Mortality Reduction by Heart Rate Characteristic Monitoring in Very Low Birth Weight Neonates: A Randomized Trial

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Objective To test the hypothesis that heart rate characteristics (HRC) monitoring improves neonatal outcomes.

Study design We conducted a two-group, parallel, individually randomized controlled clinical trial of 3003 very low birth weight infants in 9 neonatal intensive care units. In one group, HRC monitoring was displayed; in the other, it was masked. The primary outcome was number of days alive and ventilator-free in the 120 days after randomization. Secondary outcomes were mortality, number of ventilator days, neonatal intensive care unit stay, and antibiotic use.

Results The mortality rate was reduced in infants whose HRC monitoring was displayed, from 10.2% to 8.1% (hazard ratio, 0.78; 95% CI, 0.61-0.99; $P = .04$; number needed to monitor = 48), and there was a trend toward increased days alive and ventilator-free (95.9 of 120 days compared with 93.6 in control subjects, $P = .08$). The mortality benefit was concentrated in infants with a birth weight <1000 g (hazard ratio, 0.74; 95% CI, 0.57-0.95; $P = .02$; number needed to monitor = 23). There were no significant differences in the other outcomes.

Conclusion HRC monitoring can reduce the mortality rate in very low birth weight infants. *(J Pediatr 2011; ■:■-■).*

See editorial, p •••

Late-onset neonatal sepsis is an important cause of morbidity and mortality in very low birth weight infants in a neonatal intensive care unit (NICU).[1] Signs of illness are subtle and non-specific, so a clear-cut clinical presentation may appear only late in the course,[2] by which time severe illness is present.

Reduced variability and transient decelerations in heart rate (HR) may be present in the hours to days before diagnosis of late-onset proven or clinical neonatal sepsis.[3] Although the precise mechanisms are not known, one possibility is vagal activation in response to infection via the cholinergic anti-inflammatory pathway.[4-6] These abnormal HR characteristics (HRC) in response to systemic infection and inflammation have been characterized mathematically, and the resulting HRC index is the fold-increase in risk of sepsis during the next 24 hours.[7,8] This risk assessment is estimated with an externally validated multivariable logistic regression that is derived from calculations of the SD of the heartbeat intervals with novel measures of sample asymmetry[9] and sample entropy.[10-12] The index can be computed in real time and displayed continuously at the bedside.

Monitoring the HRC index score in high-risk premature infants might result in improved outcomes through early warning of subacute potentially catastrophic illnesses characterized by systemic inflammation, such as sepsis. Such early warning might lead to patient-specific testing and intervention including, in some cases, early antibiotic therapy. To test this hypothesis, we performed a two-group, parallel, individually randomized controlled clinical trial.

Methods

Very low birth weight (<1500 g birth weight) infants at 9 hospitals were randomized to display or not to display the HRC monitor results. This was a parallel

CTO	University of Virginia Clinical Trials Office
DSMB	Data Safety Monitoring Board
ELBW	Extremely low birth weight
HR	Heart rate
HRC	Heart rate characteristics
MPSC	Medical Predictive Science Corporation
NICU	Neonatal intensive care unit

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Trial is registered at ClinicalTrials.gov (NCT00307333).

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group study with one-to-one randomization. Initially, all inborn and outborn babies with birth weights <1500 g were eligible. After enrolling 257 infants, we further restricted inclusion criteria for outborn infants to include only very low birth weight infants who had been transferred to a study center at <33 weeks post-menstrual age. This modification was made to exclude the rare more mature very low birth weight infant at low risk for neonatal sepsis who was transferred to a study center for a short (often surgical) stay. The initial University of Virginia institutional review board did not require informed consent. After the first 186 infants were enrolled there, informed consent was added to the protocol at the request of a National Institutes of Health Special Emphasis group.

From April 2004 to May 2010, we randomized 3003 infants. The study was conducted in the NICUs of the University of Virginia, Wake Forest University, University of Alabama at Birmingham, Vanderbilt University, University of Miami/Jackson Memorial Hospital, Greenville South Carolina Hospital System, Winnie Palmer Children's Hospital (Orlando, Florida), and Pennsylvania State University. Institutional review boards approved the protocol at each study site.

