Does Time to Positivity of Blood Cultures Predict Causative Pathogens and Survival in Neonatal Sepsis? A Retrospective Cohort Study from Indonesia

Agung Dwi Wahyu Widodo[1,2*], Putu Bagus Dharma Permana[3], Arina Setyaningtyas[4] and Manik Retno Wahyunitisari[2]

[1]Integrated Medical Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

[2]Department of Clinical Microbiology, Dr. Soetomo General Hospital, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia

[3]Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

[4]Division of Pediatrics Emergency and Intensive Care, Department of Pediatrics, Dr. Soetomo General Hospital, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

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*****Corresponding author: agungimunologi@gmail.com

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Abstract

Objectives: In the blood culture procedure for neonatal sepsis, time to positivity (TTP) reflects the pathogenic bacterial load and the time required for an empirical antibiotic regimen to be given prior to definitive treatment. This study aims to identify the differences in TTP among causative pathogens and its predictive value for the overall survival of neonates with sepsis at a tertiary healthcare center in Indonesia.

Methods: A retrospective cohort study was conducted from January 2020 to August 2022 at Dr. Soetomo General Hospital, Surabaya, Indonesia. Neonates with blood culture-proven neonatal sepsis were included in the analysis. TTP was defined as the time between the acceptance of a blood culture specimen from the neonatal intensive care unit (NICU) and reports of positive culture growth by the laboratory.

Results: Across 125 cases, the median TTP was 58.08 h (IQR 24.48), of which 41.6% of blood cultures were positive by 48 h, 86.4% by 72 h, and 98.4% by 96 h. A significantly shorter TTP was exhibited by the three major gram-negative organisms (*Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*) compared to coagulase-negative Staphylococci (CoNS). The mortality rate of neonatal sepsis was 49.6% for the duration of this study period. On the Cox multivariable regression model, a shorter TTP was an independent prognostic factor to predict mortality in the entire cohort (HR 0.985; 95% CI [0.973–0.998]) and gram-negative sepsis cohort group (HR 0.983; 95% CI [0.968–0.999]).

Conclusion: TTP predicts different causative pathogens and the overall survival of neonatal sepsis cases from a tertiary healthcare facility in Indonesia.

Keywords: *Neonatal Sepsis, Bacterial Sepsis, Time To Positivity, Blood Culture.*

Introduction

Neonatal sepsis imposes a substantial burden on the healthcare systems of lower-and-middle income countries (LMIC). Reports have previously estimated a global mortality rate of 17.6% for early-onset sepsis (EOS) and 16.4% for late-onset sepsis (LOS), respectively.[1] In LMICs, neonates with clinically suspected sepsis or those supported by laboratory findings are associated with a significant increase of all-cause mortality risks by five and nine-fold, respectively.[2] Evidence from Vietnam showed that several clinical and laboratory factors were associated with higher risks of neonatal sepsis mortality, which include extremely low-birth-weight, hyperglycemia, thrombocytopenia, leukopenia, sclerema, base excess <-20 mEq/L, and serum lactate >4 mmol/L.[3] Elevated neutrophil-lymphocyte-ratio (NMR) has also been reported as an independent prognostic factor of neonatal sepsis mortality (HR 7.52; p=0.001), with 80% sensitivity and 65.8% specificity for the cut-off value of 7.65.[4,5] Under a resource limited setting, the use of these predictors is crucial to determine appropriate management and prognosis of neonatal sepsis patients.

Blood culture is a gold standard diagnostic procedure for neonates with clinically suspected neonatal sepsis.[6] Since the introduction of continuous blood culture monitoring devices of over 30 years ago, the term time to positivity (TTP) has been commonly used by clinicians to indicate the time lapsed from initial culture incubation to a report of positive growth signal on the instrument.[7–9] Generally, TTP provides indirect representation of bacterial load and/or rate of virulence of a certain bloodstream infection, implying that causative pathogens with higher growth rates will be detected at an earlier rate during the procedure.[9–11] A systematic review and meta-analysis suggested that shorter TTP serves as a crucial predictor of overall survival and septic shock in bloodstream infections.[12] TTP also played an important role in determining the duration of empirical antibiotics administration following clinical diagnosis of sepsis and blood specimen collection.[13] Both microbiological and clinical characteristics were recognized as determinant factors of TTP, but the interpretation of its association differs: from a microbiological standpoint, a faster or slower TTP indicates the inherent growth capacity of the organism, whereas clinically, it reflects differences in the inoculum originally present in the blood.[14]

However, it is crucial to highlight that TTP is also heavily associated with commonly overlooked confounding factors found in the clinical practice, primarily those related to logistics (transportation, administration, opening hours, etc.).[9] Therefore, although the evaluation of TTP in neonatal sepsis have been thoroughly explored on numerous reports, the lack of evidence originating from LMICs and the aforementioned reasons may potentially reveal notable discrepancies of results.[15–19] In this study, we aim to observe the TTP of neonatal sepsis cases from a tertiary healthcare center in Indonesia, as well as analyzing its differences among different causative pathogens and its prognostic capability to predict overall survival.

Methods

A retrospective cohort study was conducted at Dr. Soetomo General Hospital, a referral healthcare center for neonatal sepsis in the Eastern Region of Indonesia. All neonates with bacteriological confirmation of neonatal sepsis from the neonatal intensive care unit (NICU) from January 1st, 2020 to August 31st, 2022 were included in this study. The identification of neonatal sepsis cases was based on the electronic medical records under the International Classification of Diseases-10 (ICD-10) code P36.

Retrospective identification was conducted on all initial blood cultures collected after birth, in which organisms were isolated and subsequently confirmed as the causative pathogens of neonatal sepsis. Blood specimens for culture were collected with a minimum volume of 1 mL prior to any administration of empirical antibiotics. The microbiological culture procedure was conducted at the Clinical Microbiology Laboratory of Dr. Soetomo General Hospital using the automated and continuous detection systems of BD BACTEC. In this study, the time to positivity (TTP) was defined as the time lapsed (hours) between the acceptance of a blood culture specimen from the neonatal intensive care unit (NICU) and reports of positive culture growth by the laboratory. For neonates that underwent multiple blood culture procedures, only the initial blood culture will be analyzed for this study.

