL) and RALIS alert HOL for LOS [blood, urine]: $\text{RALIS Alert HOL} = 54.2 + 0.72 * \text{Culture HOL}$. b) Regression line equation for LOS and/or NEC: $\text{RALIS Alert HOL} = 62.9 + 0.72 * \text{Culture HOL}$. The shaded region represents the 95% confidence interval for the regression line.

Figure 2. Contribution of individual vital signs to RALIS output

Panel (a) demonstrates RALIS output for a control infant vitals abnormalities, such as desaturations, but no score over 5 generating an alert throughout the monitoring period.

Panel (b) demonstrates RALIS for a case of LOS+NEC. There is an alert, clinical suspicion for infection with blood culture sent (later positive for *S. warneri*), and necrotizing enterocolitis treated until beyond the monitoring period.

Figure 3. ROC Curve for Logistic Regression Model in Cross Validation Analysis

The AUC of the final derivation model in the testing dataset using model parameter estimates yielded an AUC of 0.8985.

Table 1Demographic and clinical characteristics of the patient sample[±]

Variable	Total Sample	Control	Expanded LOS (blood, CSF, urine, ETT)	LOS (blood, urine)	NEC	Culture Negative LOS	False Positive Culture
	N=161	n=93	n=41	n=31	n=9	n=14	n=13
Positive Culture?		No	Yes	Yes	Yes/No	No	Yes
Antibiotic course?		No	Yes	Yes	Yes	Yes	No
Gestational age, mean weeks (SD)	28.8 (2.4)	30.0 (1.7)	26.7 (2.3) *	27.2 (2.3) *	29.3(2.4)	28.9(2.5)	27.4 (1.1) *
Birth weight, grams (SD)	1189.6(25.5)	1351.2(259.7)	931.6 (248.9) *	985.6 (253.4) *	1112.2(304.1) *	1068.9(330.3) *	977.3 (241.3) *
Gender, n (%)							
Male	88 (55)	48 (52)	26 (63)	20 (65)	5 (55)	6 (43)	8 (62)
Female	73 (45)	45 (48)	15 (37)	11 (36)	4 (44)	8 (57)	5 (38)
Multiple gestation	55 (34)	35 (38)	11 (27)	8 (26)	3 (33)	6 (43)	3 (23)
Chorioamnionitis	14 (9)	5 (5)	7 (17) *	5 (16) *	1 (11)	1 (7)	1 (8)
Bronchopulmonary dysplasia	39 (24)	7 (8)	20 (49) *	14 (45) *	4 (44) *	6 (43) *	6 (46) *
Prolonged rupture of membranes, >18 hours	46 (29)	25 (27)	13 (32)	11(36)	3 (33)	4 (29)	4 (31)

[±]Variables reported take into account multiple episodes of sepsis evaluation among and between patients, resulting in a total observation count of N=161 among the 155 patients followed. NEC cases overlap with LOS (positive blood culture, n=2) and culture-negative LOS (n=7).

* Indicates comparison group is significantly different from control group at 0.05 significance level.

No significant gestational age or birth weight differences between LOS (blood, urine, ETT) and LOS (blood, urine).

Table 2RALIS output[‡]

Variable	Control	Expanded LOS (blood, CSF, urine, ETT)	LOS (blood, urine)	NEC	LOS (blood, urine) and/or NEC	Culture Negative LOS	False Positive Culture
<i>n</i>	93	41	31	9	38	14	13
RALIS alert associated with blood culture sent, n (%)	19 (21)	33 (81)	25 (81)	8 (89)	32 (84)	11 (79)	8 (62)
Sensitivity, %	N/A	84	81	89	84	N/A	N/A
Specificity		80	80	80	80		
Positive Predictive Value		63	57	30	63		
Negative Predictive Value		93	93	99	93		

[‡]Variables reported take into account multiple episodes of sepsis evaluation among and between patients, resulting in a total observation count of N=161 among the 155 patients followed. NEC cases overlap with LOS (positive blood culture, n=2) and culture-negative LOS (n=7).

Sensitivity, specificity, positive predictive value, and negative predictive value calculated with condition and control patients by 2x2 table.

Table 3

Vital signs of infant with LOS preceding clinical suspicion

	Temperature (°C)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)	Intervals with bradycardic episode (#)	Intervals with desaturation episode (#)
		mean (std dev)			
48 hour baseline period, 5–6 days before RALIS alert	37.0 (0.29)	157 (15)	45 (9)	2	1
36 hour period before RALIS alert	36.9 (0.25)	166 (9)	54 (11)	6	2
52 hour period between RALIS alert and clinical suspicion	36.9 (0.24)	169 (10)	58 (9)	3	14