Heart Rate Characteristics Monitoring

We used the HeRO monitoring system provided by Medical Predictive Science Corporation (MPSC; Charlottesville, Virginia) in a special study mode that linked randomization status to display features. Before the study, the system received 510(k) US Food and Drug Administration clearance for reporting the HRC index as a measure of reduced variability and transient decelerations. The monitors displayed an index showing the fold-increase in the risk of developing neonatal sepsis in the next 24 hours, as derived and verified from data collected before the study at the University of Virginia and Wake Forest University.[7] Values were updated hourly and included a graph of the 5-day trend, thus permitting the clinicians to quickly evaluate changes in the index. Although physicians, nurse practitioners, and bedside nurses were instructed that a rising HRC monitoring score should lead to a bedside evaluation and consideration for blood tests, specific actions were not required by the study protocol. All patients received conventional bedside vital sign and waveform monitoring. For control patients, the HRC monitor results were recorded, but not displayed. For infants whose HRC monitoring results were displayed, the HRC index was shown on separate monitors.

Outcomes

The primary outcome measure was the number of days alive and off the ventilator in the 120 days after randomization,[13,14] calculated as 120 days minus the number of days on ventilator minus number of days deceased. We also evaluated 4 secondary outcomes: in-hospital mortality, number of ventilator days, length of NICU stay, and number of days on antibiotics. Follow-up at 120 days was made via telephone and mail. We also evaluated outcomes at the time of discharge from the NICU or the hospital, for which data certainty was complete. Results did not differ for these two approaches. We noted dates and times of blood cultures drawn for the suspicion of sepsis, and the use and type of all parenteral antibiotics not including acyclovir or fluconazole used for antifungal prophylaxis.

Database Management

Research staff at each institution entered dates and times of death, mechanical ventilation, antibiotic doses, and NICU and hospital admission and discharge in a dedicated computerized database overseen by the University of Virginia Clinical Trials Office (CTO) and with study software developed by MPSC in accordance with a 1999 US Food and Drug Administration Guidance.[15] Personnel from the CTO collected patient enrollment and death data directly from the study centers every week via fax, verified every death from the medical record, and made yearly site-monitoring visits to compare the database and clinical records of a random 10% selection of the study infants. In addition, CTO personnel regularly submitted quality-checking database queries. Study site personnel made required corrections in the database for patients discharged within the past 6 months. Thereafter, MPSC made the corrections to the database records, following a written request from the site personnel or from the CTO. Audit trails were maintained for all database entries and for corrections to originally reported data. Raw data were submitted for analysis by the independent statistician (G.S.) for DSMB (Data Safety Monitoring Board) meetings and for the final report.

Sample Size

We reasoned that an increase of 2.0 days of life without mechanical ventilation was clinically meaningful, and we estimated that a total sample size of 3000 was sufficient to test this hypothesis with $\alpha = 0.05$ and $1\text{-}\beta = 0.90$. The independent statistician (G.S.) reported interim analyses to the DSMB after each increment of 300 completed patients, excluding cases with missing outcome data. The latest report prepared for DSMB review was submitted February 2010, described outcomes of the first 2400 randomized patients, and presented an analysis corrected for all the interim analyses. The mortality difference approached statistical significance toward the end of the DSMB review, but never crossed the threshold for stopping the trial.

Randomization and Masking

Patients were first stratified by birth weight at 1000 g and then randomized in 8-subject blocks at each site by using computer-generated sequences. Once randomized, HRC monitor data were displayed immediately for the study group and masked for the control group. Research coordinators and study physicians recruited participants and obtained informed parental consent. NICU healthcare personnel were aware of the assignment to the study group, had continuous access to HRC monitoring results when displayed, and were masked to the HRC index of the control infants. There were no other protocol-defined differences in the care.

Statistical Methods

The statistical significance of differences between the control and study group baseline characteristics were assessed using single factor analysis of variance tests to compare means for continuous variables, and chi-square tests to compare proportions. The statistical significance of differences between groups in mean number of days alive and off the ventilator, days in NICU, ventilator days and days on antibiotics was tested using single factor analysis of variance. Differences in survival rates between the study and control groups were assessed with proportional hazards regression analysis and the log-rank test statistic, with both left truncation and right censoring. Cases in the analysis are left truncated on the basis of the study enrollment date (date of consent) and right censored on the basis of the end of follow-up (either date of death, discharge from hospital, or discharge from NICU). Hazard ratios are reported with 95% CIs. The significance of the difference in rates of blood cultures was tested with a large sample Z-test. All statistical test results were considered to be significant at the P value <.05 threshold. All calculations were performed with SAS software version 9.2 (SAS Institute, Cary, North Carolina). The trial is registered at ClinicalTrials.gov (NCT00307333).