Information regarding clinical characteristics of neonatal sepsis was retrieved from the electronic medical records which fulfilled the following eligibility criteria: 1) neonates were delivered inside the healthcare facility; 2) complete maternal history records; and 3) the first identified case of neonatal sepsis in each patient. The following data were

extracted for each neonate: sex, gestational age, birth weight, mode of delivery, sepsis onset, presence of birth asphyxia, neonatal outcome (mortality), and risk factors associated with maternal history (premature rupture of membranes [PROM], preeclampsia, antenatal corticosteroids, urinary tract infection [UTI], anemia of pregnancy).

Continuous variables were presented as median with interquartile range (IQR) and categorical variables were presented as frequency (n) and percentages (%). The comparison of neonatal and maternal characteristics with overall survival was conducted categorically using the chi square test, while the Mann-Whitney test was applied for the continuous comparison of TTP with overall survival. The distribution of causative pathogens was listed along with their respective median (IQR) TTP as well as the proportion of positive cultures at 24, 48, 72, and 96 hours. Kaplan-Meier curves were generated to plot the proportion of positive cultures by time, compare the TTP between different species, and identify overall survival based on different TTP ranges. The comparison between curves was conducted using the log-rank test. To determine the prognostic capability of TTP and other variables for overall survival, a backward-stepwise Cox multivariate regression model was implemented. Variables were included in the model based on a score statistic of less than 0.05, and any variables with a score statistic greater than 0.25 were excluded. The measures of risk for this model were expressed as a hazard ratio (HR) with its respective 95% confidence interval (CI). Statistical significance was determined if p-value <0.05. All statistical analyses were conducted with SPSS software ver. 23 and GraphPad Prism 9.5.1.

Results

During the January 2020 to August 2022 period, 569 neonates were clinically diagnosed with neonatal sepsis and received treatments in the NICU of Dr. Soetomo General Hospital. 243 outpatient neonates, 11 neonates with incomplete medical records, and 49 neonates that did not undergo blood culture confirmation were excluded from the analysis. From the 266 eligible neonates in which blood specimens were taken for culture, 141 of them showed no growth in blood cultures or were either determined as contaminants (53.0%). Hence, 125 neonatal blood cultures and their associated clinical records were retrieved to be included in our analysis (Table 1). The majority of the study cohort consisted of late-onset sepsis cases (76.8%), male sex (52.8%), gestational age of 32–<37 weeks (46.4%), and birth weight of 1500–<2500 (37.6%). Maternal and perinatal histories identified a number of cases of birth asphyxia (12.8%), caesarean delivery (78.4%), premature rupture of membranes (PROM) (16.0%), preeclampsia (45.6%), antenatal corticosteroid use (51.2%), intrapartum fever (4.8%), maternal UTI (2.4%), anemia of pregnancy (30.4%). Across the bacteriologically confirmed cases of neonatal sepsis, the time to positivity of blood cultures ranges from 15.12 h to 143.03 h, with a median of 58.08. The all-cause mortality rate for the study cohort was 49.6%, with a median length of stay of 22 days.

Table 1: Characteristics of the Study Cohort (N=125).

Characteristic	**n (%)**
Late-onset sepsis	96 (76.8%)
Male	66 (52.8%)
Gestational age (weeks), median (IQR)	33 (5)
Gestational age (weeks)	
<28	15 (12.0%)
28-<32	35 (28.0%)
32-<37	58 (46.4%)
≥37	17 (13.6%)
Birth weight (g), median (IQR)	1450 (1000)
Birth weight (g)	
<1000	24 (19.2%)
1000-<1500	39 (31.2%)
1500-<2500	47 (37.6%)
≥2500	15 (12.0%)
Birth asphyxia	16 (12.8%)
Caesarean delivery	98 (78.4%)
Maternal history	
PROM	20 (16.0%)
Preeclampsia	57 (45.6%)

Antenatal corticosteroid	64 (51.2%)
Intrapartum fever	6 (4.8%)
Maternal UTI	3 (2.4%)
Anemia of pregnancy	38 (30.4%)
Time to positivity (h), median (IQR)	58.08 (24.48)
Time to positivity	
<48 h	50 (40.0%)
48–72 h	55 (44.0%)
>72 h	20 (16.0%)
Length of stay (day), median (IQR)	22 (26)
All-cause mortality	62 (49.6%)

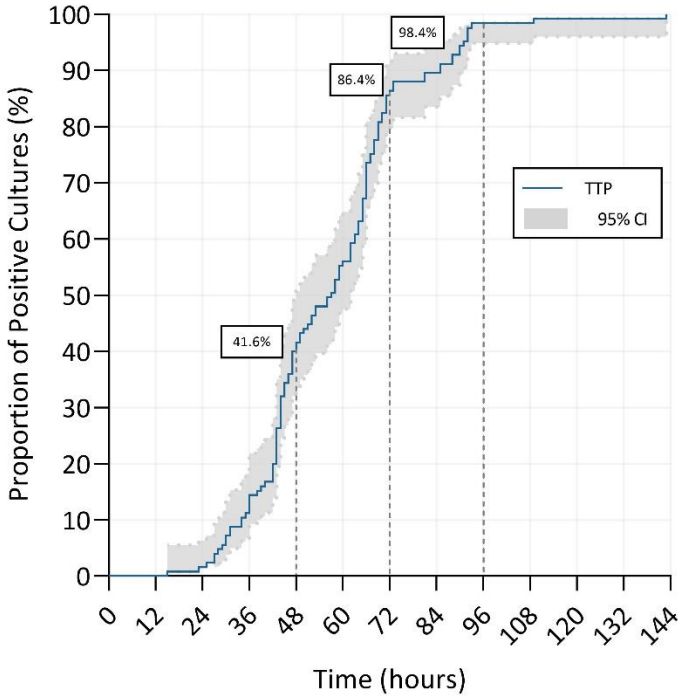
In this study, gram-negative bacteria accounted for the majority (62.4%) of pathogen growth from blood cultures (Table 2). The most commonly isolated gram-negative organisms were *Klebsiella pneumoniae* (47.4%), *Acinetobacter baumannii* (20.5%), and *Enterobacter cloacae* (14.1%). No fungal species were isolated from the blood cultures. Across the whole sample, 41.6% of blood cultures were positive by 48 h, 86.4% by 72 h, and 98.4% by 96 h (Fig. 1A). Gram-negative bacteria showed a relatively lower median TTP than gram-positive bacteria (47.76 vs 66.24 h). The lowest TTP was identified in a blood culture showing the growth of *Lelliottia amnigena* biogroup 2 at 15.12 hours. Conversely, blood cultures that yield *Pseudomonas spp*. had the longest median TTP at 95.28 hours. There are 4 neonatal blood cultures with more than 1 pathogen being grown and isolated, which include the combination of 1) *Staphylococcus sciuri* (CoNS) and *Stenotrophomonas maltophila*; 2) *Serratia plymuthica* and *Enterobacter cloacae*; 3) *Klebsiella pneumoniae* and *Escherichia coli*; as well as 4) *A. baumannii* and *S. aureus*. These polybacterial cultures had a median TTP of 59.52 hours, placing it between the median TTP of gram-negative and gram-positive bacteria. Marked differences in TTP were identified among the four major causative pathogens of neonatal species (p=0.01) (Fig. 1B). However, these differences were only found on four subgroup comparisons between *K. pneumoniae* and CoNS (p=0.027), *A. baumannii* and CoNS (p=0.011), *E. cloacae* and CoNS (p<0.0001), and *E. cloacae* and *A. baumannii* (p=0.0397) (Fig. 2).

