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Original article

Early time to positivity in blood cultures as an indicator of mortality in very low birth weight neonates – A retrospective observational cohort study

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Abstract

Introduction: Neonatal sepsis is an important cause of mortality. Blood culture is the gold standard for the diagnosis. Time to positivity (TTP) in blood cultures may help in early optimization of treatment in sick neonates.

Methods: This was an observational study. Data records were collected for all neonates who were screened for both early- and late-onset sepsis. BacT/Alert system was used for culture detection. For every positive culture, TTP was calculated as the difference between loading time and detection time. Primary outcome was correlation of TTP with mortality due to culture-proven sepsis in gestational age ≤ 34 weeks.

Results: 151 VLBW neonates with culture-proven sepsis were included in the study over a period of 22 months. Gram-negative organisms were the predominant isolates, with *Klebsiella pneumoniae* being the commonest organism. Median TTP was significantly lower in the mortality group (9 hours, IQR 5-24) versus the survivor group (20 hours, IQR 9-78) with a p-value < 0.001. Early-onset sepsis was significantly higher in the mortality group (60%) versus the no-mortality group (27.9%). On multivariate analysis, TTP was the only factor significant in the neonatal mortality amongst the cohort with a p-value < 0.001 and AOR 1.54, 95% CI 1.22-1.93, indicating higher odds of death in neonates with a shorter duration of TTP.

Conclusions: TTP is an important predictor of neonatal mortality. Neonates with shorter TTP had higher odds of neonatal mortality due to sepsis. Thus, TTP can be used as a guide for optimal and judicious treatment in neonatal sepsis.

Keywords

Neonatal sepsis, time to culture positivity, mortality.

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Introduction

Neonatal sepsis is an important cause of mortality, morbidity and associated long-term sequelae both in developed and developing countries [1]. It is the second major cause of mortality among neonates, killing more than one million neonates annually [2]. Neonatal infections account for around 26-50% of neonatal mortality [3]. Globally, of the three million annual neonatal sepsis cases (2,202/100,000 live births), India has the highest incidence of clinical sepsis (17,000/100,000 live births) [4]. There is often a clinical dilemma in the diagnosis of sepsis in newborn babies. Early diagnosis and treatment are the key to survival of these sick babies. Culture is the gold standard for the diagnosis. However, in the best centres, culture positivity rates are quite low and variable (10-15%) [5, 6]. Gram-negative organisms remain the commonest cause of neonatal sepsis in the developing countries [7]. One of the factors which can help optimize the antibacterial treatment is early identification of the organism in the blood. Literature on time to positivity (TTP) in blood cultures has been available for 25 years due to continuous monitoring blood-culture instruments. It is defined as the time from the start of incubation to a positive signal [8]. TTP provides indirect information on the biomass, which is a function of the bacteraemic load and the microbial growth rate. Thus, the lower the TTP (i.e. the faster the bottle shows positive), the higher is the inoculum in the blood and/or the higher the growth rate [8]. TTP of blood helps in identifying the bacteria, which would help to optimize treatment. This would help in reducing antibiotic overuse and resistance.

The objective of the present study was to study the role of TTP of blood cultures in predicting mortality of neonates admitted to the Neonatal Intensive Care Unit (NICU).

Materials and methods

Patients and setting

This was a retrospective observational cohort study performed in a single centre tertiary care NICU, with a yearly admission rate of approximately 900 neonates. All blood cultures obtained from NICU patients born with very low birth weight (VLBW) with suspected sepsis, and submitted to the hospital's microbiology laboratory between 1st January 2018 and 31st October 2019 (22 months), were included.

Data collection

In the microbiology laboratory, data records were collected for the NICU patients who were screened for both early- and late-onset sepsis during the study period. The data collected were time and date of collection, date and hour of sample loading, date and hour of sample detection and isolate(s) cultured for every sample. All clinical, laboratory and demographic data were obtained from the past records of the patients. All data were entered in the spreadsheet program Excel® 2016 (Microsoft®). For every positive culture, TTP was calculated as the difference between detection time and loading time.

Microbiological culture techniques

During the study period, the non-quantitative BacT/Alert system was used to automatically identify positive samples with a colorimetric, CO_2-reactive sensor. Blood culture bottles were inoculated with 1 ml of blood and incubated at 37°C for a maximum of 6 days. All positive samples were examined by Gram stain, and cultured aerobically and anaerobically. Species identification was performed by conventional biochemical methods (Pattyn et al., 1990; Ieven et al., 1995) [9, 10].