Subgroup Analysis

We assessed the heterogeneity of treatment effect for the prespecified subgroup of extremely low birth weight (<1000g, ELBW) infants, who are at highest risk for mortality. The statistical significance of difference in treatment effect was assessed in the Cox proportional hazards regression model by testing significance of the interaction term combining effects of the intervention and ELBW status.

Results

We screened 5995 infants for eligibility (Figure 1). A total of 3003 infants were randomized from April 2004 through May 2010. A total of 2989 infants were included in the analysis. Before unblinding and analysis, we excluded 14 randomized infants because of birth weight ≥1500 g (4 in the study group, one in the control group), error in randomization (3, all in the study group), consent withdrawn (two in the study group, one in the control group), or sustained cardiac arrhythmia (two in the study group, one in the control group). There were no significant differences between the control and study groups for birth weight, gestational age, study site, sex, race, Apgar score, admission diagnosis (Table), or any other characteristic measured at enrollment.

Infants meeting the eligibility criteria for the trial were enrolled at average age 3.8 days. They were observed beginning on the day of enrollment until the date of their discharge from the hospital or until death, as long as 120 days after enrollment. An average of 61 days of inpatient data was available for each infant. There were no statistically significant differences in the number of days of follow-up available between patients in the study group and patients in the control groups.

Heart Rate Characteristics Monitoring

Figure 2 shows a screen display of the HRC monitor for a patient before the trial. The *top panel* is a 5-day plot of the fold-increase in risk of illness in the next 24 hours, with 1-fold representing the average risk. In this infant, between October 22 and October 23, there was a rise to 4-fold risk. The *bottom panel* is 30 minutes of HR at the time of rising HeRO score, noted by the yellow line. There are very pronounced abnormal HRC of reduced variability and transient decelerations. This is the scenario central to the use of the monitor. The risk of illness had been low—<1-fold the average risk of illness in the subsequent 24 hours—and then rose. The suggested reaction was to examine the infant. Mild feeding intolerance developed in this infant near the time of the HRC peak, and a blood culture at that time grew *Staphylococcus aureus*.

Primary and Secondary Outcomes

Infants whose HRC were displayed had an average of 2.3 more days alive and off the ventilator (P = .083, Table). There were 274 deaths (9.5%) in the 120-day follow-up period: 152 infants (10.2%) in the control group and 122 infants (8.1%) in the study group, with an absolute risk reduction of 2.1%. The infants whose HRC monitoring results were displayed had a 22% lower relative hazard of inpatient death (hazard ratio = 0.78, 95% CI, 0.61-0.99; P = .04). Assuming that our 120-day follow-up was complete for known survivors, the results are the same (hazard ratio = 0.77; 95% CI, 0.61-0.99; P = .04). No center found a higher mortality rate in the infants whose HRC monitoring results were displayed. We found no statistically significant differences in the groups in the mean number of days on ventilator for survivors, mean number of days in NICU, or mean number of days on antibiotics (Table). Figure 3, A presents a plot of the survival probability functions for each group of very low birth weight infants in the period of available follow-up.

Subgroup Analysis: Extremely Low Birth Weight Infants

We assessed whether the effect of HRC monitoring on in-hospital mortality rate differed between ELBW infants and infants with birth weight between 1000 g and 1500 g. This subgroup analysis was justified by the stratification categories defined in the study design. The statistical significance for a difference was assessed in the proportional hazards regression model by including an interaction term between group assignment and ELBW status. The Table summarizes the subgroup analysis results. In the stratified analysis, the 1513 ELBW infants whose HRC monitoring results were displayed had a 26% lower relative hazard of death (hazard ratio = 0.74; 95% CI, 0.57-0.95; P = .02). A test for interaction showed no statistically significant difference in the effect of HRC monitoring on survival of

