Heart Rate Characteristics and Clinical Signs in Neonatal Sepsis

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ABSTRACT: To test the hypothesis that heart rate characteristic (HRC) monitoring adds information to clinical signs of illness in diagnosing neonatal sepsis, we prospectively recorded clinical data and the HRC index in 76 episodes of proven sepsis and 80 episodes of clinical sepsis in 337 infants in the University of Virginia NICU more than 7 d old. We devised an illness severity score based on clinical findings and tests relevant to sepsis. Point scores were derived from coefficients of multivariable regression models, and we internally validated a total score. We determined relationships of the HRC index with individual clinical signs, laboratory tests, and the total score. We found highly significant correlations of the clinical score and individual clinical signs with the HRC index. The clinical score and HRC index added independent information in predicting sepsis, and were similar in clinical and proven sepsis. The clinical score and the HRC index rose before sepsis, and the HRC index rose first. We conclude that clinical signs of illness and HRC monitoring add independent information to one another in the diagnosis of neonatal sepsis. (Pediatr Res 61: 222–227, 2007)

Acute clinical deterioration of infants in the NICU occurs frequently, especially in those of very low birth weight (<1500 g, VLBW), and late-onset bacterial sepsis is a common cause (1). Early diagnosis, before obvious clinical signs of illness, is an important goal but is difficult to achieve. The clinical signs of neonatal sepsis are neither specific nor uniform. Fanaroff et al. in the NICHD Neonatal Research Network found that increasing apnea, feeding intolerance, abdominal distension or guaiac-positive stools, increased respiratory support, lethargy and hypotonia were the most common presenting signs of sepsis, but none were found to have high predictive accuracy (2).

Diagnosis of late-onset neonatal sepsis is difficult even when infants appear ill because blood cultures have a substantial false-negative rate, especially when based on small volumes of blood (3–7). Accordingly, the Centers for Disease Control recognizes “clinical sepsis” in infants, which requires only signs of illness with antibiotic therapy but no positive blood culture (8). The NICHD Neonatal Research Network found that both proven sepsis and clinical sepsis were associated with neurodevelopmental impairment in extremely low birth weight (<1000 g, ELBW) infants (9). The common denominator is the systemic inflammatory response syndrome (SIRS), the result of the host response to infectious and noninfectious insults (10). Clearly, new approaches to the early diagnosis of both proven and clinical neonatal sepsis are required.

Before physician suspicion of sepsis and SIRS, neonates have reduced heart rate variability and transient decelerations similar to the findings in distressed fetuses (11,12). Heart rate measures optimized to detect these abnormal heart rate characteristics (HRC) (11,13–15) were used to develop an HRC index at one NICU and then validated at another as a predictor of neonatal infection and death (16–18).

Laboratory tests such as the I:T ratio add predictive information independent of the HRC index (19). Clinicians also have ready access to many other sources of information about the status of the infant such as the general appearance, vital signs, apnea, oxygen requirement, and feeding tolerance. Our goal in this work was to determine whether the HRC index added to the clinical information that is already available to physicians in the diagnosis of late-onset neonatal sepsis.

Accordingly, we developed a clinical illness score that was specifically relevant to the diagnosis of neonatal sepsis. The method for development followed that of the Richardson score (20) for neonatal mortality or prolonged ventilation. Thus armed, we examined the relationship between HRC and objective measures of clinical illness. The major hypothesis was that HRC monitoring and clinical signs provide independent information in the early diagnosis of late-onset clinical and proven neonatal sepsis.

METHODS

Patient population. We studied all admissions to the University of Virginia NICU from August 2001 to July 2003 who were 7 or more days of age and had 7 or more days of HRC monitoring. The clinical research protocol

Abbreviations: HRC, heart rate characteristics; I:T ratio, ratio of immature to total neutrophils; VLBW, very low birth weight; WBC, white blood cell count
was approved by the Human Investigation Committee of the University of Virginia.

