Early-onset neonatal sepsis is associated with a high heart rate during automatically selected stationary periods

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Short title: Heart rate and early onset sepsis

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Abstract

Aim: This study examined the heart rate variability characteristics associated with early onset neonatal sepsis in a prospective, observational controlled study.

Methods: Eligible patients were full-term neonates hospitalised with clinical signs that suggested early onset sepsis and a C reactive protein of > 10 mg/L. Sepsis was considered proven in cases of symptomatic septicaemia, meningitis, pneumonia or enterocolitis. Heart rate variability parameters (n=16) were assessed from five, 15 and 30 minutes stationary sequences automatically selected from electrocardiographic recordings performed at admission and compared with a control group using the U-test with post-hoc Benjamini-Yekutieli correction. Stationary sequences corresponded to the periods with the lowest changes of heart rate variability over time.

Results: A total of 40 full-term infants were enrolled, including 14 with proven sepsis. The mean duration of the cardiac cycle length was lower in the proven sepsis group than in the control group (n=11), without other significant changes in heart rate variability parameters. These durations, measured in five minutes stationary periods, were 406 (367-433) milliseconds in proven sepsis group versus 507 (463-522) milliseconds in the control group ($p<0.05$).

Conclusion: Early onset neonatal sepsis was associated with a high mean heart rate measured during automatically selected stationary periods.

Key words: heart rate, heart rate variability, neonatal sepsis, newborn infant, signal processing

Key notes

- This prospective observational controlled study assessed heart rate variability in order to stratify patients for the risk of early neonatal onset sepsis (EOS).
- The patients we included were full-term neonates hospitalised with clinical signs that suggested early onset sepsis and a C reactive protein of > 10 mg/L.

- Proven EOS was associated with a high heart rate measured from automatically selected stationary sequences without significant changes in heart rate variability.

INTRODUCTION

Preventative measures have successfully decreased the incidence of early neonatal-onset sepsis (EOS), but identifying neonates with clinical signs of sepsis and a high likelihood of EOS remains an important challenge (1). These neonates are usually identified on the basis of perinatal risk factors and diagnostic tests that have a poor predictive accuracy, leading to inappropriate antibiotherapy decisions. There is therefore a need to develop new approaches to stratify the risk of EOS (1-3).

Heart rate variability (HRV) reflects the multiple interactions required for normal cardio-vascular function. In sepsis, induced inflammation of all the components of the control/regulatory systems can be altered, including receptors, effectors, autonomic nervous system and central influences. Different approaches have been used to select the most appropriate HRV indices to diagnose or stratify the associated risk of sepsis. HRV is a useful non-invasive, real-time marker for the diagnosis of sepsis (2,4-6). Moorman *et al* proposed the use of selected HRV measurements to identify neonatal late-onset sepsis in premature infants. This approach resulted in a decrease in sepsis-related infant mortality in a randomised controlled trial (5,7), but this was associated with an increase in the number of blood cultures and antibiotic treatments. A lack of specificity was also suggested in a monocentric retrospective study (8).

In this study, that focused on a population with a high prevalence of EOS, we investigated possible changes in HRV patterns recorded in stationary conditions in full-term neonates during the early stages of EOS (9) in order to distinguish between infected and uninfected infants. We therefore compared patients with proven sepsis to a control group.

METHODS

Patients

We carried out a prospective, observational controlled study comparing full-term neonates considered to have proven sepsis with a control group in the Neonatology Department of the National Pediatric Hospital in Hanoi Vietnam, from November 2011 to July 2012. Informed consent was obtained from the parents of the infants. No additional blood examinations were performed for the study.