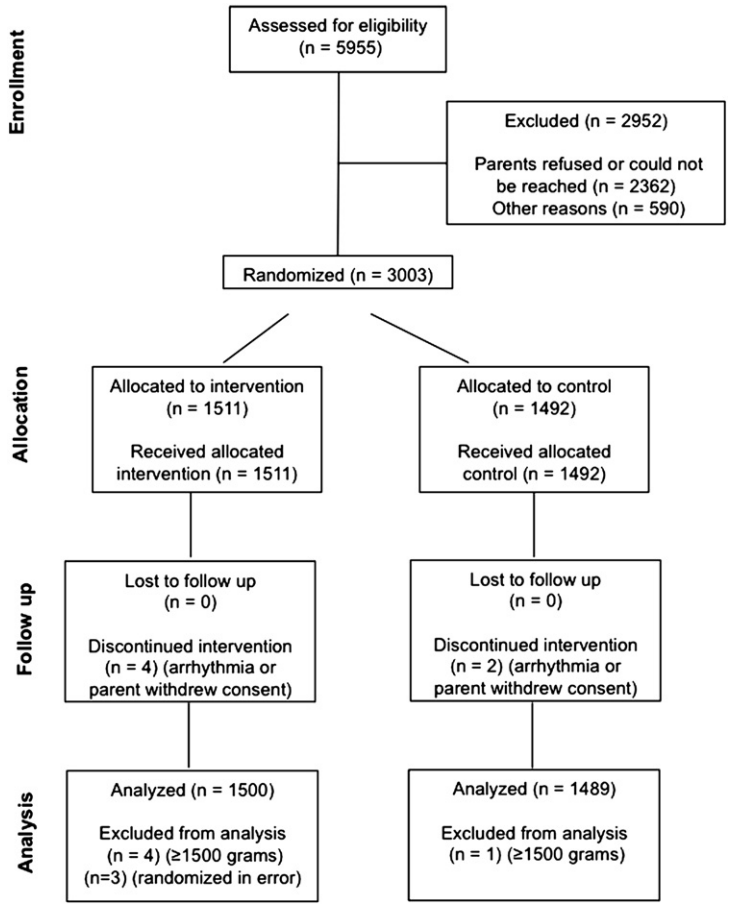

Figure 1. Consolidated Standards of Reporting Trials diagram.

ELBW infants compared with infants with birth weights >1000 g (P = .22). We attribute this finding to the disproportionately large number of deaths in the ELBW group. The 233 deaths in the ELBW infants represented 85% of deaths in study participants. In ELBW infants, 17.6% (133 of 757) in the control group died, as compared with 13.2% (100 of 756) in the HRC monitored group, an absolute risk reduction of 4.4%. No other subgroup analysis was conducted.

Is the Mortality Benefit of Heart Rate Characteristics Monitoring Related to Sepsis?

HRC monitoring is predicated on the idea that neonatal sepsis is preceded by subclinical reduced variability and transient decelerations of HR. Thus, we explored the idea that the mortality benefit in this trial was caused by early diagnosis of neonatal sepsis. We plotted the observed risk of culture-proven sepsis in the next 24 hours as a function of the risk that was predicted by the HRC index. Figure 3, B shows the resulting predictiveness curve,[16,17] which shows the distribution of observed risk of sepsis in the next 24 hours binned in deciles (*open circles*) and the HRC index (*line*) plotted from lowest to highest. This display combines features of both risk assessment and diagnostic classification across the entire range of possible results, improving on standard concepts of sensitivity and specificity. We found good agreement of the observed and expected risks, affirming the validity of the HRC index as a risk measure for imminent sepsis.

The incidence of proven sepsis was not different in the infants whose HRC monitoring results were displayed (358/1500 compared with 379/1489, P = .34). To test the possibility that altered detection or management of sepsis contributed to the mortality benefit, we compared the effect of HRC monitoring on outcomes of infants who had at least one sepsis episode. We found that the mortality rate in the 30 days after the first episode of proven sepsis was 10.0% in the infants whose HRC monitoring results were displayed compared with 16.1% in the control infants, an absolute risk reduction of 6.1% (36/358 versus 61/379, P = .01).

We also tested the possibility that HRC monitoring led to more sepsis work-ups and courses of antibiotics. Infants whose HRC monitoring results were displayed had 10% more blood cultures drawn for the suspicion of sepsis (1.8 per month compared with 1.6, P = .05) and 5% more days on antibiotics (15.7 compared with 15.0, P = .31, Table).