Table 2: TTP by Species (N=125).

Species	**n (%)**	**Median (IQR)**	**TTP (h)**			
			Positive at 24 h (%)	**Positive at 48 h (%)**	**Positive at 72 h (%)**	**Positive at 96 h (%)**
Gram-negative	78 (62.4%)	47.76 (22.56)	2.56%	50.00%	89.74%	97.44%
Klebsiella pneumoniae	37 (47.4%)	48.00 (22.32)	0%	48.65%	89.19%	97.30%
Acinetobacter baumannii	16 (20.5%)	58.32 (23.28)	6.25%	43.75%	93.75%	100%
Enterobacter cloacae	11 (14.1%)	46.32 (16.32)	0%	63.64%	100%	100%
Escherichia coli	5 (6.4%)	53.28 (23.76)	0%	40.00%	80.00%	100%
Klebsiella ozaenae	2 (2.6%)	45.12 (0.72)	0%	100%	100%	100%
Pseudomonas spp.	2 (2.6%)	95.28 (13.68)	0%	0%	0%	50%
Achromobacter spp.	1 (1.3%)	42.48	0%	100%	100%	100%
Aeromonas caviae	1 (1.3%)	29.52	0%	100%	100%	100%
Serratia marcescens	1 (1.3%)	52.8	0%	0%	100%	100%
Serratia plymuthica	1 (1.3%)	57.36	0%	0%	100%	100%
Lelliottia amnigena	1 (1.3%)	15.12	100%	100%	100%	100%
Gram-positive	43 (34.4%)	66.24 (13.92)	0%	23.26%	74.42%	100%
CoNS	36 (83.7%)	66.72 (12.96)	0%	22.22%	75.00%	100%
Staphylococcus aureus	2 (4.7%)	60.96 (1.44)	0%	0%	100%	100%
Bacillus spp.	2 (4.7%)	54.96 (18.48)	0%	50.00%	50.00%	100%
Streptococcus agalactiae	1 (2.3%)	30.00	0%	100%	100%	100%

Enterococcus faecalis	1 (2.3%)	65.04	0%	0%	100%	100%
Corynebacterium	1 (2.3%)	93.60	0%	0%	0%	100%
Polybacterial	4 (3.2%)	59.52 (25.92)	0%	25.00%	75.00%	100%

A)



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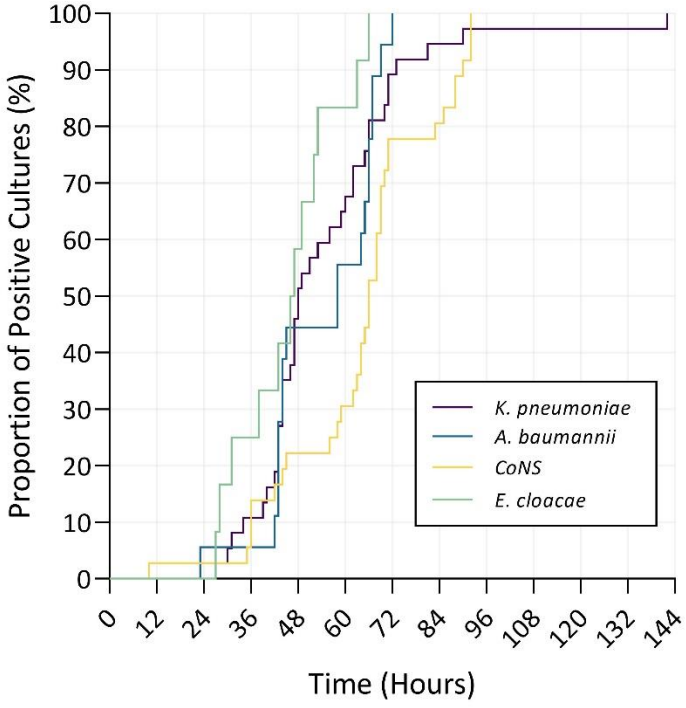

Figure 1. A) Kaplan-meier curve of the proportion of positive blood cultures by time (hours). B) Kaplan-meier curves of the positive blood cultures by time (hours) across different species (p=0.01).

