ORIGINAL ARTICLE

CLINICAL PREDICTION OF EARLY ONSET SEPSIS IN PRETERM NEONATES

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ABSTRACT
The aim of the study was to analyze and identify risk factors for the development of early onset sepsis in preterm neonates and to develop a clinical prognostic model.
Materials and methods: A retrospective cohort study included 152 newborns with birth weight from 1000 to 2500 g, who were treated in the neonatal intensive care units of medical institutions in the Poltava region. Among 152 children, 121 had clinical and laboratory symptoms of infection, which were regarded as manifestations of early onset sepsis, the rest of the children (n = 31) had no manifestations of infection.
Results: According to the results of multiple stepwise logistic regression analysis, the predictive model has been developed. It included gestational age, visual changes of placenta, Apgar score at the 1st minute, the level of monocytes more than 6.5%, the history of abortions and premature rupture of membranes. The diagnostic characteristics of the developed model had high: sensitivity – 82.2%, specificity – 93.55%, positive predictive value – 97.98%, negative predictive value – 58%.
Conclusions: The prognostic model developed by us, which showed high diagnostic characteristics, includes information on maternal risk factors, the state of the newborn immediately after birth, and biomarkers of infection (C-reactive protein and monocyte count). Therefore, we believe that when interpreting biomarkers, the decision to prescribe antibiotics should be based on the presence of maternal risk factors and clinical symptoms of infection in the prematurely born child, which may be nonspecific

   KEY WORDS: premature infants, early onset neonatal sepsis, risk factors, diagnostic markers

INTRODUCTION

Early onset sepsis (EOS) in premature infants is the condition that has a potentially huge impact on early and long-term adverse developmental outcomes in children. Over the past decades, improvements in obstetric and neonatal services have contributed to a significant reduction in mortality, but practically did not affect the incidence of neonatal sepsis. Between 1995 and 2005 the number of cases of severe sepsis in newborns has doubled (from 4.5 to 9.7 per 1000 newborns) [1], inversely proportional to gestational age at birth and can reach 60% in the most immature infants [2]. Probably, the improvement in the survival rates of children with very low birth weight [3], as well as the presence of polymorphism of key genes that are responsible for the formation of the body's response to bacterial infection [4], led to an increase in the population of newborns susceptible to the development of intrauterine infections. Neonatal sepsis is the cause of death or severe disability in 39% of children, even with the timely initiation of antibiotic therapy [5]. In Ukraine, infections of the perinatal period, congenital pneumonia, sepsis rank III-IV in the structure of morbidity and mortality in newborns. The true frequency of this pathology among prematurely born children is unknown, since there are no clear criteria for determining the diagnosis in this cohort of children, as well as modern bacteriological methods for determining the blood culture.

To this day, the diagnosis of sepsis in premature babies remains controversial [6], since the accuracy of the key criteria for sepsis established by the Pediatric Consensus [7] has not been determined in this cohort. Symptoms and signs of sepsis in premature infants are heterogeneous, so it is difficult for a doctor to correctly and timely diagnose and assess the severity. In these cases, a conflicting decision-making dilemma arises. On the one hand, there is a desire to minimize the prescription of antibiotics, and on the other, there is strong evidence that the timely administration of antibiotics saves lives [8].

Prematurely born children represent a very heterogeneous cohort due to the peculiarities of the course of the antenatal, intrapartum periods and the period of early adaptation. Knowledge of reliable risk factors, as well as biomarkers of sepsis, can become an information basis for ensuring the decision-making process in this situation.

3370 studies were devoted to the study of biochemical criteria of sepsis, in which the diagnostic value of 178 sepsis biomarkers was studied, but today only two are widely used – the C-reactive protein (CRP) and the procalcitonin test (PCT) [9]. However, a recent meta-analysis [10] casts doubt on the diagnostic value of these markers, and subsequent controlled studies of PCT [11, 12] presented conflicting results. Based on the foregoing, today it is relevant to search for EOS markers in premature babies in order to predict the development of their disease and timely prescribe antibiotic therapy.