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Table. Characteristics and outcomes

	Infants whose HRC monitoring results were displayed (n = 1500)	Control (n = 1489)	*P* value
Characteristics			
Mean birth weight, g (SD)	999 (283)	986 (289)	.22
Mean gestational age, weeks (SD)	28 (2.8)	28 (2.8)	.54
Male sex, n (%)	791 (52.7)	767 (51.5)	.49
Race*			.56
Non-Hispanic white, n (%)	785 (52.3)	727 (48.8)	
Non-Hispanic black, n (%)	533 (35.5)	563 (37.8)	
Hispanic, n (%)	144 (9.6)	154 (10.3)	
Other or unknown, n (%)	38 (2.5)	45 (3.0)	
Apgar score < 3 at 5 minutes, n (%)	59 (3.9)	54 (3.6)	.66
ELBW <1000 g, n (%)	756 (50.5)	757 (50.8)	.84
Outcomes			
Days alive and off the ventilator, mean (SD)	95.9 (35.1)	93.6 (37.8)	.08
Hazard ratio for inpatient death (95% CI)†	0.78 (0.61 to 0.99)	1.28 (1.01 to 1.62)	.04
Days on ventilator, mean (SD)	12.9 (22.8)	13.0 (23.3)	.91
Days in NICU, mean (SD)	59.6 (33.7)	58.7 (34.5)	.47
Days on antibiotics, mean (SD)	15.7 (19.4)	15.0 (19.1)	.31
Days on ventilator for survivors, mean (SD)	12.2 (22.4)	12.3 (23.3)	.91
Subgroup analysis of mortality			
Hazard ratio for inpatient mortality: birth weight <1000 g	0.74 (0.57 to 0.95)	1.36 (1.05 to 1.76)	.02
Hazard ratio for inpatient mortality: birth weight >1000 g	1.11 (0.60 to 2.06)	0.89 (0.49 to 1.66)	.73
Interaction term for ELBW			.22

*A single *P* value is given for the race comparisons.
†Each hazard ratio is relative to the other group.

Discussion

In this large, simple randomized trial,[18] we tested the hypothesis that HRC monitoring, which detects the abnormal reduced variability and transient decelerations that often precede late-onset neonatal sepsis, can improve outcomes in very low birth weight infants. The 2.3-day increase of the composite primary outcome measure of days alive and not on a ventilator in the infants whose HRC monitoring results were displayed was not statistically significant ($P = .08$). We found, however, a clinically significant 22% relative reduction in mortality rate in infants whose HRC monitoring