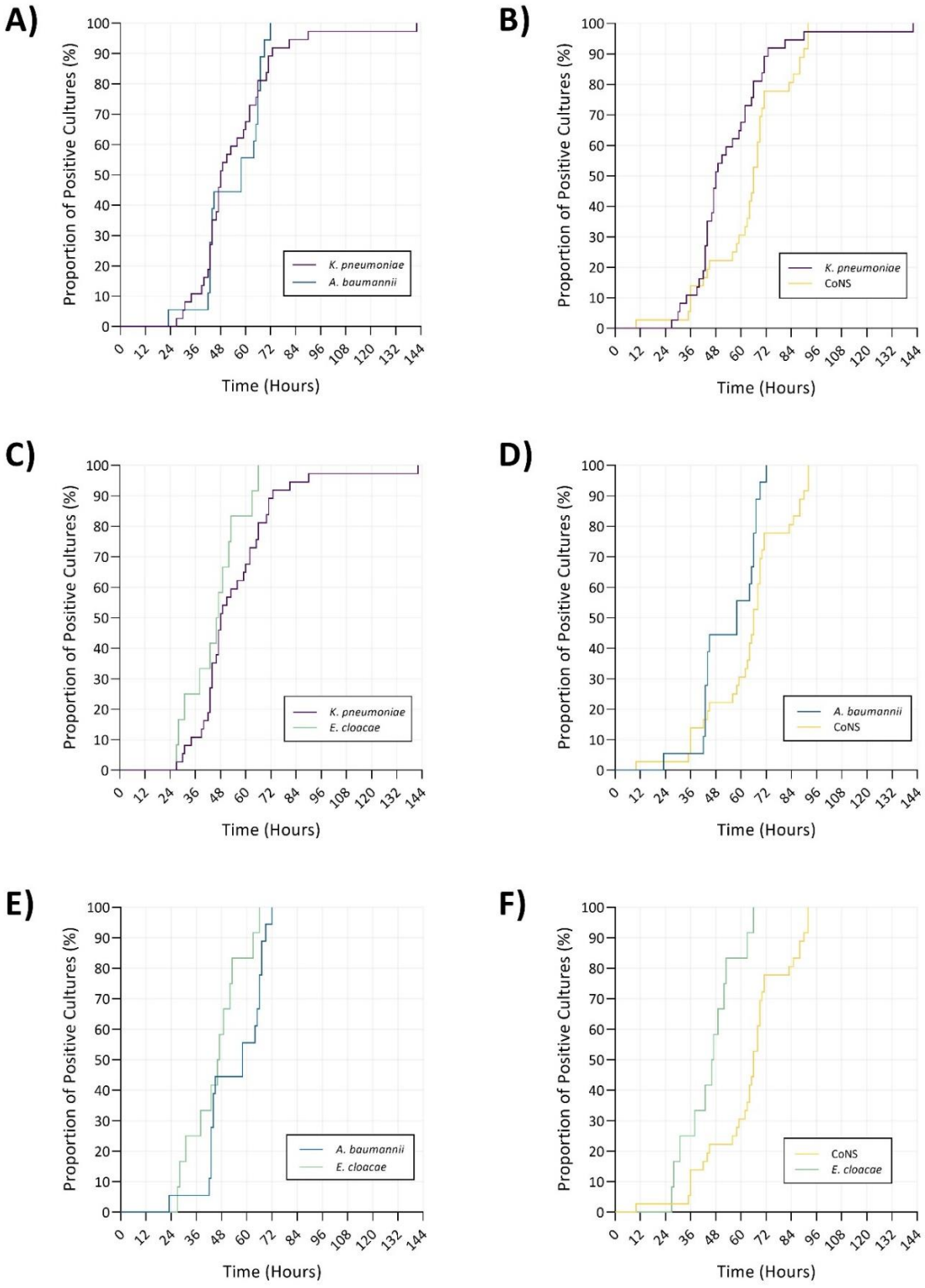

Figure 2. Kaplan-meier curves of TTP by species subgroups. A) *K. pneumoniae* and *A. baumannii* (p=0.8126). B) *K. pneumoniae* and CoNS (p=0.027). C) *K. pneumoniae* and *E. cloacae* (p=0.096). D) *A. baumannii* and CoNS (p=0.011). E) *A. baumannii* and *E. cloacae* (p=0.0397). F) CoNS and *E. cloacae* (p<0.0001).

Significant differences were identified between gestational age (p=0.020), birth weight (p=0.026), birth asphyxia (p=0.007), antenatal corticosteroid use (0.040), and length of stay (p<0.0001) with overall survival among the entire study cohort (Table 3). Neonates with monobacterial gram-negative sepsis had a significantly lower rate of survivability (p<0.0001), whereas 100% mortality was observed in all four cases of polybacterial culture. Although no differences were found in the Mann-Whitney test, significant differences in the Kaplan-Meier curve were identified among the three categorical groupings of TTP (<48 h, 48-72 h, >72 h; p=0.0364) (Fig. 3). Two Cox multivariable regression models were created for the entire cohort and for neonates with gram-negative sepsis (Table 4). Five variables were included in the regression model of the entire cohort, including sepsis onset, sex, gestational age, birth asphyxia, and time to positivity. Late-onset sepsis and longer TTP were independent predictors for higher survivability (HR 0.423; 95% CI [0.229-0.781]; p=0.006 and HR 0.985; 95% CI [0.973-0.998]; p=0.027, respectively). Conversely, the presence of birth asphyxia increases the hazard of mortality by 4 times among patients with bacteriologically confirmed neonatal sepsis (HR 4.095; 95% CI [2.095-8.005]; p<0.0001). On the gram-negative sepsis model, four variables were included in the equation: sepsis onset, gestational age, birth asphyxia, and time to positivity. Gestational age <28 weeks and birth asphyxia were associated with mortality in gram-negative neonatal sepsis (HR 3.472; 95% CI; [1.065–11.319]; p=0.039 and HR 6.662; 95% CI [2.495–17.784]; p=0.000, respectively), while late-onset sepsis was independently predictive of overall survival (HR 0.356; 95% CI [0.171–0.742]; p=0.006]). A longer TTP in gram-negative neonatal sepsis was also marginally predictive of overall survival, with a 1.7% increase in odds for every 1 h increase in TTP (HR 0.983; 95% CI [0.968–0.999]; p=0.033).

Table 3: Comparison of Factors with Overall Survival (N=125).