**Clinical and HRC index database.** We prospectively recorded clinical
and respiratory support into a relational events database (Microsoft
Access), and laboratory results were available from an electronic archive.
Each hospital course was divided into 6-h blocks beginning at midnight (n =
41,769), and the HRC index and clinical score were noted for each block.
Healthcare personnel were not aware of the result of the HRC monitoring.

The HRC index reflects the degree of reduced variability and transient
decelerations in the prior 12 h. We record RR intervals in sets of 4,096,
approximately 20 to 25 min in duration, depending on the heart rate. For each
we calculate SD, sample asymmetry (the characteristic abnormality is in-
creased asymmetry due to more decelerations and fewer accelerations (13))
and sample entropy (SampEn, and the characteristic abnormality is a reduc-
tion due to outliers (14,15,21)). We select the median SD and sample
asymmetry values over the preceding 12 h, and the 10th percentile lowest
SampEn value. These are used in a regression expression relating these
parameters to episodes of clinical illness that was developed at one center and
validated at another (16). The result is the probability of clinical illness in the
next 24 h. It is divided by the average probability and presented as the
fold-increase in risk of imminent clinical illness.

**Proven sepsis and clinical sepsis events.** We defined proven sepsis as
clinical signs of sepsis and a positive blood culture prompting five or more
days of antibiotic therapy. We defined clinical sepsis as clinical signs of sepsis
with a negative blood culture prompting five or more days of antibiotic
therapy (9). Recurrent episodes of sepsis were considered to be distinct if they
occurred 7 or more days apart.

**Clinical illness score.** We (1) identified candidate findings of neonatal
sepsis based on clinical experience, (2) made internally-validated regression
models relating these findings to proven and clinical sepsis (22), and (3) used
coefficients of the models to make an integer-based score (23).

The candidate findings that appeared in the final score were: Severe apnea
requiring positive pressure ventilation or 50% increase in apnic episodes
over 24 h in an extubated infant stable for three days; increased ventilatory
support and \( F_{O_2} \) by 25%; temperature instability (>38°C or <36.2°C) twice
in 8 hours; lethargy or hypotonia; feeding intolerance (feedings held for >24
h) in an infant tolerant of advancing or full feeds for 3 days; immature/total
neutrophil (I:T) ratio >0.2; white blood cell count >25,000 or <5,000/mm\(^3\);
hyperglycemia (>180 mg/dL).

Other findings that were considered but did not remain sufficiently asso-
ciated with sepsis after taking all the other findings into account were: New
onset thrombocytopenia (<100,000/mm\(^3\)); severe hypotension requiring vol-
ume or pharmacologic support; increased support on nasal CPAP, nasal
cannula or hood oxygen.

We assigned clinical illness points a duration of 12 hours or until a follow-up
normal laboratory result or a change in the type of ventilatory support occurred.

**Statistical analysis.** We used multivariable logistic regression modeling
adjusted for repeated measures and a Wald \( \chi^2 \) test of the hypothesis that
variables added independent information (16,17). Internal validation of the
predictive logistic regression models was performed using a bootstrapping
methodology that has been recommended as more efficient over traditional
split-sample approaches (22). Predictions for each infant were calculated
based on coefficients estimated using a training set of all other infants. We
evaluated the correlation of HRC measures with clinical signs using boot-
strapped estimates of the \( p \)-value. Confidence intervals for the correlation
coefficients, also determined by bootstrap, were <0.03.

