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BIOCHEMICAL CHANGES OCCURING IN NEONATES WITH SEPSIS

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Abstract: This retrospective study aims to analyze the relationship between biochemical changes occurring in newborns with sepsis proven by positive blood culture (BC) and possible correlations with 1 min Apgar score, 5 min Apgar score, gestational age (GA), and birth weight (BW). We included in the study all infants ≤7 days of life with positive BC that were admitted to the Neonatal Intensive Care Unit (NICU) and Neonatology Department (ND) of the County Emergency Clinical Hospital of Târgu Mureş, a tertiary level hospital, between 2014-2018. The analyzed parameters are: day of life for blood sampling (0-7 days of life), gender, Apgar score (1 and 5 minute), GA, BW, urea, creatinine, total bilirubin, direct bilirubin, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), c-reactive protein (CPR), bacteria involved, empiric antibiotics administered before blood sampling, temperature of the newborn on the day of BC. We found there is a statistically significant negative correlation between 1 and 5 min Apgar score and creatinine, between GA and urea and also between BW, GA and Direct Bilirubin. We found a statistically significant positive correlation between BW, GA and GPT.

Keywords: neonate, septicemia, bacteremia, biochemistry, CRP.

1. Introduction

According to World Health Organization (WHO) in 2017, globally 2.5 million children died in the first month of life, approximately 7000 newborn deaths every day with about 1 million dying on the first day and close to 1 million dying within the next 6 days.

The most common causes are: preterm birth, intrapartum-related complications (birth asphyxia, lack of breathing at birth), infections and birth defects. The vast majority of newborn deaths take place in low and middle-income countries (WHO, 2018). Annual neonatal mortality rates (NMRs, the probability of dying during the first 28 days of life) vary widely across the world, but west and Central Africa and South Asia had the highest NMRs while Western Europe has the lowest NMRs in 2017 (Hug et al., 2019).

Neonatal sepsis is defined as a systemic infection (positive culture of blood, urine or cerebrospinal fluid) occurring in infants at ≤ 28 days of life. According to the time of onset of the disease, neonatal sepsis may be classified in early onset (EOS, defined as a positive culture during ≤3 days of life) and late onset (LOS, a positive culture >3 days of life) (Simonsen et al., 2014).

2. Materials and Methods

The study was approved by the Ethics Committee of the County Emergency Clinical Hospital of Târgu Mureş and it follows the Helsinki Declaration principles.

A 5 years retrospective study, from 2014-2018, was performed in June 2019, to evaluate the relationship between biochemical changes occurring in newborns with sepsis proven by positive blood culture (BC) and possible correlations with 1 min Apgar score, 5 min Apgar score, gestational age (GA), birth weight (BW).

Data were collected of all infants ≤7 days of life with positive BC that were admitted to the Neonatal Intensive Care Unit (NICU) and Neonatology Department (ND) of the County Emergency Clinical Hospital of Târgu Mureş, a tertiary level hospital.

The data were obtained by accessing the H3 electronic medical database and the laboratory records. Tracked parameters:

- general data: day of life that the blood sampling was performed (0-7 days of life), gender, Apgar score (1 and 5 minute), GA, BW
- biochemical data: urea, creatinine, total bilirubin, direct bilirubin, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), c-reactive protein (CPR)
- microbiological data: positive BC, bacteria involved, empiric antibiotics administered before blood sampling, temperature at blood sampling for BC.

We set a maximum of 2 days between the biochemical analyses and the date of blood sampling for the BC.

Patients excluded:

- Microorganism considered to be contaminants: *Methylobacterium* spp., *Streptococcus mitis, Streptococcus oralis, Micrococcus luteus, Ochrobactrum anthropi* and the association over 3 types of germs;
- A positive BC with coagulase-negative staphylococci (CoNS) and when in the comments of the microbiologist it was specified contamination, possible contamination or skin flora.

The data analysis included descriptive statistics elements (frequency, percentage, confidence interval 95%, mean, median, standard deviation) and inferential statistics. The D'Agostino & Pearson test was applied to determine the distribution of the analyzed data series. The Pearson correlation coefficient, respectively Spearman, was calculated. The significance threshold chosen for p was 0.05. The statistical analysis was performed using the GraphPad Prism 7 utility, the Trial variant.