Eligible patients were non-asphyxiated full-term infants born at 37-41 weeks of gestation, with a birth weight of 2500-4000g, an Apgar score of more than six at five minutes, clinical signs suggestive of EOS and a C-reactive protein concentration of > 10 mg/L before seven days of age. They were all included in the study during the first hours following admission. The clinical signs were: an abnormal temperature of less than 36.5°C or more than 37.5°C, respiratory distress with respiratory rate of more than 60 breaths per minute or a Silverman score of more than three, an haemodynamic instability with persistent tachycardia (>160 bpm) or bradycardia (<100 bpm) or prolonged capillary refilling time (>4 seconds), neurologic signs including hypotonia, hypertonia, lethargy, convulsion, and gastrointestinal signs including feeding intolerance and abdominal distension. The exclusion criteria were: cardiac conduction abnormalities, ischaemic-hypoxic encephalopathy and intracranial haemorrhage. The infants were classified into two groups: the proven and non-proven sepsis groups. They were considered to have proven sepsis in cases of clinically symptomatic septicaemia with at least one positive blood culture, meningitis defined as neurologic signs associated with a white blood cell count ≥ 100/mm^3 in cerebrospinal fluid - with or without a positive culture - pneumonia defined as respiratory distress associated with an abnormal chest X ray or enterocolitis presenting with gastrointestinal signs associated with significant abnormalities on abdominal X ray. The control group of quasi-healthy, full-term infants was recruited during the study period from neonates hospitalised in the same hospital. They were eligible to be included in the control

group if they were admitted for maternal indications following an uncomplicated Caesarean section without general anesthesia or for simple neonatal jaundice with a total bilirubinemia concentration of less than 200 µmol/L and with a C reactive protein concentration of less than 5 mg/L without phototherapy indication.

Study protocol

An anonymized 24-hour electrocardiogram recording was acquired soon after inclusion for all patients in a standardised environment - side position, minimal sound or tactile stimuli - with a sampling frequency of 1000 Hz using a SpiderView Holter, (ELA Medical, Montrouge France). Clinical signs, blood cell counts and C-reactive protein concentration were recorded.

HRV analysis

Custom-built signal processing tools, designed with the Matlab software 6.0.0.42a, release 12 (The MathWorks Inc, Natick, Massachusetts, USA), were used, as previously described (4,10,11). Each 24-hour Holter recording was processed manually to extract the first segment of 180 minutes, corresponding to a period of low levels of ECG noise with no knowledge of the patient's characteristics, no access to clinical data or the group they belonged to. QRS complexes were detected automatically with a modified version of the Pan and Tompkins algorithm (12), including filter coefficients specifically adapted for neonates (13). Each RR intervals time series - used to study the fluctuations in the interval between heart beats - that we obtained was checked manually and an automatic algorithm was applied to select the most stationary segments of five, 15 and 30 minutes duration, based on the criteria proposed by Gonzalez (14). The different time durations were tested to determine the optimal period duration to discriminate between groups.

The HRV parameters studied were chosen by taking into account previous publications regarding the diagnosis of sepsis, which showed changes in heart rate, changes in the distribution of cardiac cycle lengths, decrease in the complexity/regularity, alterations in the fractal organisation and changes in the frequency domain analysis (LF/HF ratio) (2-4,7,15). The time-domain analysis of HRV was based on the extraction of the mean RR duration, the standard deviation (SD), which reflects the magnitude of global HRV, and the square root of the mean squared differences of successive RR intervals (rMSSD), which measures short-term beat-to-beat variability and mainly reflects parasympathetic control. The distribution of RR intervals was assessed by calculating the skewness and kurtosis of each RR series. We estimated the complexity and regularity of RR series by approximate entropy (ApEn) and sample entropy (SampEn) measurements (4). The RR series, resampled at 10 Hz, was also subjected to frequency domain analysis, through autoregressive estimation of the power spectrum and integration for the low-frequency (LF) 0.02-0.2 Hz, and high-frequency (HF) 0.2-2 Hz spectral bands. The LF/HF ratio was used as an index of sympathovagal balance. Poincaré plot analysis was then performed for further analyses of short-term (SD1) and long-term (SD2) HRV in the selected RR series. Acceleration capacity (AC) and deceleration capacity (DC) were computed as previously described (16). The scale invariance was tested through the detrended fluctuation analysis technique. The fluctuations were characterised by a self-similarity parameter (α) representing the long-range fractal correlation properties of the signal. We evaluated the fractal scaling exponent α1 from 4 to 40 beats, and α2 from 40 to 1,000 beats (4,10).

