Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Can Base Excess be Used for Prediction to Early Diagnosis of Neonatal Sepsis in Preterm Newborns?

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Competing interests: The authors have declared that no competing interests exist.

Abstract. ***Background:*** **Neonatal sepsis remains an important and potentially life-threatening clinical syndrome and a major cause of neonatal mortality and morbidity. The aim of this study to investigate whether values of base excess before the onset of clinical signs and symptoms of sepsis indicate infection in the early diagnosis of neonatal sepsis.**
Methods: **In this study, a total of 118 infants were enrolled. The infants were classified into two groups: group 1 (sepsis, n=49) and group 2 (control, n=69). Blood gas analysis investigated for the screening of neonatal sepsis.**
Results: **A total of 49 newborns with neonatal sepsis and 69 healthy controls were enrolled. Comparison of markers of sepsis revealed C-reactive protein, interleukin-6 level to be significantly higher and pH, pCO_2, HCO_3, and base excess values to be significantly lower in newborns with sepsis compared healthy controls ($p<0.01$). The optimum cut-off value in the diagnosis of neonatal sepsis was found to be -5 mmol/L for base excess. Sensitivity, specificity, positive predictive value and negative predictive value of this base excess cut-off for neonatal sepsis were 75, 91, 86 and 84% respectively.**
Conclusion: **This is the first study to determine the relationship between the decreased value of the base excess and early stage of neonatal sepsis. If the value of base excess <-5 mmol/L without an underlying another reason, may need close follow up of infants for neonatal sepsis and it may help early diagnosis.**

Keywords: Base excess; Newborn; Sepsis.

Citation: Arayici S., Kadioglu Simsek G., Canpolat F.E., Oncel M.Y., Uraş N., Oguz S.S. Can base excess be used for prediction to early diagnosis of neonatal sepsis in preterm newborns? Mediterr J Hematol Infect Dis 2019, 11(1): e2019014, DOI: http://dx.doi.org/10.4084/MJHID.2019.014

Published: March 1, 2019 **Received: August 16, 2018** **Accepted: January 19, 2019**

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Introduction. Neonatal sepsis (NS) remains an important and potentially life-threatening clinical syndrome and a major cause of neonatal mortality and morbidity, particularly in preterm infants. The risk of sepsis increases with decreasing birth weight and gestational age.[1,2] Early diagnosis and adequate antibiotic treatment are required because of the high rates of mortality and morbidity. The spectrum of NS symptoms in preterm infants ranges from nonspecific or subtle findings to fulminant septic shock. Due to subtle or nonspecific signs and symptoms, NS is difficult to diagnose in the early period.[2,3]

Additionally, clinical signs associated with normal physiological disturbances and those of sepsis can overlap. Diagnosis is made by clinical and laboratory findings. Blood culture is the gold standard laboratory technique for diagnosis, but results may take 48-72 h, and false-negative results may occur. Several markers

such as C-reactive protein (CRP), interleukins (ILs), procalcitonin (PCT), and immunoglobulins, have been used to diagnose sepsis.[4,5] However, there is no suitable marker for diagnosis of NS, particularly in the early period.

Sepsis is associated with many clinical features, including acidosis. Metabolic acidosis results from a variety of common etiologies, including lactic acidosis, hyperchloremic acidosis, renal failure, and ketoacidosis. Anaerobic respiration begins, and metabolic acidosis develops when an imbalance between oxygen supply and demand. Acidosis can be determined from direct blood gas analysis by examining base excess. Although there are a variety of other causes of metabolic acidosis, the early identification of those infants with tissue dysoxia may facilitate the early diagnosis of NS.[6-10]

This study aimed to investigate whether base excess values before the onset of clinical signs and symptoms of sepsis indicate infection in the early diagnosis of NS.

Materials and Methods. The study was conducted in the NICU at Zekai Tahir Burak Maternity Teaching Hospital, Turkey. This unit has 150 incubators and serves as a referral Level III NICU, with approximately 3000 newborn admissions per year. This single-center, retrospective study was conducted between June 2013 and November 2013 after approval from the local ethics committee.

Participants and definitions. Cases with a gestational age ≤ 32 weeks and/or a birth weight ≤ 1,250 g were included in the study.