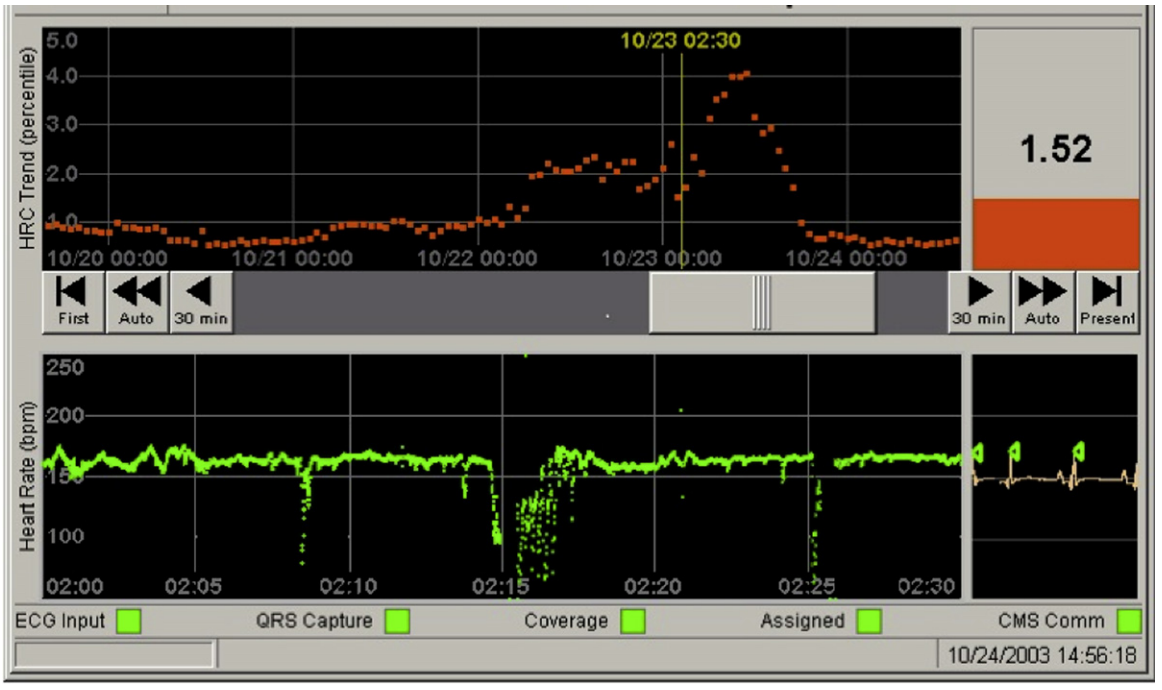

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Figure 2. Screen display of the monitor as used in the study.

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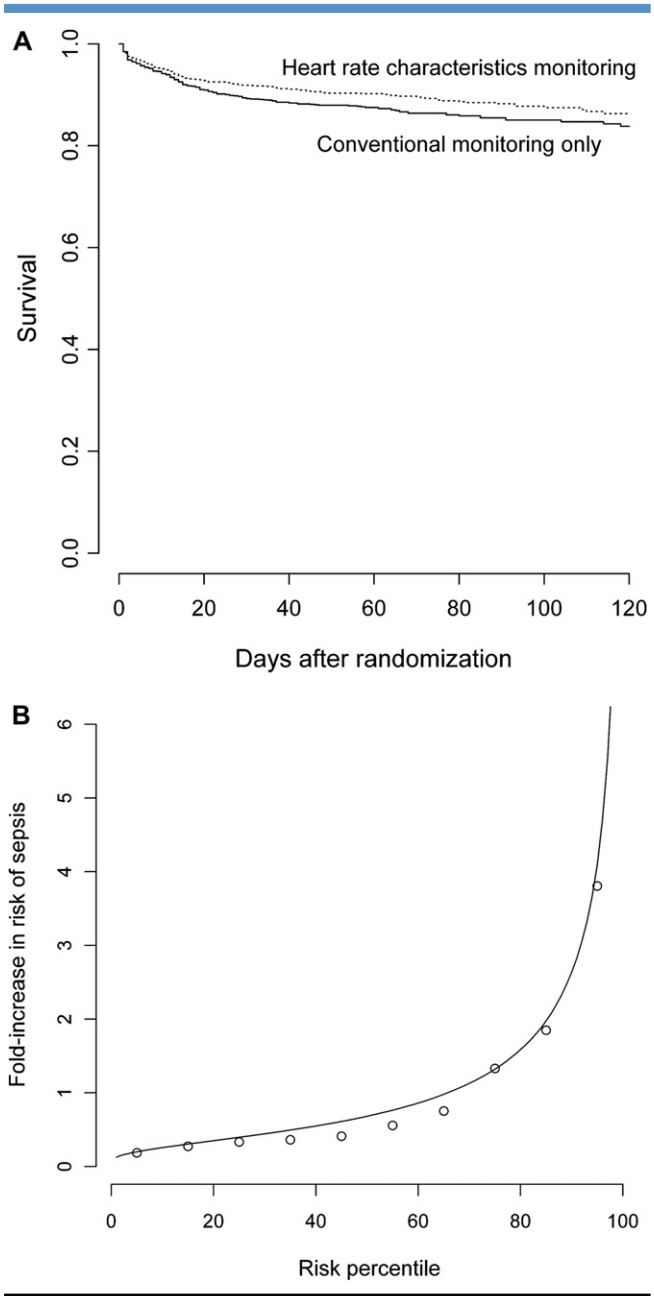

Figure 3. **A,** Survival of very low birth weight infants as a function of time in the infants whose HRC monitoring results were displayed and conventional monitoring-only group. **B,** Predictiveness curve of the HRC index as a risk model and classifier for neonatal sepsis. The *solid line* represents 2M HRC index values normalized by the average risk of 0.62% and plotted from lowest to highest with units of fold-increase in risk. The *open circles* are the proportion of infants per decile with proven sepsis in the next 24 hours.

results were displayed ($P = .04$), from 10.2% to 8.1%. There were no significant differences in the number of days in NICU or days on antibiotics.