Characteristic	**Survivor**	**Non-survivor**	**RR (95% CI)**	**p-value**
Onset				
Early-onset	10 (34.5%)	19 (65.5%)	2.342 (0.986-5.562)	0.050
Late-onset	53 (55.2%)	43 (44.8%)	ref.	
Sex				
Male	36 (54.5%)	30 (45.5%)	0.703 (0.347-1.423)	0.327
Female	27 (45.8%)	32 (54.2%)	ref.	
Gestational age (weeks)				
<28	2 (13.3%)	13 (86.7%)	7.312 (1.249-42.813)	
28 - <32	21 (60.0%)	14 (40.0%)	0.750 (0.233-2.412)	0.020*
32 - <37	31 (53.4%)	27 (46.6%)	0.980 (0.332-2.894)	
≥37	9 (52.9%)	8 (47.1%)	ref.	
Birth weight (g)				
<1000	7 (29.2%)	17 (70.8%)	2.125 (0.555-8.140)	
1000 - <1500	18 (46.2%)	21 (53.8%)	1.021 (0.309-3.369)	0.026*
1500 - <2500	31 (66.0%)	16 (34.0%)	0.452 (0.139-1.470)	
≥2500	7 (46.7%)	8 (53.3%)	ref.	
Birth Asphyxia				
Yes	3 (18.8%)	13 (81.3%)	5.306 (1.430-19.683)	0.007*
No	60 (55.0%)	49 (45.0%)	ref.	
Mode of delivery				
Vaginal	11 (40.7%)	16 (59.3%)	1.644	0.257

			(0.693-3.902)	
Caesarean	52 (53.1%)	46 (46.9%)	ref.	
PROM				
Yes	9 (45.0%)	11 (55.0%)	1.294 (0.495-3.381)	0.598
No	53 (51.4%)	51 (48.6%)	ref.	
Preeclampsia				
Yes	27 (47.4%)	30 (47.4%)	1.250 (0.618-2.530)	0.535
No	36 (52.9%)	32 (47.1%)	ref.	
Antenatal corticosteroid use				
Yes	38 (59.4%)	26 (40.6%)	0.475 (0.233-0.970)	0.040*
No	25 (41.0%)	36 (59.0%)	ref.	
Intrapartum fever				
Yes	3 (50.0%)	3 (50.0%)	1.017 (0.197-5.243)	1.000
No	60 (50.4%)	59 (49.6%)	ref.	
Maternal UTI				
Yes	0 (0%)	3 (100%)	N/A	0.119
No	63 (51.6%)	59 (48.4%)		
Anemia of pregnancy				
Yes	20 (52.6%)	18 (47.4%)	0.880 (0.410-1.886)	0.742
No	43 (49.4%)	44 (50.6%)	ref.	
Gram staining				
Monobacterial gram-positive	32 (74.4%)	11 (25.6%)	ref.	
Monobacterial gram-negative	31 (39.7%)	47 (60.3%)	4.411 (1.940-10.030)	<0.0001*
Polybacterial	0 (0%)	4 (100%)	N/A	
TTP (h), median (IQR)[#]	62.64 (28.08)	50.28 (21.66)	N/A	0.137
Time to positivity				
<48 h	23 (46.0%)	27 (54.0%)	3.522 (1.110-11.176)	
48-72 h	25 (45.5%)	30 (54.5%)	3.600 (1.148-11.288)	0.056
>72 h	15 (75.0%)	5 (25.0%)	ref.	
LoS (day), median (IQR)[#]	10 (18)	29 (27.2)	N/A	<0.0001*

**Statistical significance, p<0.05*
[#]Mann-Whitney test
RR: relative risk; CI: confidence interval; ref: reference category

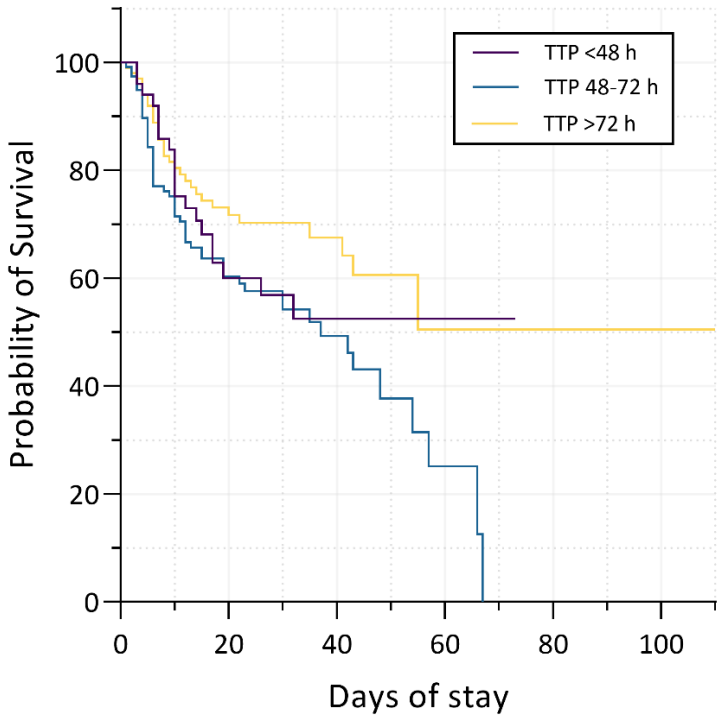

Figure 3. Kaplan-meier curves of overall survival based on different range of TTP (p=0.0364)

Table 4: Cox Proportional Hazard Regression Model of Overall Survival.