A diagnosis of clinical sepsis required the presence of at least three of the following: bradycardia (<100/min), tachycardia (>200/min), hypotension, hypotonia, seizure, apnea, tachypnea, cyanosis, respiratory distress, poor skin color and perfusion, feeding difficulty, irritability, and lethargy in addition to laboratory results showing elevated levels of CRP or interleukin-6 (IL-6). Late-onset sepsis (LOS) was defined as sepsis that occurred after the first 72 h of age. Patients with culture positivity were considered to have proven sepsis.[11]

Patients with LOS (group 1) were further divided into two subgroups based on whether they had proven (group 1a: newborns with positive blood cultures, clinical findings in agreement with the diagnosis, and elevated IL-6 and/or CRP levels during the clinical course) or clinical sepsis (group 1b: newborns with clinical findings of infection, plus a significant rise in IL-6 and/or CRP levels during the clinical course, but with negative blood cultures). The control group (group 2) consisted of healthy newborns without sepsis. Infants in the control group had normal physical examination findings and were matched as much as possible in demographic characteristics to those in the proven and clinical sepsis groups.

Methods. Blood gas analysis values in the sepsis group taken 12-24 h before the onset of signs and symptoms of sepsis were evaluated. Hemodynamic findings (heart rate, mean arterial pressure, urine output), actual weight, serum sodium level, and total fluid intake were recorded simultaneously. Hematocrit, complete white blood count (WBC), platelet, CRP, and IL-6 levels as well as blood cultures, which were performed after the appearance of symptoms and signs of sepsis, were also recorded.

Blood gas analysis values and hemodynamic and laboratory findings were recorded at similar times in the control group and compared with those in the sepsis group.

Exclusion criteria were defined as congenital heart disease, heart failure, renal failure, inborn errors of metabolism, chromosomal aberrations, patients with respiratory acidosis, and the presence of definite causes resulting in lactic acidosis such as seizure.

Blood samples for culture were taken from patients with a diagnosis of sepsis before antibiotic therapy. Urine and cerebrospinal fluid cultures were taken when clinically indicated. Blood culture was not taken from healthy controls. The Bactec microbial detection system (Becton-Dickinson, Sparks, MD) was used to detect positive blood cultures. Two-blood culture positivity was required to confirm *Staphylococcus epidermidis* sepsis.

All capillary blood samples were analyzed using in a RAPIDlab 1265 (Siemens Diagnostic Product Corporation, Los Angeles, CA) blood gas analyzer.

Serum concentrations of CRP were measured by a Tinaquant CRP (Latex) high sensitive immune turbidimetric assay on a Roche Modular P analyzer (Roche kit, Roche Diagnostics, Mannheim, Germany) according to manufacturer instructions. Plasma levels of IL-6 were determined by IL-6 solid phase, enzyme labeled, chemiluminescent sequential immunometric assay on an IMMULITE 2000 analyzer (Siemens Diagnostic Product Corporation, Los Angeles, CA) as per manufacturer instructions.

Statistical Analyses. Statistical analyses were performed using the SPSS software version 20. Categorical variables between groups were analyzed using the chi-squared test. Comparison of mean between two groups was examined using a t-test where the data fit a normal distribution, and the Mann–Whitney U test where the data was non-normal. ROC analysis was used to determine the power of variables to differentiate groups, and the area under the curve (AUC) was calculated; significant cut-off levels were calculated using a Youden index. A *p*-value of less than 0.05 was considered indicative of statistical significance.

Results. The study group included 118 patients, 49 with sepsis and 69 controls. The demographic characteristics of the study population are summarized in **Table 1**. Gestational age, birth weight, gender, and mode of delivery were similar in groups 1 and 2 ($p > 0.05$).

The most frequently isolated microorganisms were *Staphylococcus epidermidis* (50%), *Staphylococcus aureus* (12%), *Enterobacter cloacae* (10%), *Klebsiella pneumoniae* (6%), *Escherichia coli* (6%), *Pseudomonas aeruginosa (2%)*.

Table 2 shows the hemodynamic and metabolic status of the infants in each group. No significant differences in hemodynamic findings, total fluid intake, serum sodium levels, hematocrit, or platelet levels were observed between the sepsis and control groups ($p > 0.05$). However, significant differences were observed for WBC, pH, HCO_3, base excess, CRP, and IL-6 levels between the sepsis and control groups ($p < 0.05$).