Outcome measures

Primary outcome measure was to find out whether odds of mortality are related to TTP in the neonates' born VLBW in the NICU, negating the effects of other significant parameters which may affect the outcome measure of mortality in this group of neonates.

Statistical analysis

The data were entered into Microsoft® Excel® sheet version 2016, and analysis was done by using IBM® SPSS® version 24. Continuous variables were presented as mean ± standard deviation in normal distribution and median and interquartile range (IQR) in skewed distribution. Categorical variables were expressed in frequency and percentages. Continuous variables were compared between survival and non-survival by performing independent t-test for normalised data and Mann-Whitney test for non-normalised data. Categorical variables were compared by Chi-square test. For small numbers, Fisher's exact test was used wherever applicable. Multiple logistic regressions were performed to identify significant risk factors of mortality. Adjusted odds ratio (AOR) and 95% confidence interval (CI) were calculated. P-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the Institutional Ethics Committee.

Results

Over a period of 22 months (January 2018-October 2019), we had 1,862 NICU admissions. 868 neonates were VLBW. We sent a total of 1,063 blood cultures in VLBW during this period, of which 151 (14.3%) neonates were identified as culture-proven neonatal sepsis. Gram-negative organisms were predominant isolates (81.5%), 13.2% were Gram-positive isolates and 5.3% were fungal isolates. (**Tab. 1**) Amongst the Gram-negative isolates, *Klebsiella pneumoniae* (31.1%) was the most common, followed by *Acinetobacter baumannii* (15.2%) (**Fig. 1**).

Table 1. Type of organisms obtained.

Gram-negative organisms	123 (81.5%)
Gram-positive organisms	20 (13.2%)
Fungi	8 (5.3%)
Total	151 (100%)