Statistical analysis

We used Statistica 10 software (Statsoft, Tulsa, Oklahoma, USA,) Comparisons between the proven sepsis and control groups were based on chi-square and Mann-Whitney U tests. An adjustment for multiple comparisons of 16 HRV parameters was performed using Benjamini-Yekutieli correction. Spearman's rank correlation coefficient was calculated for the pooled

proven and non-proven sepsis groups, to assess associations between HRV indices, C reactive protein concentrations and blood leukocytes. The control group was not included in this because the haemogram analysis was not performed systematically. Results are expressed as medians (interquartile range). A p value of < 0.05 was considered statistically significant.

RESULTS

Clinical data

The clinical data is summarised in Table 1. During the study period, 186 neonates were admitted to the National Pediatric Hospital in Hanoi for suspected EOS. Of these, 61 were full-term and 125 were premature infants. We did not include 21 full-term neonates due to parental refusal (n=10) or technical issues due to the recording system being unavailable or poor signal quality (n=11). A total of 40 full-term infants were included - 14 in the proven-sepsis group and 26 in the non-proven sepsis group - and 31 of them had received their first dose of antibiotic before their blood collection. All the included neonates had blood cultures taken. The control group consisted of 11 quasi-healthy full-term neonates. There was no significant difference in terms of sex ratio, chronological age and birth weight between these groups and the control group. The clinical signs at inclusion are summarised in Table 1. The diagnoses in the proven sepsis group were three cases of *Escherichia coli* septicaemia, five cases of meningitis – including four with *Escherichia coli* identified on the cerebrospinal fluid culture – five cases of pneumonia and one case of enterocolitis. One patient with meningitis died four days after birth.

HRV analysis

The values of HRV parameters obtained in the control group from five minutes stationary sequences with the different methods used are listed below and the analysis appears in Table 2. The results in time the domain analysis were mean 507 (463-522) msec, SD 26 (15-37) msec and rMSSD 13 (4-23) msec. The distribution of the RR intervals were kurtosis 3.7 (2.9-4.6) and

skewness 0.30 (-0.13-0.71). The complexity and regularity analysis were ApEn 1.02 (0.82-1.19) and SampEn 0.058 (0.033-0.069). The results of the Poincaré plot analysis were SD1 9.3 (2.7-16) msec and SD2 36 (21-51) msec. The results of the frequency domain analysis were LF 205 (18-284) $msec^2 \times 10^3$, HF 55 (4-115) $msec^2 \times 10^3$ and LF/HF 3.7 (2-6). The acceleration and deceleration capacities were AC: 1.33 (0.27-1.80) and DC: 1.44 (0.45-2.28). The results of the fractal analysis were α1 1.20 (1.08-1.29) and α2 0.90 (0.71-1.19).

The mean RR intervals, measured in five, 15 and 30 minutes on automatically selected stationary sequences, were lower in the proven sepsis group than in the other groups. The mean RR intervals were not modified by the duration of the automatically stationary sequence studied with a median of the standard deviation of seven msec (4-14) between the three periods. The ApEn, assessed from five and 15 minutes, and the AC and DC, assessed from 30 minutes stationary sequences, were also lower in the proven sepsis group using the U test, but this difference disappeared after correction for multiple comparisons. The other HRV parameters didn't differ between groups.

The mean RR intervals were generally correlated ($p<0.05$) with leukocyte counts at admission ($r = 0.47$, 0.51 and 0.56 for five, 15 and 30 minutes sequences, $p<0.01$) but not with C-reactive protein values.

DISCUSSION

This study of Vietnamese full-term neonates showed that EOS was associated with: (i) a significant increase in the mean heart-rate measured during automatically selected stationary periods, (ii) non-significant changes in ApEn with a tendency to low values during the five and 15 minutes sequences and AC and DC with a tendency to low values on 30-minute sequence and (iii) no other changes in HRV.