3. Results and discussions

We identified 694 BC performed on first 7 days of life on neonates, which of 88 (12.68%) are positive, 26 (3.74%) contaminated and 62 (8.94%) true positive BC. In our group 24 (38.70%) neonates are from the NICU and 38 (61.30%) are from the ND. 13 (20.96%) of newborns with sepsis died in hospital. **Table 1** contains the description of the studied group.

In our group, 32 (51.61%) of neonates had EOS and 30 (48.39%) had LOS. The bacteria being identified and the antibiotics used, if it were the case, are presented in **Table 2**. The most common empirical choices of antibiotics for the treatment of neonatal sepsis were Aminoglycoside (in EOS 5, 71.42%; in LOS 7, 22.58%) and Penicillin (in EOS 4, 57.14%; in LOS 12, 38.70%) and for both categories.

Table 1. The description of the studied group

Parameter (unit of measure, number of values	*Mean±SD[a]*	*Median*	*Normal range[e]*
Age (days, 62)	3.5±2.31	3	
Gestational age (weeks, 62)	35.13±4.44	36	
Birth weight (grams, 62)	2482±1050	2530	
1 min APGAR score (62)	7.22±2.28	8	
5 min APGAR score (62)	8.06±1.83	9	
Temperature at blood collection (°C, 62)	36.98±0.65	37	
Urea (mg/dL, 47)	56.25±39.37	43.98	9-14
Creatinine (mg/dL, 50)	0.78±0.39	0.66	0.17-0.85
Total Bilirubin (mg/dL, 48)	7.24±6.08	6.11	0-12.6
Direct Bilirubin (mg/dL, 43)	0.80±0.53	0.57	0-0.6
GOT[b] (U/L, 52)	60.97±48.48	48	0-110
GPT[c] (U/L, 50)	35.34±48.96	16	0-60
CRP[d] (mg/L, 50)	82.10±92.98	53.71	0-5

Note: a - Standard Deviation; b - aspartate aminotransferase; c - alanine aminotransferase; d - c-reactive protein; e - normal clinical biochemistry reference ranges for neonates in Clinical Laboratory of County Emergency Clinical Hospital of Târgu Mureş (data from the manufacturer and the literature)

Table 2. The bacteria identified and the antibiotics used

Parameter		*Frequency*	*Percentage*	*Confidence interval (95%)*
Gender	Female	23	37.10%	25.16%-50.31%
	Male	39	62.90%	49.69%-74.84%
Positive bacteria	*Streptococcus*	3	4.84%	1.01%-13.50%
	Staphylococcus	20	32.26%	20.94%-45.34%
	Stenotrophomonas maltophilia	2	3.23%	0.39%-11.17%
	Escherichia coli	10	16.13%	8.02%-27.67%
	Enterococcus	9	14.52%	6.86%-25.78%
	Listeria	2	3.23%	0.39%-11.17%
	Klebsiella	7	11.29%	4.66%-21.89%
	Candida	7	11.29%	4.66%-21.89%
	Serratia	2	3.23%	0.39%-11.17%
	Acinetobacter	2	3.23%	0.39%-11.17%
Empiric antibiotics administered before blood sampling	Aminoglycoside	12	50.00%	29.12%-70.88%
	Penicillin	16	66.67%	44.68%-84.37%
	Carbapenem	5	20.83%	7.13%-42.15%
	Cephalosporin	3	12.50%	2.66%-32.36%
	Polymyxin	4	16.67%	4.74%-37.38%
	Fluoroquinolone	1	4.17%	0.11%-21.12%
Blood sampling on treatment	No	38	61.29%	48.07%-73.40%
	Yes	24	38.71%	26.60%-51.93%

Of the 24 newborns receiving antibiotic empirical therapy, 16 (66.66%) of them had associations of drug classes (14, 58.33% received association with 2 classes and 2, 8.33% association with 3 classes). The most common association of drug classes was Penicillin with Aminoglycosides (7, 43.75%).

In **Table 3** correlations between 1 min Apgar score, 5 min Apgar score, GA, BW and biochemical parameters are presented.