Table 1. Definition and ranks of prognostic variables associated with early onset sepsis in preterm infants

Prognostic variables	Definition and ranks	Prognostic variables	Definition and ranks
Somatic status of mother			
Smoking	0 - no, 1 - yes	Kidneys diseases	0 - no, 1 - yes
Mother`s age	years	Diabetes mellitus	0 - no, 1 - yes
Cardiovascular diseases	0 - no, 1 - yes	Thyroid gland diseases	0 - no, 1 - yes
		Oncology	0 - no, 1 - yes
Prenatal and intrapartum factors			
Abortions in anamnesis	0 - no, 1 - yes	Number of labor in mother	Units.
Miscarriages	0 - no, 1 - yes	Duration of I period of delivery	minutes
Stillbirths	0 - no, 1 - yes	Duration of II period of delivery	minutes
Hestosis	0 - no, 1 - yes	Placenta`s detachment	0 - no, 1 - yes
Spontaneous abortion threat	0 - no, 1 - yes	Cord entanglement	0 - no, 1 - yes
Anemia	0 - no, 1 - yes	Cesarian section	0 - no, 1 - yes
Fetoplacental insufficiency	0 - no, 1 - yes	Cesarian section due to hestosis	0 - no, 1 - yes
Polihydramnion	0 - no, 1 - yes	Breech or foot presentation	0 - no, 1 - yes
Olygohydramnion	0 - no, 1 - yes	Multiple pregnancy	0 - no, 1 - yes
Colpitis	0 - no, 1 - yes	Mother's illness during childbirth	0 - no, 1 - yes
Premature rupture of amniotic fluid	0 - no, 1 - yes	Visual changes in placenta	0 - no, 1 - yes
The duration of the waterless gap	hours		
Medical, demographic and clinical factors			
Weight	gramms	Masculine sex	0 - no, 1 - yes
Gestational age	weeks	Physical development <10 percentiles	0 - no, 1 - yes
Evaluation using Apgar scale		**Primary resuscitation**	
Apgar on 1st min.	scores	Need in resuscitation	0 - no, 1 - yes
		Oxygenotherapy	0 - no, 1 - yes
Apgar on 5th min	scores	Trachea intubation	0 - no, 1 - yes
		Artificial lung ventilation	0 - no, 1 - yes
Laboratory indicators			
CRP	mg/l	Bands	%
		Platelets	10^9/л
Blood glucose	mmol/l	Monocytes	%
WBC	10^9/l	Monocytes> 6,5%	0 - no, 1 - yes
Neutrophils	10^9/l	Urea	mmol/l
Immature vs mature granulocytes ratio	Units		

THE AIM

To analyze and identify risk factors for the development of early-onset sepsis in preterm neonates and to develop a clinical prognostic model with high diagnostic characteristics.

MATERIALS AND METHODS

A retrospective cohort study was carried out, which included 152 newborns with birth weight from 1000 to 2500 g, who were treated in the neonatal intensive care units (NICU) of medical institutions in the Poltava region. Of the 152 children, 121 had clinical and laboratory symptoms of infection, which were regarded as manifestations of EOS,

the rest of the children (n = 31) had no manifestations of infection. Clinical symptoms of sepsis included the presence of tachycardia, tachypnea, desaturations, apnea, perfusion disorders, seizures, shock manifestations; laboratory signs of infection – the number of leukocytes is more than $20x10^9$ or less than $5x10^9$ and an increased level of C-reactive protein. Confirmation of early onset sepsis was due to the presence of a positive blood culture, but this was not a mandatory criterion for inclusion in the main group.

We considered 52 candidate predictive variables associated with the development of EOS (Table 1). Previously identified reliable factors associated with the development of EOS, with a simple logistic regression analysis, were

Table 2. Frequency of individual risk factors for antenatal and intrapartum periods and medical and demographic characteristics of children included in the survey