**RESULTS**

**Clinical and HRC database.** Demographics of the patient
population are shown in Table 1. We prospectively recorded

<table>
<thead>
<tr>
<th>Total</th>
<th>&lt;1500 g</th>
</tr>
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<tbody>
<tr>
<td>No. of infants</td>
<td>337</td>
</tr>
<tr>
<td>Birth weight</td>
<td>1,460 (910, 2, 539)</td>
</tr>
<tr>
<td>Gestational age</td>
<td>30 (27, 35)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>56</td>
</tr>
<tr>
<td>Illness events</td>
<td></td>
</tr>
<tr>
<td>Positive blood culture (infants)</td>
<td>76 (63)</td>
</tr>
<tr>
<td>Negative blood culture (infants)</td>
<td>80 (63)</td>
</tr>
</tbody>
</table>

Results are presented as median (25th, 75th percentiles) or episodes (patients).

more than 57,000 clinical observations in 337 infants. There
were 76 episodes of proven sepsis and 80 episodes of clinical
sepsis. As expected, the majority of cases of sepsis occurred in
VLBW infants.

**Descriptions of illustrative cases.** Figure 1 shows graphical
displays of clinical data and HRC index for the hospital
courses of three infants. The top half of each panel shows the
presence of selected clinical signs, and the shade of the bar
reflects the severity of the sign and the clinical point score
associated with it. The bars representing blood cultures have
duration equal to the antibiotic therapy. The bottom half
shows the HRC index (solid line) and the clinical score
dashed line).

Figure 1A shows the benign course of an infant born at
gestational age 29 wk and birth weight 1,149 g. After the first
several days of life, there were no major clinical events or
major HRC abnormalities during the nearly 8 wk course. The
infant was intubated for about 1 wk and on other oxygen
support until day 45. Blood and urine cultures were drawn late
on day 20 because of increasingly frequent episodes of apnea
followed by temperature instability. Laboratory results of
WBC, I:T ratio, platelet count blood glucose were normal.
Cultures were negative, and antibiotics were administered
for 2 d.

Figure 1B shows the course of an infant of gestational age
29 wk and birth weight 1,285 g who had an episode of late-onset neonatal sepsis. On day 18, Klebsiella sepsis was
diagnosed and manifest by feeding intolerance and need for
intubation and mechanical ventilation. On day 21, he failed
extubation and required re-intubation. On day 40, there was
an increase in the clinical score due to temperature instability,
frequent apnea and feeding intolerance, but this was not
accompanied by a rise in HRC index, positive blood culture,
prolonged antibiotic therapy or abnormal laboratory tests.

Panel C shows the course of another infant, gestational age
26 wk and birth weight 715 g, who had an episode of
late-onset neonatal sepsis. On day 30 there was an episode of
Klebsiella sepsis with hyperglycemia and need for intubation
and mechanical ventilation. On day 107, an abrupt rise and fall
of the HRC index marks the time of bilateral inguinal herni-
orrhaphy and circumcision under anesthesia.

From inspection of graphical records of individual patients,
we hypothesized that both HRC and clinical signs report on
neonatal sepsis. If the hypothesis is true, then HRC and
clinical illness should rise before the clinical diagnosis, HRC
and at least some clinical signs should be correlated, and HRC
and clinical signs should predict sepsis.

**A clinical score for neonatal sepsis.** The performance of
predictive models depended on the time window of the training
set. Clinical signs are the usual means for suspecting the
diagnosis of neonatal sepsis, and are thus clustered around
episodes of illness. If the time window for model-training
included the time of diagnosis (that is, the time of the blood
culture), model performance was improved. If the time win-
dow was just before, but did not include the time of the blood
culture, signs were sparse and model performance deterio-
rated. In either case, there was highly significant association
with feeding intolerance, increased frequency and severity of
apnea, abnormal I:T ratio, need for increased support on mechanical ventilator, and temperature instability with the diagnosis of sepsis. Hyperglycemia and abnormal WBC were significantly associated with sepsis only when the time window included the time of the blood culture. Conversely, the finding of hypotonia and lethargy was significantly associated with sepsis only when the time window preceded the blood culture. Based on the regression coefficients, we assigned the point score shown in Table 2. Systematic analysis of changing each coefficient confirmed that the final score was optimal.