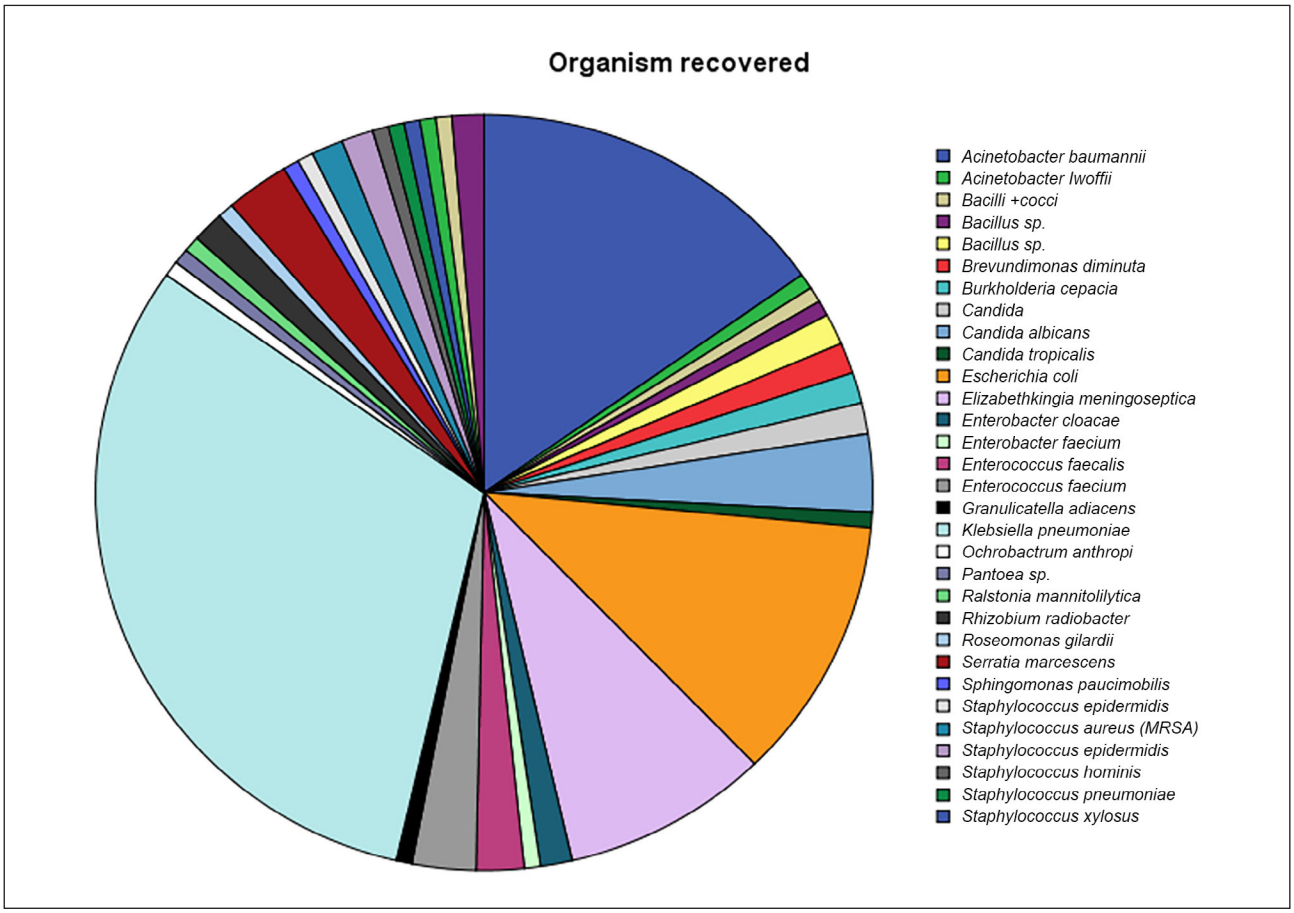

Figure 1. Depicting all the isolated organisms as pie chart.

Baseline characteristics

Baseline characteristics are presented in **Tab. 2**.

Out of total 151 culture-positive neonates, survival was seen in 73.5% and 26.5% died. The mean gestational age in the mortality group was 30.59 (± 1.88) weeks, and in the survival group was 31.38 (± 1.50) weeks, and the difference was statistically significant. The mean birth weight in the mortality group versus the survival group was 1,099 (± 211) versus 1,194 (± 204) grams and the difference was significant. This suggests that more preterm and smaller babies had higher mortality. Parameters such as mode of delivery, sex, SGA status, and antenatal steroids were similar in both survival and mortality groups. Need for resuscitation was higher in the mortality group (35%) compared to the survival group (10.8%) (relative risk [RR] 0.33; 95% CI 0.14-0.81). Incidence of preterm premature rupture of membranes (PPROM) (70% and 19.8%) and chorioamnionitis (55% and 6.3%) was significantly higher in the mortality group versus those who survived.

Outcomes

Outcomes are presented in **Tab. 3**.

Median TTP was significantly lower in the mortality group (9 hours, IQR 5-24) versus the survivor group (20 hours, IQR 9-78) with a p-value < 0.001. Incidence of Gram-negative sepsis was 97.5% amongst the mortality group and 75.7% amongst the survivors, which was statistically significant. Early-onset sepsis had significantly higher mortality (60% versus 27.9%). Incidence of intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD) was similar in both the groups. Amongst commonly used markers for neonatal sepsis, thrombocytopenia was significantly higher in the mortality group (65%) versus the survivor group (40.5%) (RR 0.36; 95% CI 0.17-0.78). Incidence of C-reactive protein

Table 2. Baseline characteristics of the study groups.

	Survival group (n = 111 [73.5%])	Mortality group (n = 40 [26.5%])	p-value or RR (95% CI)
Gestational age at birth, weeks, mean (SD)	31.38 (± 1.50)	30.59 (± 1.88)	**0.020**
Mean birth weight, g, mean (SD)	1,194 (± 204)	1,099 (± 211)	**0.019**
Males	53 (47.7%)	18 (45%)	1.11 (0.54-2.3)
Lower segment Caesarean section	98 (88.3%)	35 (87.5%)	0.92 (0.3-2.8)
Small for gestational age	66 (59.5%)	29 (72.5%)	0.56 (0.25-1.22)
Resuscitation as bag and mask and above (Yes)	12 (10.8%)	14 (35%)	**0.33 (0.14-0.81)**
Antenatal steroids	96 (86.5%)	34 (85%)	1.13 (0.4-3.14)
PPROM	22 (19.8%)	28 (70%)	**0.10 (0.04-0.24)**
Chorioamnionitis	7 (6.3%)	22 (55%)	**0.05 (0.02-0.15)**

Data are presented as n (%) if not otherwise indicated.
CI: confidence interval; PPROM: preterm premature rupture of membranes; RR: relative risk; SD: standard deviation.

Table 3. Difference of outcomes across the two groups.