	Entire Cohort (N=125)			Gram-Negative Sepsis (N=78)		
Variables	**Mortality (%)**	**HR (95% CI)**	**P-value**	**Mortality (%)**	**HR (95% CI)**	**P-value**
Sex						
Male	36 (54.5%)	ref.	ref.			
Female	27 (45.8%)	1.612 (0.904–2.875)	0.105			
Onset						
Early-onset	19 (65.5%)	ref.	ref.	14 (70.0%)	ref.	ref.
Late-onset	43 (44.8%)	0.423 (0.229–0.781)	0.006*	33 (56.9%)	0.356 (0.171–0.742)	0.006*
Gestational Age						
<28 weeks	13 (86.7%)	1.893 (0.737–4.865)	0.185	9 (90.0%)	3.472 (1.065–11.319)	0.039*
28 - < 32 weeks	14 (40.0%)	0.704 (0.290–1.711)	0.438	11 (52.4%)	0.793 (0.264–2.386)	0.680
32 - <37 weeks	27 (46.6%)	1.317 (0.583–2.978)	0.508	22 (61.1%)	1.769 (0.648–4.832)	0.266
≥37 weeks	8 (47.1%)	ref.	ref.	6 (54.5%)	ref.	ref.
Birth asphyxia	13 (81.3%)	4.095 (2.095–8.005)	<0.0001*	7 (87.5%)	6.662 (2.495–17.784)	<0.0001*
Time to positivity (h)	50.28 (21.66) #	0.985 (0.973–0.998)	0.027*	47.76 (17.76)#	0.983 (0.968–0.999)	0.033*

**Statistical significance, p<0.05*
[#]Data presented as median (IQR)
HR: hazard ratio; CI: confidence interval; ref: reference category

Discussion

In comparison to prior studies, our findings revealed a higher median TTP of 58.08 hours, in which the majority of positive cultures were identified between 48 and 72 hours. For instance, a study by Abdelhamid (2017) reported a median TTP of 21.1 hours for pathogenic organisms causing neonatal sepsis.[19] Another study conducted on early-onset neonatal sepsis demonstrated that 68% of positive blood cultures were identified within 24 hours, 94% within 36 hours, and 97% within 48 hours.[20] One important factor that needs to be considered is this study took into account the time in which blood specimens enter the laboratory to be processed (inoculation and incubation), thereby allowing a more clinically applicable interpretation of the results. Since most studies define TTP from the start of incubation time, the usage of cut-off values established from single-center studies may not be suitable as its value is greatly influenced by technical matters (transportation and laboratory logistics). An illustrative instance of this line of reasoning is evidenced in a study carried out by Cobos-Trigueros *et al*. (2014), which reported significant difference of TTP for *Candida glabrata* in Barcelona, Spain and Cologne, Germany (80.8 h vs 53.4 h, respectively); presumably as a result of the unequal distribution of cases and loading time between the two centers.[21] This further underscores the impact of the geographical context in which the study was undertaken, given the inherent constraints imposed by the limited availability of laboratory facilities and human resources in LMICs.[22,23] Additionally, our study also highlights that recommendations on empirical antibiotic withdrawal after 24 or 36 h presented by previous reports may not be applicable in this resource-limited setting.[15,16,18,20] The reasons underlying this practice are inherently complex, as healthcare providers may need to administer empirical antibiotics until the blood culture results are available for review.[24,25] In spite of this, it is also crucial to emphasize that empirical antibiotic administration has been shown to elongate the length of stay in the NICU[26,27] and alter the normal body microbiome[28,29] during critical developmental period of an infant. Hence, the measurement of TTP can provide valuable information for the antibiotic stewardship team to consider in developing an antibiotic de-escalation strategy.

Variations of TTP have been identified among the pathogens causing neonatal sepsis in this study. All gram-negatives (*K. pneumoniae*, *A. baumannii*, and *E. cloacae*) exhibited a significantly lower TTP compared to the only gram-positives (CoNS). A previous study conducted on patients with neutropenia highlighted similar differences between both groups of pathogens, showing that monomicrobial gram-positive and CoNS bacteremia had 2.47 and 3.92 times higher risks of yielding TTP longer than 24 h, respectively.[30] Furthermore, the study demonstrated that all monomicrobial gram-negative aerobic bacteremia cases had a TTP of less than 24 h, with 48% higher odds of yielding positivity within 16 h.[30] Another study demonstrated that the median TTP of suspected EOS and LOS due to monobacterial gram-positive organisms were longer than those caused by monobacterial gram-negative organisms (23.1 h vs 17 h).[19] However, it may be inappropriate to generalize the differences solely based on Gram staining, as the variations could be attributed to the level of specific species. An earlier study exploring this approach showed that even among gram-negative bacilli, a significantly shorter TTP was identified between infections caused by *E. coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Citrobacter spp.*, and *Aeromonas spp.* Compared to those caused by *Proteeae*, *Salmonella spp.*, *Serratia spp.*, *P. aeruginosa*, and other non-fermenters.[14] In our study, a significant difference in TTP among gram-negative isolates was only identified on one comparison between *E. cloacae* and *A. baumannii* (p=0.0397). Nevertheless, it should also be emphasized that determining CoNS as causative gram-positive pathogens required extensive consideration as it is commonly associated as contaminants and therefore were excluded in the analysis of TTP in prior studies.[16–18,31] A study conducted on 0 to 90-day infants from an emergency department showed a significantly shorter TTP of pathogenic organisms compared to contaminants and stated that an incubation period of 36 h was adequate to detect 100% blood culture for significant pathogens.[32] Additionally, Huggard *et al*. (2021) identified that shorter TTP is associated with gram-negative neonatal sepsis and a 15.5% increase in the odds of isolating a pathogenic organism compared to contaminants (including CoNS).[16] Further adjustment on both patient-specific and culture-specific factors also suggested that the odds of obtaining TTP >36 h were 14 times higher for CoNS (aOR 14.60; 95% CI [6.98–30.58]; p<0.001).[18]

This is the first study that evaluates the prognostic potential of blood culture TTP for mortality in culture-proven neonatal sepsis cases. In this study, a longer TTP marginally predicts a better prognosis for the overall survival of neonates with neonatal sepsis. The risk of mortality was reduced by 1.5% and 1.7% for every increase of 1 h in TTP

both in the entire cohort and gram-negative sepsis, respectively. In line with these findings, a study conducted over a 14-year period in Ireland showed a comparatively lower neonatal sepsis mortality rate of 0.403 per 1000 live births, in which the TTP were consistently observed under 24 h.[16] A previous report conducted on children with *Pseudomonas aeruginosa* bacteremia also showed the prognostic role of TTP ≤18 h to predict in-hospital mortality (OR 5.88; 95% CI [1.21–21.96]; p=0.035).[33] In gram-positive neonatal sepsis, TTP ≤12 and ≤17 h have also been previously associated with in-hospital mortality among children with *Streptococcus pneumoniae* and *Staphylococcus aureus* bacteremia, respectively.[34,35]