Predictive variables	Children with EOS	Children without EOS	OR 95% CI	p
Abortion, n (%)	40 (33,06)	4 (12,9)	3,3 (1,09-10,17)	0,035
Duration of the II stage of labor (hours), M ± m	23,9 ± 1,39	31,1 ± 1,83	0,9 (0,86-0,99)	0,037
Premature rupture of membranes, n (%)	73 (60,33)	8 (25,81)	4,4 (1,81-10,57)	0,001
Caesarean section due to fetal distress, n (%)	40 (33,33)	5 (16,13)	2,6 (0,92-7,19)	0,073
Mother's illness during childbirth, n (%)	39 (32,23)	5 (16,13)	2,47 (0,88-6,92)	0,085
Visual changes in the placenta, n (%)	25 (20,66)	1 (3,23)	7,8 (1,01-60,10)	0,013
Birth weight (grams), M ± m	1973,9±8,36	2232,2±64,2	0,9 (0,98-0,99)	0,014
Gestational age, M ± m	32,59 ± 0,2	35,19± 0,19	0,4 (0,24-0,53)	<0,001
Apgar scale at 1 minute, M ± m	6,08 ± 0,14	7,35 ± 0,09	0,1 (0,05-0,31)	<0,001
Apgar scale at 5 minutes, M ± m	6,77 ± 0,10	7,77 ± 0,13	0,3 (0,16-0,49)	<0,001
Laboratory indicators:				
CRB, M ± m	7,10±0,84	4,28±1,10	1,5 (1,07-2,03)	0,017
Monocytes, M ± m	7,008±0,30	5,32±0,57	1,2 (1,03-1,35)	0,018
Monocytes> 6.5%, n (%)	66 (54,55)	8 (25,81)	3,4 (1,43-8,32)	0,006

Notes: CRP – C-reactive protein, OR – odds ratio, CI - confidence interval, M - median, m - standard error.

Table 3. Distribution of variables in stepwise multiple logistic regression model

Variables	β	m	OR	95% CI	Area under ROC	% of ROC increase
Gestational age	-0,786	0,236	0,455	0,286-0,724	0,8661	
Premature rupture of membranes	0,989	0,609	2,689	0,815-8,871	0,8777	1,3
Visual changes in the placenta	1,699	1,410	5,470	0,345-86,74	0,8922	1,7
Monocyte count> 6.5%	0,944	0,647	2,571	0,722-9,148	0,9036	1,3
Apgar score for 1 min.	-2,076	0,744	0,125	0,029-0,538	0,9370	3,7
History of abortion	1,193	0,865	3,298	0,604-17,99	0,9377	0,1

subsequently subjected to multiple logistic regression analysis to identify reliable risk factors and include them in the clinical prognostic model.

A 5% significance level was used to include each variable in the model. After the final model was created, the area under the ROC (receiver operating characteristic) curve was calculated.

Constant variables are represented as mean and standard error, categorical variables as absolute numbers and percentages. Student's test was used to compare independent samples and χ^2 test to compare proportions. All statistical analyzes were performed using the STATA v11 software package for Windows (StataCorp, Texas, USA).

RESULTS

Information from the case histories of 152 children who were included in the study was analyzed taking into account certain criteria. The average gestational age of the examined children was 33.14 ± 2.27 weeks, the average body weight at birth was 2026.9 ± 509.13 g. Among all the studied prenatal risk factors that could affect the development of EOS in children, only the presence of abortion and premature rupture of the amniotic membranes in the mother were significantly associated with development of EOS (table 2).

Among the factors of the intrapartum period, the duration of the second stage of labor, the operation of a cesarean section due to fetal distress, the mother's illness during childbirth, and visual changes in the placenta were reliable. Among the clinical and demographic factors, the weight of the child at birth, gestational age, and Apgar score at 1 and 5 minutes were significant.

As for the course of the disease, the need for the use of mechanical ventilation was in 71 (58.68%) infants, drug support for hemodynamics – in 47 (38.84%). Food intolerance was found in 62 (51.4%) infants, episodes of arterial hypotension – in 50 (41.2%), early jaundice – in 29 (23.97%), convulsions – in 9 (7.44%), oliguria – in 8 (6.6%) and hemorrhagic syndrome – in 4 (3.31%) children.

Of the laboratory parameters, only CRP and the level of monocytes were significantly associated with the development of EOS in premature babies. As presented in Table 2, gestational age, visual changes in the placenta, Apgar scale at 1 minute, monocyte level more than 6,5%, history of abortion and premature rupture of membranes have the greatest influence on the development of EOS in premature infants. Therefore, these variables were included in the final predictive model chosen by stepwise multiple logistic regression analysis (Table 3).
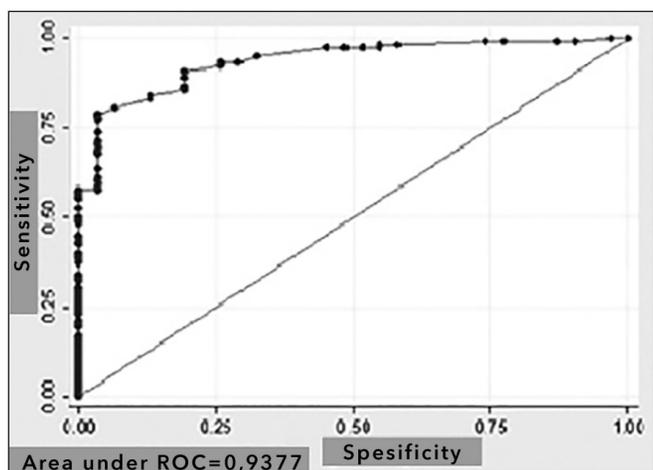
Fig. 1. ROC-curve of clinical prognostic model for the development of early onset sepsis in premature infants