Several clinical findings commonly associated with sepsis did not appear in the final score. Severe hypotension is certainly a sign of sepsis, but occurred very infrequently in this population – only 3% of infants with sepsis had this sign. Thus it failed to reach sufficient significance in our scheme to warrant a point. New-onset thrombocytopenia is also a finding in sepsis, but did not reach sufficient association with sepsis after taking everything else into account.

**HRC index and clinical score increase at the time of neonatal sepsis.** Figure 2 shows the mean HRC index and clinical scores as a function of time relative to the 156 episodes of proven or clinical neonatal sepsis. The plots have been scaled to allow comparison of the changes near the time of sepsis. Asterisks mark data points that differed significantly from 24 h prior \((p < 0.05, t\text{-test})\). The HRC index rises first, and shows significant increases over the 24 h before clinical suspicion.

### Table 2. Point score for imminent neonatal sepsis

<table>
<thead>
<tr>
<th>Signs</th>
<th>Points</th>
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</thead>
<tbody>
<tr>
<td>Feeding intolerance (feedings held for greater than 24 hours) in an infant who had been tolerating advancing or full feeds for three days</td>
<td>2</td>
</tr>
<tr>
<td>Severe apnea requiring positive pressure ventilation</td>
<td>2</td>
</tr>
<tr>
<td>50% increase in number of apneic episodes over a 24 hour period in an infant who had been extubated and stable for three days</td>
<td>2</td>
</tr>
<tr>
<td>Immature/total neutrophil (I:T) ratio greater than 0.2</td>
<td>2</td>
</tr>
<tr>
<td>Increase in ventilatory support and (F_{\text{O}_2}) by 25% from baseline</td>
<td>1</td>
</tr>
<tr>
<td>Lethargy or hypotonia</td>
<td>1</td>
</tr>
<tr>
<td>Temperature instability (&gt;38°C or &lt; 36.2°C); two episodes within an eight hour period</td>
<td>1</td>
</tr>
<tr>
<td>Hyperglycemia (&gt;180 mg/dL)</td>
<td>1</td>
</tr>
<tr>
<td>Abnormal white blood cell count (&gt;25,000 or &lt; 5,000)</td>
<td>1</td>
</tr>
</tbody>
</table>

**HRC correlates with clinical signs of sepsis.** The mean HRC index, taken over the entire hospital course for each infant, was highly significantly correlated with the mean clinical score \((r = 0.55, n = 337 \text{ infants, } p < 0.001)\). In addition, the 6-hourly HRC index measurements \((n = 41,769)\) were highly significantly associated with the corresponding 6-hourly clinical scores, with correlation coefficient \(r = 0.27\) (95% confidence interval 0.24–0.30). Each of the candidate signs was significantly correlated with the HRC index \((p < 0.01)\). The highest correlation was with increased ventilatory support, with \(r = 0.24\) (95% confidence intervals 0.21–0.27). Correlations with the laboratory tests led to \(r\) values between 0.14 and 0.16, and correlations with the other signs led to \(r\) values between 0.04 and 0.07.

**HRC and clinical scores predict sepsis.** Individually, both the clinical score and the HRC index were predictive of sepsis in the next 24 h (ROC areas 0.62 and 0.67, respectively, \(p < 0.001)\). Knowledge of both led to higher association with imminent sepsis (ROC area 0.70, \(p < 0.001)\), and the HRC index added significantly to the clinical score \((p < 0.001)\). The performance of models that included data from the time of diagnosis was better, with ROC areas of 0.80 (clinical score), 0.82 (HRC index), and 0.85 (combined).
Figure 3. Odds ratios for clinical findings and the HRC index in the prediction of clinical and proven sepsis in the next 24 h. Horizontal lines are 95% confidence limits. The inset above shows the time window for training the models – the 24 h preceding, but not including, the time of the blood culture obtained for suspicion of sepsis (BC).