Additionally, our study also found that EOS, extremely preterm birth (gestational age <28 weeks), and birth asphyxia were associated with worse outcomes in neonatal sepsis cases. It is noteworthy to emphasize that both prematurity and intrapartum complications (such as birth asphyxia) are the two most common causes of neonatal death worldwide; thus rendering the outcome not necessarily surprising.[36,37] A systematic review also suggested that neonatal mortality was estimated at a higher rate for EOS compared to LOS, presumably attributed to a higher incidence of other risk factors in those groups (prematurity and low-birth-weight).[1] Previous studies have highlighted that maternal history of pre-eclampsia/eclampsia and antepartum hemorrhage were significantly associated with mortality in neonatal sepsis.[38] Higher mortality was also associated with a maternal history of abortions, abnormal placenta, and PROM.[39] However, the current study did not find any significant association between maternal history and neonatal sepsis mortality. It is important to note that invasive procedures such as mechanical ventilation and total parenteral nutrition, which have been shown to increase the risk of poor outcomes in neonatal sepsis[40], were not accounted for in this study due to the insufficient information available in the medical records.

While our study provides valuable insights, it is important to acknowledge its limitations. Firstly, the sample size of this study was relatively small, despite applying a total sampling frame. To increase robustness, a longer cohort period would have greatly benefited the research. Secondly, the study didn't account for several potential confounding factors that may impact neonatal survival. This limitation arises from incomplete documentation in the medical records on information regarding the use of perinatal antibiotics, the type of empirical antibiotics for neonates, and other therapeutic modalities, the use of nutrition (TPN or enteral feeding), use of isotopes, and oxygen/mechanical ventilation. Thirdly, there is a lack of data on the time to bottle load following blood specimen collection, which would vary on each sample due to the availability of human resources on that period of time. Lastly, the survival analysis of TTP was conducted solely on the entire cohort and gram-negative sepsis cases, whereas it would have been ideal to perform this analysis for every neonatal sepsis cases caused by a specific pathogen (species). These limitations should be taken carefully into consideration when interpreting the findings of this study.

Conclusion

Yes. Time to positivity (TTP) predicts different species of causative pathogens and the overall survival of neonatal sepsis in a tertiary healthcare facility in Indonesia. *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter cloacae* are the three most common gram-negative pathogens causing neonatal sepsis in this study and exhibited significantly shorter TTP compared to the most common gram-positive pathogens, Coagulase-negative Staphylococci (CoNS). *E. cloacae* also showed a significantly shorter TTP compared to *Acinetobacter baumannii*. A shorter TTP, early-onset neonatal sepsis (EOS), and birth asphyxia were independent prognostic factors for in-hospital mortality for the entire cohort and the gram-negative sepsis cohort. Additionally, extremely preterm birth (gestational age <28 weeks) was also associated with mortality in gram-negative neonatal sepsis.

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References

1. Fleischmann C, Reichert F, Cassini A, et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. Arch Dis Child. 2021 Jan;106(8):745–52.

2. Milton R, Gillespie D, Dyer C, et al. Neonatal sepsis and mortality in low-income and middle-income countries from a facility-based birth cohort: an international multisite prospective observational study. Lancet Glob Heal [Internet]. 2022 May 1;10(5):e661–72. Available from: https://doi.org/10.1016/S2214-109X(22)00043-2

3. Toan ND, Darton TC, Huong NHT, et al. Clinical and laboratory factors associated with neonatal sepsis mortality at a major Vietnamese children's hospital. PLOS Glob Public Heal [Internet]. 2022 Sep 2;2(9):e0000875. Available from: https://doi.org/10.1371/journal.pgph.0000875

4. Xia X, Wang Y, Xie M, Qiu S, Zhou J. Elevated neutrophil - to - monocyte ratio as a prognostic marker for poor outcomes in neonatal sepsis. Heliyon [Internet]. 2022;8(10):e11181. Available from: https://www.sciencedirect.com/science/article/pii/S2405844022024690

5. Sumitro KR, Utomo MT, Widodo ADW. Neutrophil-to-Lymphocyte Ratio as an Alternative Marker of Neonatal Sepsis in Developing Countries. Oman Med J. 2021 Jan;36(1):e214.

6. Puopolo KM, Benitz WE, Zaoutis TE. Management of Neonates Born at ≥35 0/7 Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics. 2018 Dec;142(6).

7. Rogers MS, Oppenheim BA. The use of continuous monitoring blood culture systems in the diagnosis of catheter related sepsis. J Clin Pathol. 1998 Aug;51(8):635–7.

8. Weinstein MP, Towns ML, Quartey SM, et al. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis an Off Publ Infect Dis Soc Am. 1997 Apr;24(4):584–602.

9. Lamy B. Blood culture time-to-positivity: making use of the hidden information. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2019 Mar;25(3):268–71.

10. George BJ, Horvath LL, Hospenthal DR. Effect of inoculum size on detection of Candida growth by the BACTEC 9240 automated blood culture system using aerobic and anaerobic media. J Clin Microbiol. 2005 Jan;43(1):433–5.

11. Haimi-Cohen Y, Vellozzi EM, Rubin LG. Initial concentration of Staphylococcus epidermidis in simulated pediatric blood cultures correlates with time to positive results with the automated, continuously monitored BACTEC blood culture system. J Clin Microbiol. 2002 Mar;40(3):898–901.

12. Hsieh YC, Chen HL, Lin SY, Chen TC, Lu PL. Short time to positivity of blood culture predicts mortality and septic shock in bacteremic patients: a systematic review and meta-analysis. BMC Infect Dis. 2022 Feb;22(1):142.

13. Lambregts MMC, Bernards AT, van der Beek MT, Visser LG, de Boer MG. Time to positivity of blood cultures supports early re-evaluation of empiric broad-spectrum antimicrobial therapy. PLoS One. 2019;14(1):e0208819.

14. Martínez JA, Pozo L, Almela M, et al. Microbial and clinical determinants of time-to-positivity in patients with bacteraemia. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2007 Jul;13(7):709–16.