The results showed that each additional week of gestational age reduces the risk of early onset sepsis (β = -0.786), and the inclusion of this factor alone has a significant prognostic value (the area under the ROC curve is 0.8661). Subsequent incremental addition of each predictive variable increases the area under the ROC and accordingly enhances the predictive characteristics of the model. Thus, the operational characteristics of our final model, which include gestational age, maternal history of abortion, premature rupture of membranes, visual changes in the placenta, Apgar score at 1 minute, monocyte count> 6.5%, were found to be quite high, namely: the sensitivity was 82.2%, the specificity was 93.55%, the positive predictive value was 97.98%, the negative predictive value was 58%, the area under the ROC curve was 0.9377 (Fig. 1).

DISCUSSION

The results of the study showed that the clinical picture of EOS is highly variable. Other authors also pay attention to nonspecific symptoms of sepsis [13-17]. Most of the symptoms related to the cardiovascular system (tachycardia / bradycardia, hypotension, poor perfusion) [18-22], the respiratory system (apnea, cyanosis, tachypnea, the need for respiratory support, and increased oxygen support) [18,23], central nervous system (lethargy, hypotension and seizures), temperature changes (increase or decrease) and nutritional problems indicate the presence of difficulties in making a diagnosis [8, 18]. A complex of antenatal (premature rupture of membranes, history of abortion), intranatal (visual changes in the placenta), clinical and demographic factors (gestational age of the child, low score on the Apgar scale) and some biomarkers are reliably associated with the data obtained on the development of early onset sepsis in prematurely born children. (level of CRP, monocytes). It should be noted that of all the biomarkers that were studied in the study, only CRP and the number of monocytes had significant value, and such well-known markers as the number of leukocytes, the proportion of immature cells,

and a low number of platelets were not particularly significant. Hornik et al. [24, 25], who retrospectively studied the diagnostic value of the total number of leukocytes ($5,000 / mm^3$ -$19,000 / mm^3$), the absolute number of neutrophils ($\geq 1,500 / mm^3$) and the ratio of immature neutrophils to mature (<0.2) in more than 200 000 premature and term infants concluded that these indicators are not acceptable for identifying infants with early or late neonatal sepsis. As for the number of immature forms of leukocytes, according to the results of the study by Van der Meer et al. [23], there are wide intra- and interlaboratory variations in the interpretation of neutro phyla (15-72%, SD, 11%) and their immature forms (4-64%, SD, 11%). Most authors consider thrombocytopenia, hyperglycemia or hypoglycemia to be appropriate [18, 23, 8, 9, 22]. A positive culture is considered the "gold standard" for defining infection, although culture-positive clinical sepsis is rarely diagnosed in all age groups [27]. In this study, a positive blood culture was found in a minimum number of children (18.5%). Other authors also write about similar trends. In a study by Garges it was shown that in 38% of newborns (GA less than 34 weeks) with culture-positive bacterial meningitis, the blood culture was negative [26]. It should be borne in mind that a false negative result of a blood culture may be due to a low number of bacteremia colonies in newborns or their absence in case of insufficient blood volume collected for research. Therefore, it is currently believed that if a child has negative cultures of blood or other loci, but has symptoms of suggested infection, the child should be diagnosed with clinical sepsis [27].

CONCLUSIONS

The prognostic model developed by us, which showed high diagnostic characteristics, includes information on maternal risk factors, the state of the newborn immediately after birth, and biomarkers of infection (CRP and monocyte count). Therefore, we believe that when interpreting biomarkers, the decision to prescribe antibiotics should be based on the presence of maternal risk factors and clinical symptoms of infection in the prematurely born child, which may be nonspecific.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis,

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