Figure 4. Odds ratios for clinical findings and the HRC index at the time of diagnosis of clinical and proven sepsis. Horizontal lines are 95% confidence limits. The inset above shows the time window for training the models – the 6 h preceding and 18 h following the time of the blood culture obtained for suspicion of sepsis (BC).

HRC index in diagnosing the symptomatic infant. The major finding is that clinical signs and tests are less useful in the time window preceding sepsis, because they are present much less often. This is expected – once findings are present, we expect the diagnosis to be suspected and tested for promptly. Here we categorized the HRC index into low: 70% lowest values, less than 1-fold increase in risk; intermediate: 70th to 90th percentile values, 1- to 2-fold increase (not shown); and high-risk: 10% highest values, more than 2-fold increase groups (19).

Odds ratios for prediction and diagnosis of neonatal sepsis are shown in Figures 3 and Figure 4. They were determined from multivariable regression models taking all the variables into account. In Figure 3, the time window for training multivariable regression models was the 24 h before the event. In Figure 4, the time window was a 24-h period including the time of diagnosis, and the evaluation is of clinical findings and

HRC index

Figure 5. HRC index measurements in the 6 h before the diagnosis of clinical sepsis (n = 77 of 80 episodes) or proven sepsis (75/76) compared with control measurements (n = 211). B. Clinical scores. Neither HRC measurements nor clinical scores differ between clinical and proven sepsis, though both are highly significantly higher than control.

Figure 6. A risk assessment card for neonatal sepsis based on clinical signs and HRC monitoring.
clinical score and HRC monitoring. When neither is measured, the fold-increase in risk of illness is 1.0. The clinical score alone differentiates infants across a spectrum of risk, as does the HRC index. Knowledge of both allows greater refinement in the estimation of risk. For example, an infant with a clinical score of 0 generally has lower risk of illness, but the concurrent finding of a high-risk HRC index identifies a subset with 2.5-fold increase in risk. For infants with 2 points or more, that is, with clinical findings of illness, knowledge of the HRC index adds little and even a low-risk HRC index does not cancel out the clinical presentation. This is in keeping with the idea that the HRC index is adjunctive to clinical information, and is not a standalone substitute for medical personnel.

**DISCUSSION**

We studied heart rate characteristics (HRC) and objective findings of clinical illness in infants in a tertiary care NICU. The major findings were (1) a validated HRC index was correlated with clinical signs of illness and abnormal laboratory tests as well as a composite score of clinical illness severity, and (2) the HRC index added significantly to clinical signs and abnormal laboratory tests in predicting imminent sepsis.

The mechanism by which sepsis leads to reduced variability and transient decelerations of heart rate is not known, though we speculate that circulating cytokines may interfere with normal signal transduction in sinus node pacemaker cells. There is substantial evidence that sepsis causes large increases in the circulating levels of many cytokines (24), and that these changes are evident before the clinical diagnosis of illness in newborn infants (25). Sepsis is not the only illness in the NICU that alters cytokine levels, and we know that the HRC index also increases with illnesses such as necrotizing enterocolitis, intracranial hemorrhage and respiratory failure, and at the time of surgery. Sepsis remains the focus of our work with HRC monitoring as it is common, and it seems reasonable that earlier diagnosis and therapy might improve outcome.

**Inclusion of infants with negative blood cultures in studies of neonatal sepsis.** Stoll et al. (9) in the NICHD Neonatal Research Network recently reported on neurodevelopmental impairment (NDI) in extremely low birth weight (<1000 g, ELBW) infants. The major finding was that infants who had one or more episodes of infection had a 50% increase in the already high rate of NDI. Importantly, NDI was equally increased in infants with “clinical sepsis,” that is, sepsis-like illness warranting five or more days of antibiotics despite negative blood cultures. This finding justifies inclusion of these patients in evaluating novel means for early diagnosis of neonatal sepsis, and it is reasonable to suspect that some of them had bacterial sepsis that was not detected by blood culture, an imperfect test. Certainly the NICHD Neonatal Research Network clinicians must have believed the clinical presentation more than the blood culture, as prolonged courses of antibiotics were administered. Our findings of similar HRC and clinical signs in proven and clinical sepsis are consistent with our earlier work (11,16).