Acute kidney failure (AKF) is a common clinical problem in NICUs. According to Mathur et al. (2006), in India, renal failure occurred in 26% neonates with sepsis and Low birth weight is an important risk factor for the development of AKF, a significantly higher number of babies with AKF weighed less than 2500 gm. The mortality was three times higher in neonates with AKF. In Turkey, Agras et al. (2004) found a frequency of 3.4% AKF in the NICU, the premature newborns constituting 31.1% of the cases. The most common condition that contributed to AKF that they found was asphyxia (40.0%) followed by sepsis/metabolic disease (22.2%) and feeding problems (17.8%). In another study, also conducted in Turkey, the prevalence of neonatal AKF was 8.4%. The common cause of AKI was respiratory distress syndrome, followed by sepsis, asphyxia, dehydration, congenital anomalies of the urinary tract, congenital heart disease, and medication. In that case, the overall mortality rate was 23.8% (Bolat et al., 2013). In Egypt, 40.7% of the AKI cases were born after full-term pregnancy while 59.3% were pre-term babies. The predisposing factors for AKI were sepsis (63%), respiratory distress syndrome (55.6%), mechanical ventilation (51.9%), peri-natal asphyxia (18.5%), dehydration (14.8%), surgical operation (11.1%), congenital heart disease (7.4%), sub-galeal hematoma (3.7%), polycythemia (3.7%) and intra-ventricular hemorrhage (3.7%) (Youssef et al., 2015). Although the prevalence and mortality rate are different depending on the hospital, the causes remain roughly the same and sepsis is found everywhere. We found a statistically significant negative correlation between a high 1 and 5 min Apgar score and a low value of creatinine. We have also found a statistically significant negative correlation between a high GA and a low value of urea.

Hepatic pathology is common among newborns with sepsis. Jaundice is a well-known complication of sepsis or nonbacterial infection. Sepsis and bacterial infection are responsible for up to 20% of cases of jaundice in patients of all ages in a community hospital setting (Whitehead et al., 2001). Sepsis is more likely to manifest with jaundice in infants and children than in adults. Various mechanisms that can lead to hyperbilirubinemia alone during systemic infection are hemolysis, hepatic dysfunction, cholestasis (Chand and Sanyal, 2007). We found that a high BW and a high GA is significantly negative correlated with a low value of Direct Bilirubin. Another cause of neonatal jaundice is urinary tract infection (UTI). Shahian et al. (2012) found 12.5% of the asymptomatic jaundice neonates with the onset of unconjugated hyperbilirubinemia in the first week of life, and suggested that urine culture should be considered as a part of the diagnostic evaluation of jaundice neonates >3 days of life with an unexplained etiology (Shahian et al., 2012). On the other hand, Oswari et al. (2013) found that serum gamma-glutamyltransferase (GGT) and AST values can be used to predict the prognosis of patients with sepsis-associated cholestasis (Oswari et al., 2013). Our results show that there is a positive statistical correlation between BW, GA and GPT, a high BW or a high GA is correlated with a high GPT value.

Table 3. Correlations between independent variables (1 min Apgar score, 5 min Apgar score, Gestational age, Birth weight) and biochemical parameters

1 min Apgar score			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.08372	-0.3698 to 0.2169	0.5758
Creatinine	-0.3012	-0.5407 to -0.01638	0.0336*
Total Bilirubin	0.1412	-0.1574 to 0.4161	0.3385
Direct Bilirubin	0.02178	-0.2889 to 0.3283	0.8897
GOT	0.02761	-0.2550 to 0.3058	0.8460
GPT	0.2574	-0.03110 to 0.5063	0.0712
CRP	-0.1415	-0.4110 to 0.1508	0.3271
5 min Apgar score			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.1393	-0.4174 to 0.1626	0.3503
Creatinine	-0.2826	-0.5262 to 0.003939	0.0468*
Total Bilirubin	0.1140	-0.1843 to 0.3930	0.4404
Direct Bilirubin	-0.1084	-0.4037 to 0.2072	0.4888
GOT	0.01448	-0.2672 to 0.2939	0.9189
GPT	0.2335	-0.05643 to 0.4872	0.1026
CRP	-0.1801	-0.4435 to 0.1118	0.2106
Birth weight			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.2740	-0.5266 to 0.02312	0.0624
Creatinine	-0.008219	-0.2937 to 0.2786	0.9548
Total Bilirubin	-0.007929	-0.2994 to 0.2849	0.9573
Direct Bilirubin	-0.5542	-0.7369 to -0.2961	0.0001*
GOT	0.09467	-0.1910 to 0.3656	0.5044
GPT	0.5324	0.2905 to 0.7104	0.0001*
CRP	-0.2504	-0.5007 to 0.03856	0.0795
Gestational age			
	r[a]	**Confidence interval (95%)**	***p*[b]**
Urea	-0.2938	-0.5420 to 0.001547	0.0450*
Creatinine	-0.06826	-0.3477 to 0.2223	0.6376
Total Bilirubin	-0.01890	-0.3093 to 0.2747	0.8985
Direct Bilirubin	-0.4443	-0.6622 to -0.1571	0.0028*
GOT	0.1060	-0.1800 to 0.3754	0.4546
GPT	0.4558	0.1950 to 0.6563	0.0009*
CRP	-0.1143	-0.3878 to 0.1777	0.4292