Early-onset neonatal sepsis is a life-threatening disease and its incidence is generally estimated to be about one per 1,000 live births in developed countries (17). This incidence varies from 6.5

to 23 per 1,000 in Africa and from 7.1 to 38 per 1,000 in Asia (16 per 1,000 in Vietnam) (18-20). It is therefore plausible that differences in the course and epidemiology of neonatal early onset sepsis exist between countries. However, it would have been difficult to carry out a study of this type in a country with a low incidence of EOS. Many infants included were born outside study hospital and it was therefore not possible to correlate the results with antenatal events and to estimate the influence of the delay from onset of sepsis signs on the results. Despite the strict environmental control that was applied to all patients during the ECG recording, we cannot exclude the possibility that some differences in the quality or quantity of interventions between the proven-sepsis and quasi-healthy groups may have had an impact on the HRV. As the number of patients was relatively low, with only 14 patients in the proven sepsis group, we could not reach formal conclusions about the approximate entropy or the AC and DC, which tended to differ between the groups but did not reach a significant p value. However, from the present results it appears unlikely that these parameters would have been discriminant enough to support a medical decision.

A study by Escobar et al stratified the early-onset sepsis risk in neonates ≥ 34 weeks gestation from a database that included 350 cases with early onset sepsis and 1,063 controls One of the vital signs that the authors identified was tachycardia, which was defined as a heart rate ≥ 160 bpm or mean cardiac cycle length ≤ 375 msec over a period of ≥ two hours, obtained from paper charts or inpatients electronic records during the first 24 hours after birth (3). Another study suggested that fetal tachycardia during the second stage of labour should be used as a sign of infection (21). The present study evaluated mean heart rate on stationary sequences, which were defined by the stability of the mean duration and the variance of the RR duration. These automatically selected sequences corresponded to periods with homogeneous HRV and, therefore, corresponded to periods with limited environmental influences, which were likely to reflect the patients' intrinsic condition. In this study we observed a higher mean heart rate in the proven sepsis values, of around 150 beats per minute, than in the control group, of around 120

beats per minute. Moreover, we found that mean RR intervals were correlated with leukocyte counts on admission. These results confirm the potential interest of an accurate evaluation of heart rate in the evaluation of early onset sepsis risk and suggest that the setting of a threshold could be optimised by the use of an automatic standardised method.

In this study the global HRV, measured by SD, as well as the short-term (rmssd, HF, SD1) and long-term (LF, SD2) fluctuations measured by conventional methods were not significantly modified following sepsis. This differs from other findings reported on adults or animals. In adults, a decrease in SD before the clinical diagnosis and treatment of sepsis has been reported (9,22). In fetal lambs, an increase in SD was observed after the injection of a lipopolysaccharide endotoxin (23). Studies on paediatric patients, adult humans and animals have also resulted in different conclusions with regard to short-term and long-term variabilities. These discrepancies may be accounted for by many factors, including sepsis severity, time since disease onset, age, species and treatments.

AC and DC were quantified from the method called phase rectified signal averaging, which is more resistant to artefacts and non-stationarities than the conventional methods. AC and DC are mainly considered to represent an integrated quantification of the sympathetic and vagal activities. Both AC and DC were found to be low in the 30-minute stationary periods recorded in the proven-sepsis group, but this result didn't persist after multiple comparisons correction. As the correction increased the risk of false negatives, and because we had a relatively low number of patients with confirmed EOS, we cannot exclude that a decrease in AC and DC did exist but did not reach the significant threshold.

Similarly, we observed a decrease in ApEn in the proven sepsis group, which was not confirmed after multiple comparisons correction. A low ApEn indicates a periodic behavior, namely a decrease in complexity that reflects a low level of adaptability, with the deactivation of regulation

mechanisms within the cardiovascular system. This tendency in our study was in agreement with the decrease in ApEn reported in all studies of adult (9) or neonatal sepsis (4,24) and by the results of experimental studies based on LPS endotoxin injections (25).