**A clinical illness score relevant to neonatal sepsis.** Neonatal sepsis is suspected because of clinical signs of illness. When signs are severe enough, prolonged antibiotic therapy is administered even if the blood culture is negative; this is “clinical sepsis.” Thus using a clinical score to predict neonatal sepsis is circular – since the signs are the diagnosis, they will never fail as a diagnostic test. Laboratory tests are also problematic, as they are much more frequently available near the time of sepsis (19). A more stringent test of the utility of clinical signs in early diagnosis is to analyze them before the clinical diagnosis of sepsis, when subtle signs might be attributed to benign causes. This is the approach we used in developing the HRC index, where the outcome of interest was the 24 h before, but not including, the quarter-day in which the diagnosis was made.

Here we developed and validated internally a clinical score following a method similar to that used by Escobar and coworkers to produce the Richardson score for neonatal mortality or prolonged ventilation (20). We began with clinical signs that we knew were associated with sepsis, and optimized their weighting. As expected, these signs were more predictive of sepsis when observed at the time of diagnosis. There were, however, highly significant associations in the preclinical phase as well. The most predictive findings were feeding intolerance, hypotonia and lethargy, abnormal I:T ratio, severe or increased apnea, need for increased support on mechanical ventilator, and temperature instability. The finding that I:T ratio was the most robust independent predictor of sepsis confirms our recent finding in a superset of this population (19).

We found a small increase in the clinical score in the 24 h before the clinical diagnosis of illness. This finding, which further validates the score, is not surprising since sepsis and SIRS are preceded by a period during which cytokines are elevated though symptoms are subtle or absent (25). This finding confirms that of Fanaroff et al. (2) that clinical signs were increased before the diagnosis of proven sepsis, and extends it by combining them into a clinical illness severity score and demonstrating similar changes before clinical sepsis. However, clinical experience teaches that the early diagnosis of neonatal sepsis and SIRS using clinical signs and laboratory findings alone is unsatisfactory, and that new diagnostic measures are badly needed (1,26).

Surprisingly, up to 40% of infants with clinical and proven sepsis had 0 points. Several factors could contribute to this finding. First, several of our signs required the presence of an abnormal clinical sign or lab test for 8 or 24 h. Thus the very acute illness would not score points. Second, infants might have had values near but not quite exceeding the threshold for points. For example, an infant with a 40% increase in apnea, 18 h of feeding intolerance, one episode of temperature instability and an I:T ratio of 0.18 would have 0 points despite an overall clinical presentation that could represent impending sepsis. Different thresholds and different tests would lead to different results, but scoring systems with hard cutoffs all suffer this shortcoming.

**HRC index is correlated with clinical signs of illness and abnormal laboratory tests.** We found that the HRC index was highly significantly associated with objective clinical findings
of illness and with abnormal test results. There were correlations with individual signs and tests, and with the composite clinical score whether measured at each 6 h time point or averaged over the entire hospital course. Correlations were moderate or weak in degree.

Summary. In summary, we have devised an internally validated point score system relevant to late-onset neonatal sepsis. We found that heart rate characteristics (HRC) monitoring is correlated with this score, adds independent information to it, and becomes abnormal before it, among infants with clinical and proven neonatal sepsis. Since the HRC index is continuous and in real time, it does not require any new contact with the patient, and is significantly associated with imminent clinical neonatal illness, we feel that it may have a favorable impact on clinical care. While it will not replace clinical assessment by the physician, blood cultures or other laboratory tests, HRC monitoring can add information to conventional measures in the early diagnosis of neonatal sepsis.

REFERENCES

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