Note: a - correlation coefficient; b - significance criterion * - significant values where $p \leq 0.05$

CRP is an acute phase reactant, a protein synthesized and secreted by the liver in response to inflammatory cytokines, specifically IL-6 (Satar and Özlü, 2012) and is commonly used for bacterial sepsis detection in neonates. Still it is not useful as an early phase infection marker and it lacks specificity (Ng and Lam, 2006). All neonates in our study had a high CRP level, the mean being 8.21 mg/dl. In their study, Zhou et al. (2016) have found a CRP level >0.8 mg/dl in neonates (39.1%) with positive blood culture results and 45.3% of them died within 7 days after birth, a higher prevalence than us (20.96%) (Zhou et al., 2016). Also, Mannan et al. (2010) found that CRP was raised in 72% of cases of neonates with positive blood culture and only in 4% of control cases, and their study concluded that CRP is the most sensitive method (93%) in culture proven sepsis, 79% in suspected sepsis and its positive predictive value in suspected sepsis amounts to 88%.

Hofer et al. (2012) found that a growing body of evidence suggests a link between GA and CRP kinetics with lower baseline CRP values and a lower CRP response to infection in preterm compared to term newborns. All correlations between all independent variables that we studied (1 and 5 min Apgar score, GA, BW) and CRP are negatively correlated, a high value of independent variable is associated with a low CRP value, but it is not statistically significant. Hofer et al. (2012) conclude that CRP has the best diagnostic accuracy when combined with another infection marker like PCT, IL-6, and IL-8, that provides a higher sensitivity during the early phases of sepsis.

The gold standard for diagnostic sepsis is BC but the CRP is also particularly useful for monitoring the response to treatment and guiding antibiotic therapy. The highest level of CRP concentrations is detected during the first day of illness but because sustained pro-inflammatory action of IL-6, production could be detected until 24 hours after treatment was started. In their study, Janković et al. (2001) found that in the case of non-adequate initial antibiotic therapy of neonatal sepsis, CRP level increases further during the second day, but if the treatment is appropriate in the second day there is a significant decrease of CRP levels. CPR level can be taken as indication for replacement of initial antibiotics during the second day of treatment of sepsis neonates. The pathogens that are involved in neonatal sepsis are different depending on the type of neonatal sepsis, EOS or LOS, and the country's degree of development. Organisms associated with EOS are Group B *Streptococcus* (GBS, in special *Streptococcus agalactiae*), *Escherichia coli* (*E. coli*) which together account for about 70% of cases, and *Streptococcus viridans*. In LOS, organisms associated are CoNS, *Staphylococcus aureus*, *Candida albicans* and *Klebsiella pneumoniae* (Shah and Padbury, 2014; Cortese et al., 2016; Resende et al., 2015). In developed countries, in EOS are dominant GBS and E. coli, and in LOS are CoNS and GBS followed by *Staphylococcus aureus* (Hyde et al., 2002; Vergnano et al., 2005). In developing countries, the pathogens associated with EOS are *E. coli*, GBS, *Enterobacter*, *Enterococcus*, *Listeria* and with LOS *Pseudomonas* spp., *Salmonella*, *Seratia*. On both, EOS and LOS, are more associated *Klebsiella*, *Acinetobacter*, *Staphylococcus aureus* and also CoNS (Vergnano et al., 2005).

The appropriate empirical antibiotic selection during neonatal sepsis is based on the likely etiologic pathogens based on epidemiologic surveillance. Cortese et al. (2016) found that for EOS, the recommended empiric therapy as 1st line is Ampicillin and an Aminoglycoside, and for LOS is Vancomycin and an Aminoglycoside. Also in our study the most used antibiotic was Aminoglycoside followed by Penicillin but for both of type of sepsis.

Conclusions

There is a statistically significant negative correlation between 1 and 5 min Apgar score and creatinine, between Gestational Age and urea, and also between Birth Weight, Gestational Age and Direct Bilirubin. The statistically significant positive correlation is between Birth Weight, Gestational Age and alanine aminotransferase.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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