	Survival group (n = 111 [73.5%])	Mortality group (n = 40 [26.5%])	p-value or RR (95% CI)
TTP, hours, median (IQR)	20 (9-78)	9 (5-24)	**< 0.001**
Gram-negative sepsis	84 (75.7%)	39 (97.5%)	**0.08 (0.01-0.60)**
Early-onset sepsis	31 (27.9%)	24 (60%)	**0.08 (0.03-0.19)**
IVH (any grade)	7 (6.3%)	5 (12.5%)	0.47 (0.14-1.6)
ROP (any stage)	38 (34.2%)	21 (52.5%)	0.47 (0.22-1.0)
NEC (any stage)	4 (3.6%)	2 (5%)	0.71 (0.12-4.0)
BPD (any stage)	6 (5.4%)	6 (15%)	3.1 (0.93-10.2)
CRP positivity	59 (53.2%)	16 (40%)	1.7 (0.81-3.5)
Neutropenia	42 (37.8%)	17 (42.5%)	0.82 (0.39-1.7)
Thrombocytopenia	45 (40.5%)	26 (65%)	**0.36 (0.17-0.78)**

Data are presented as n (%) if not otherwise indicated.
BPD: bronchopulmonary dysplasia; CI: confidence interval; CRP: C-reactive protein; IQR: interquartile range; IVH: intraventricular hemorrhage; NEC: necrotizing enterocolitis; ROP: retinopathy of prematurity; RR: relative risk; TTP: time to positivity in blood cultures.

(CRP) positivity and neutropenia was the same in both groups.

Regression analysis

Regression analysis is presented in **Tab. 4**.

Since mortality in culture-positive VLBW neonates was affected by many variables, a logistic regression analysis was done keeping it as a dependent variable and other significant factors like baseline variables (gestational age, birth weight, resuscitation, PPROM and chorioamnionitis) and significant outcome variables (TTP, Gram-negative sepsis, early-onset sepsis and thrombocytopenia) as the independent variables. On multivariate analysis, we found that TTP was the only factor significant in the neonatal mortality amongst the cohort, with a p-value < 0.001 and AOR 1.54, 95% CI 1.22-1.93, indicating that odds of death were 1.5 times higher in neonates with a shorter duration of TTP. Other variables such as Gram-negative sepsis (p-value 0.16, AOR 10.7, 95% CI 0.4-290.4), gestational age at birth (p-value 0.88, AOR 0.95, 95% CI 0.51-1.7), birth weight (p-value 0.42, AOR 1.0, 95% CI 0.99-1.01), PPROM (p-value 0.29, AOR 2.08, 95% CI 0.53-8.2), resuscitation (p-value 0.11, AOR 4.1, 95% CI 0.71-24.5), chorioamnionitis (p-value 0.07, AOR 4.2, 95% CI 0.89-20.1), early-onset sepsis (p-value 0.08, AOR 3.3, 95% CI 0.84-12.7), thrombocytopenia (p-value 0.17, AOR 2.8, 95% CI 0.64-11.9) were insignificant in predicting the mortality.

Table 4. Regression analysis of the significant factors affecting mortality across the groups.

Variables	p-value	AOR (95% CI)
TTP	**< 0.001**	**1.54 (1.22-1.93)**
Gram-negative sepsis	0.16	10.7 (0.4-290.4)
Gestational age at birth	0.88	0.95 (0.51-1.7)
Birth weight	0.42	1.0 (0.99-1.01)
PPROM	0.29	2.08 (0.53-8.2)
Resuscitation	0.11	4.1 (0.71-24.5)
Chorioamnionitis	0.07	4.2 (0.89-20.1)
Early-onset sepsis	0.08	3.3 (0.84-12.7)
Thrombocytopenia	0.17	2.8 (0.64-11.9)

AOR: adjusted odds ratio; CI: confidence interval; PPROM: preterm premature rupture of membranes; TTP: time to positivity in blood cultures.

Discussion

Sepsis is a leading cause of morbidity and mortality among neonates admitted in the NICU. Clinicians all over the world face a dilemma regarding the diagnosis of neonatal sepsis. Initial presentation of sepsis is vague and non-specific. This often results in over-treatment in the majority of cases. Delay in treatment may be associated with higher morbidity and mortality, especially in sick neonates.

Blood culture is the gold standard for diagnosis of sepsis [8]. However, in the best centres, the yield of culture positivity is quite low, ranging from 10% to 15% [5, 6]. The majority of neonatal blood cultures will remain sterile, implying unnecessary overuse of antibiotics and presumably triggering multidrug resistance [5]. This prolonged and unnecessary use of antibiotics may be associated with NEC, fungal infections and antimicrobial resistance. Hence TTP may be an important factor so as to reduce this overuse, which in turn may help in reducing costs, resistance and NEC in sick neonates. TTP is defined as the time from the start of incubation to a positive result [8]. TTP may be affected by factors such as bacterial load, type of bacteria, type of culture facility used and prior antibiotic usage [11].