Sepsis has been reported to be associated with alterations in autonomic regulation, adaptability and organisation of HRV. The results of the present study suggest that EOS was associated with a high mean heart rate when it was automatically measured in stationary condition and with a tendency to low adaptability, namely low ApEn and low AC/DC. We think that the automatic standardised method proposed to measure mean heart rate could be a useful noninvasive approach for identifying neonates at high risk of EOS, particularly through integration in multivariate predictive models.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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LIST OF ABBREVIATIONS

α1: fractal correlation properties of the signal from 4 to 40 beats

α2: fractal correlation properties of the signal from 40 to 1,000 beats

AC: Acceleration capacity

ApEn; approximate entropy

DC: deceleration capacity

ECG: electrocardiogram

EOS: early neonatal-onset sepsis

HF: power in the the high-frequency spectral band (0.2-2 Hz)

HRV: heart rate variability

LF: power in the low-frequency spectral band (0.02-0.2 Hz)

rMSSD: square root of the mean squared differences of successive RR intervals

SampEn: Sample entropy

SD: standard deviation

SD1: short-term heart rate variability measured from Poincaré plot analysis

SD2: long-term heart rate variability measured from Poincaré plot analysis

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Table 1: Baseline characteristics of the patients

	Control (*n*=11)	Non-proven sepsis (*n*=26)	Proven sepsis (*n*=14)
Sex (male/female)	9/2	18/8	9/5
Postnatal age (days)	3 (3-5)	3 (3-5)	3 (3-6)
Birth weight (g)	3,150 (2,730-3,300)	3,000 (2,700-3,370)	3,000 (2,800-3,120)
Clinical signs, n (%)			
Abnormal temperature		11 (42%)	9 (64%)
Respiratory distress		21 (81%)	11 (79%)
Hemodynamic		8 (31%)	8 (57%)
instability		18 (69%)	11 (79%)
Gastrointestinal signs		**6 (23%)**	**11 (79%) ††**
Neurologic signs			
CRP (mg/L)	**1.6 (0.5-2.9)**	**24 (20-30)**	**51 (27-107)****
Maximal CRP (mg/L)		**30 (21-42)**	**85 (46-135)****
WBC (10^3/mm^3)		16 (10.7-23.0)	14.8 (8-17.8)
Neutrophils (10^3/mm^3)		11.9 (5.9-15.3)	10.1 (5.5-14.1)
Platelets (10^9/L)		**204 (174-247)**	**63.5 (11-230)***

Values are presented as medians (interquartile range) or *n* (%).*: $p< 0.05$; **: $p < 0.01$ (Mann-Whitney U test); ††: $p < 0.01$ (chi-square test).

CRP, C-reactive protein concentration; WBC, white blood cell count; CRP concentration. WBC and neutrophil and platelet counts were performed at inclusion.

Table 2: Heart rate variability parameters

	Control (*n*=11)	Proven sepsis (*n*=14)	U test p value	Corrected p value
5min-Mean RR (ms)	507 (463-522)	406 (367-433)	0.001	0.037
15min-Mean RR (ms)	497 (467-526)	412 (370-432)	0.002	0.048
30min-Mean RR (ms)	479 (465-504)	417 (365-426)	0.003	0.040
5min-ApEn	1.02 (0.82 - 1.19)	0.69 (0.60 – 0.92)	0.012	0.139
15min-ApEn	1.04 (0.81 -1.34)	0.80 (0.54 – 0.93)	0.044	0.239
30min-AC (ms)	0.78 (0.56 - 1.16)	0.24 (0.06 – 1.53)	0.038	0.239
30min-DC (ms)	0.93 (0.59 - 1.73)	0.25 (0.10 – 1.61)	0.033	0.241

Values are presented as medians (interquartile range). The parameters presented are those with significant p values (Mann-Whitney U test) when comparing the two groups before post-hoc correction. The p values have been corrected using (Bonferini- Yekutieli BY correction). Mean RR and SD = mean and standard deviation of all normal RR intervals; rMSSD = root mean square successive difference; ApEn = approximate entropy; SampEn = sample entropy; SD1 an SD2 = short and long-term variability measured by Poincaré plot; LF = low frequency; HF = high frequency; AC = acceleration capacity; DC = deceleration capacity.