15. Arias-Felipe A, Ramírez-Berrios J, Recio-Martinez R, et al. Determining Time to Positivity of Blood Cultures in a Neonatal Unit. J Pediatric Infect Dis Soc. 2022 Dec;11(11):510–3.

16. Huggard D, Powell J, Kirkham C, Power L, O'Connell NH, Philip RK. Time to positivity (TTP) of neonatal blood cultures: a trend analysis over a decade from Ireland. J Matern neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2021 Mar;34(5):780–6.

17. Coggins SA, Harris MC, Srinivasan L. Dual-site blood culture yield and time to positivity in neonatal late-onset sepsis. Arch Dis Child - Fetal Neonatal Ed [Internet]. 2022 Sep 1;107(5):475 LP – 480. Available from: http://fn.bmj.com/content/107/5/475.abstract

18. Mukhopadhyay S, Briker SM, Flannery DD, et al. Time to positivity of blood cultures in neonatal late-onset bacteraemia. Arch Dis Child - Fetal Neonatal Ed [Internet]. 2022 Nov 1;107(6):583 LP – 588. Available from: http://fn.bmj.com/content/107/6/583.abstract

19. Abdelhamid SM. Time to Positivity and Antibiotic Sensitivity of Neonatal Blood Cultures. J Glob Infect Dis. 2017;9(3):102–7.

20. Kuzniewicz MW, Mukhopadhyay S, Li S, Walsh EM, Puopolo KM. Time to Positivity of Neonatal Blood Cultures for Early-onset Sepsis. Pediatr Infect Dis J. 2020 Jul;39(7):634–40.

21. Cobos-Trigueros N, Kaasch AJ, Soriano A, et al. Time to positivity and detection of growth in anaerobic blood culture vials predict the presence of Candida glabrata in candidemia: a two-center European cohort study. J Clin Microbiol. 2014 Aug;52(8):3082–4.

22. Ombelet S, Barbé B, Affolabi D, et al. Best Practices of Blood Cultures in Low- and Middle-Income Countries. Front Med. 2019;6:131.

23. Isaac EW, Jalo I, Difa AJ, et al. Bacterial Blood Isolates in Children: Conventional vs. Bactec Automated Blood Culture System in a Tertiary Health Centre in Gombe, North East Nigeria. Open J Med Microbiol. 2022;12(3):101–16.

24. Graus JM, Herbozo C, Hernandez R, Pantoja AF, Zegarra J. Managing antibiotics wisely in a neonatal intensive care unit in a low resource setting. J Perinatol Off J Calif Perinat Assoc. 2022 Apr;1–6.

25. Schulman J, Dimand RJ, Lee HC, Duenas G V, Bennett M V, Gould JB. Neonatal intensive care unit antibiotic use. Pediatrics. 2015 May;135(5):826–33.

26. Kopsidas I, Tsopela GC, Molocha NM, et al. Reducing Duration of Antibiotic Use for Presumed Neonatal Early-Onset Sepsis in Greek NICUs. A "Low-Hanging Fruit" Approach. Antibiot (Basel, Switzerland). 2021 Mar;10(3).

27. Sourour W, Sanchez V, Sourour M, et al. The Association between Prolonged Antibiotic Use in Culture Negative Infants and Length of Hospital Stay and Total Hospital Costs. Am J Perinatol. 2023 Apr;40(5):525–31.

28. Neuman H, Forsythe P, Uzan A, Avni O, Koren O. Antibiotics in early life: dysbiosis and the damage done. FEMS Microbiol Rev. 2018 Jul;42(4):489–99.

29. Vangay P, Ward T, Gerber JS, Knights D. Antibiotics, pediatric dysbiosis, and disease. Cell Host Microbe. 2015 May;17(5):553–64.

30. Lambregts MMC, Warreman EB, Bernards AT, et al. Distribution and clinical determinants of time-to-positivity of blood cultures in patients with neutropenia. Eur J Haematol [Internet]. 2018 Feb 1;100(2):206–14. Available from: https://doi.org/10.1111/ejh.13001

31. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal sepsis due to coagulase-negative staphylococci. Clin Dev Immunol [Internet]. 2013;2013. Available from: https://doi.org/10.1155%2F2013%2F586076

32. Lefebvre CE, Renaud C, Chartrand C. Time to Positivity of Blood Cultures in Infants 0 to 90 Days Old Presenting to the Emergency Department: Is 36 Hours Enough? J Pediatric Infect Dis Soc. 2017 Mar;6(1):28–32.

33. Xu H, Cheng J, Yu Q, et al. Prognostic role of time to positivity of blood culture in children with Pseudomonas aeruginosa bacteremia. BMC Infect Dis. 2020 Sep;20(1):665.

34. Li Q, Li Y, Yi Q, et al. Prognostic roles of time to positivity of blood culture in children with Streptococcus pneumoniae bacteremia. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2019 Mar;38(3):457–65.

35. Li Y, Li Q, Zhang G, et al. Time to positivity of blood culture is a risk factor for clinical outcomes in Staphylococcus aureus bacteremia children: a retrospective study. BMC Infect Dis. 2019 May;19(1):437.

36. World Health Organization. Newborn Health [Internet]. 2022 [cited 2022 Nov 30]. Available from: https://www.who.int/teams/maternal-newborn-child-adolescent-health-and-ageing/newborn-health/perinatal-asphyxia

37. World Health Organization. Newborns: improving survival and well-being [Internet]. 2020 [cited 2022 Nov 30]. Available from: https://www.who.int/news-room/fact-sheets/detail/newborns-reducing-mortality

38. Meshram RM, Gajimwar VS, Bhongade SD. Predictors of mortality in outborns with neonatal sepsis: A prospective observational study. Niger Postgrad Med J. 2019;26(4):216–22.

39. Leal YA, Álvarez-Nemegyei J, Velázquez JR, et al. Risk factors and prognosis for neonatal sepsis in southeastern Mexico: analysis of a four-year historic cohort follow-up. BMC Pregnancy Childbirth. 2012;12:1–9.

40. Turhan EE, Gürsoy T, Ovalı F. Factors which affect mortality in neonatal sepsis. Turk Pediatr Ars. 2015 Sep;50(3):170–5.