In our study, we studied the time for blood culture positivity as a predictor of mortality in preterm VLBW neonates. In general, the shorter the TTP, it is likely that the organism is more virulent or the bacterial load is higher, which may be associated with higher mortality. There are very few studies that have directly correlated TPP with neonatal mortality.

Lefebvre et al. documented that the mean TTP for pathogens was shorter as compared to non-pathogens, 14.40 and 23.18 hours, respectively (p < 0.001) [12]. Guerti et al. described that the median TTP for Gram-negative organisms was shorter, i.e. 11.17 hours (Q1-Q3 8.84-15.67), whereas the median TTP for Gram-positive organisms was 23.59 hours (Q1-Q3 15.29-34.58) (p = 0.001) [5]. Similar findings were made by Mendoza et al. [13]. Some recent works have also reconfirmed the fact that the majority of pathogenic bacteria are fast-growing and are isolated by 36 hours after blood culture collection in 94% of neonatal early blood cultures [14]. These findings suggest that the more aggressive the bacteria, the shorter the TTP *in vitro* cultures

and the poorer the neonatal outcomes. Guerti et al. made a similar recommendation regarding shorter TTP and if it is a premonition of adverse or fatal outcome, and therefore a trigger for extended care. They could not justify their claims as they did not consider outcome or mortality in their study [5]. The issue has been dealt with in the present study as we studied the mortality outcomes and their correlation with the TTP.

Many authors have suggested an early de-escalation of antibiotic therapy if the blood cultures are negative after 36-48 hours of incubation. This finding directs to the fact that aggressive organisms will show an earlier *in vitro* growth and are actually the ones requiring antibiotic therapy for a longer duration [5, 13, 15, 16]. Shorter TTP helps in the early identification of the organism and its sensitivity pattern would enable us to optimize the therapy in the NICU. This strategy would help in the early de-escalation of antibiotics. Decreased antibiotic use may reduce the emergence of resistant organisms, length of hospital stay in a defined neonatal population and the workload in Neonatal Units apart from cost savings [16].

Concerning the potential usefulness of TTP to guide antimicrobial therapy, most works have addressed onco-haematological patients with febrile neutropenia [17, 18], probably because these patients combine a high risk of antimicrobial selective pressure with a high risk of bloodstream infection. Puerta-Alcalde et al. [17] conducted a retrospective study where they found out that the majority of episodes with positive blood culture are positive within the first 24 hours, and growth of multidrug-resistant Gram-negative bacilli is exceptional beyond 24 hours. These results again are in line with the findings of the present study that the faster growing aggressive Gram-negative organisms relate with poorer outcomes.

A number of studies in the older patients have reported similar findings. One of the studies conducted showed that patients with earlier positive cultures were more likely to have more severe underlying diseases [19].

This finding can be attributed to the fact that with the advancing use of more and more empirical broad-spectrum antibiotics, the incidence of multidrug-resistant (MDR) organisms has increased several folds in the past decade. These resistant organisms tend to show more aggressive growth *in vivo*, replicated by a shorter TTP *in vitro*. TTP provides indirect information on the biomass, which is a function of the bacteraemia load and of the microbial growth rate: the lower the TTP (i.e. the faster the bottle shows positive), the higher is the inoculum in the blood and/or the higher the growth rate [8].

Limitations and strengths

Our study has a few limitations. First, it was a retrospective study. Secondly, there was no *a-priori* sample size estimation.

Strengths of this study include its first-of-a-kind analysis in neonatal population with culture-positive sepsis. However, a larger multi-centric study to look at the generalizability of the prediction of TTP as a predictor for neonatal mortality would be desirable.

Conclusion

In conclusion, we found that TTP is an important predictor of neonatal mortality in sick neonates. Hence aggressive and good supportive care could be started early so as to increase the chances of survival. TTP can be used as a guide to deescalate the antibiotic therapy in culture-positive neonatal sepsis to prevent mortality. Due to the ever-increasing antibiotic resistance, it is essential to know the risk factors associated with mortality, to take the necessary preventive measures against these factors, and to initiate early and correct antibiotic therapy so as to reduce morbidity and mortality in the future.

Declaration of interest

The Authors declare that there is no conflict of interest.

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