The patient population in this trial was very similar to that reported in 2002 by Stoll et al[1] from the National Institutes of Child Health and Human Development Neonatal Research Network on late-onset neonatal sepsis. Our trial produced very similar rates of mortality (9.2% in this study versus 9.6% in the earlier one), rate of sepsis (25% versus 21%), overall mortality rate in infants with sepsis (19.3% versus 18.4%), mortality rate in infants without sepsis (6.6% versus 7.2%), days on ventilator (13 versus 14), and days in the NICU (59 versus 65).[1]

Previously, we reported that reduced variability and transient decelerations occur early in the course of neonatal sepsis,[3] developed time-series measures optimized to detect these abnormal HRC,[9-11,19-22] developed a multivariable logistic regression model at the University of Virginia and validated it externally at Wake Forest University,[7] and showed that the resulting HRC index added information to laboratory tests and clinical signs.[17,23,24] Concerns remained, however, about widespread use of HRC monitoring in hospitalized infants. Because the monitoring might lead to earlier diagnosis and therapy of sepsis, it might also lead to unnecessary sepsis evaluations or excessive blood cultures or antibiotic use. To address these concerns, we randomized infants to real-world use of HRC monitoring. Rather than specify protocols for numerical interpretation or for diagnostic testing or therapy, we educated the study personnel and NICU staff in the development, meaning, and interpretation of the HRC index, and told them a rising score might indicate sepsis. Clinicians were then free to respond independently to an abnormal HRC index value. The index is presented as fold-increase in risk, allowing natural and intuitive interpretation, rather than as a value with more arbitrary units requiring thresholds and guidelines for its use.

The strengths of this study include its large sample size, patient similarities to other large studies of very low birth weight infants,[1] simple design, and clinical relevance. A debatable weakness is the lack of a mandated intervention when the HRC index rose, or a specified definition of what constituted a significant rise. The study design, however, recapitulates the intended real-world use of HRC monitoring as an additional piece of data to be used in the context of the individual infant with complex illness and high, changing risks.

The mechanism by which display of HRC monitoring results in reduced mortality was not studied. One possible explanation is that sepsis was suspected and treated earlier. To address this possibility accurately requires knowing the time of sepsis initiation in a premature infant. Because this is unknowable, we cannot measure time-to-diagnosis and directly test this important hypothesis. However, we found indirect evidence of a role for improved diagnosis and treatment of sepsis because the group of infants whose HRC monitoring results were displayed had >30% fewer deaths in the 30 days after first diagnosis of proven sepsis ($P = .01$), at the cost of a 10% increase in blood cultures ($P = .05$) and 5% in antibiotic days ($P = .31$). Alternative mechanisms include the use of HRC monitoring as an index of systemic inflammation, allowing the possibility of early diagnosis and intervention of not only sepsis, but also other infections or common noninfectious problems such as necrotizing enterocolitis.

The mechanism by which reduced variability and transient decelerations of the HR occur in neonatal illness is not

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known. A current concept is that the most severe and life-threatening aspects of sepsis are caused not by the infecting organisms but rather by an exaggerated and dysregulated immune response, the so-called systemic inflammatory response syndrome proposed originally by Bone and coworkers.[25] This has been an extremely useful framework for understanding why antibiotics are not always curative unless given very early in the course of illness and underscoring the need for improved early detection of infection. An even more current concept is that of the cholinergic anti-inflammatory pathway proposed by Tracey and coworkers,[4] a strikingly original framework for understanding how the body attempts to limit the pro- and anti-inflammatory responses to infection. Because the HR and its variability are exquisitely controlled by cholinergic activity of the vagal nerve, this point of view predicts that HR variability will be altered early in the course of severe infection. A complementary and even more general point of view has been put forward by Buchman et al[26-28] and Goldberger et al.[29,30] Their view is of the body as a complex system in the sense of non-linear dynamics and chaos theory and that physiological rhythms simplify during illness. This justifies our approach, especially the use of entropy measures.[10]

In summary, very low birth weight infants in this randomized trial who had HRC monitoring results displayed had a lower in-hospital mortality rate than very low birth weight infants who did not have these results displayed (8.1% compared with 10.2%). In ELBW infants, the mortality rate was similarly lower in the monitored group (13.2% versus 17.6%). One life was saved for every 48 very low birth weight infants monitored and for every 23 ELBW infants monitored. ■

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