No significant differences were noted between the proven and clinical sepsis subgroups in any of the laboratory parameters ($p > 0.05$). No significant difference in any variable was noted between infants with gram-negative and -positive culture positivity in the proven sepsis group ($p > 0.05$).

The optimal cut-off levels for base excess between the sepsis and control groups and between the sepsis subgroups and the control group were calculated by drawing receiver operating characteristic curves. **Table 3** shows the cut-off levels. The sepsis group and sepsis subgroups had similar cutoff levels vs. the control group. The optimal base excess cut-off level between groups 1 and 2 was −5 mmol/L. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) values for base excess were 75, 91, 86, and 84%, respectively (**Table 3**).

Table 1. Demographic Characteristics of Sepsis and Control Groups.

	Group 1 (n=49)	Group 2 (n=69)	*P*
Gestational age, weeks[a]	27.6 ± 1.9	28.2 ± 1.7	0.09
Birthweight, g[a]	993 ± 160	1007 ± 145	0.62
Male[b]	28 (56)	34 (49)	0.45
Caesarean section[b]	33 (66)	45 (65)	0.84
Apgar score (1 min)[c]	5 (3-7)	6 (3-7)	0.16
Apgar score (5 min)[c]	8 (5-9)	8 (5-9)	0.57
Antenatal steroid use[b]	43 (86)	58 (84)	0.60

[a]Mean ± SD, [b]*n* (%), [c]Median (minimum-maximum).

Table 2. Hemodynamic and Laboratory Findings of Sepsis and Control Groups.

	Group 1 (n=49)	Group 2 (n=69)	*P*
Blood gas analysis work-up day[b]	15.4 (7-30)	17.5 (5-30)	0.11
Heart rate (beats/min)[a]	146 ± 7	144 ± 6	0.22
Mean arterial pressure (mmHg)[b]	50 (35-56)	50 (33-60)	0.14
Na (mEq/L)[a]	137.7 ± 4.5	136.7 ± 3.1	0.13
Fluid (mg/kg/d)[b]	148 (130-180)	151 (120-180)	0.20
Urine output (ml/kg/h)[b]	3.2 (1.8-5.1)	3.1 (2.1-4.9)	0.98
Hematocrit (%)[b]	39.5 (28.5-52)	40.1 (30-59)	0.65
White blood cell ($x10^9$/L)[a]	15,7 ± 7,5	11,6 ± 4	0.006
Platelet ($x10^9$/L)[a]	245 ± 147	275 ± 102	0.31
pH[a]	7.27 ± 0.06	7.32 ± 0.04	<0.001
pCO2 (mmHg)[a]	42.4 ± 9.6	46.4 ± 6.8	0.009
HCO3 (mmol/L)[a]	19.4 ± 3.4	23.9 ± 3	<0.001
Base excess (mmol/L)[a]	-6.6 ± 3.9	-1.6 ± 2.6	<0.001
CRP (mg/l)[b]	54.9 (29-328)	1.2 (0.1-11)	<0.001
IL-6 (pg/ml)[b]	367 (5.4-1000)	6.3 (2-24)	<0.001

Mean ± SD, [b]Median (minimum-maximum)

Table 3. Sensitivity, Specificity, PPV, NPV, and Area under the ROC Curve for Base Excess at the Optimal Cut-off Levels Between Groups 1 and 2.

	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
Base excess	< -5	75	91	86	84	88

AUC, area under curve, NPV, negative predictive value; PPV, positive predictive value.

Discussion. Bacterial sepsis is an important problem in very low birth weight (VLBW) infants despite advances in neonatal intensive care and continues to be an important cause of morbidity and mortality.[1-3] Blood culture is the gold standard for diagnosing NS; however, 48-72 h are required to obtain results. It is important to identify infected neonates as early as possible, but the nonspecific clinical signs and the absence of good diagnostic tests are obstacles to an early diagnosis. Diagnostic markers are useful indicators of NS. Serial measurements of infection markers can improve diagnostic sensitivity, and the use of multiple markers can enhance diagnostic accuracy. Many studies have evaluated various markers.[12,13] However, sensitivity and specificity of hematological criteria such as absolute neutrophil count, platelet counts, and immature to total neutrophil ratio vary widely among studies.[4,14-16] Celik et al.[17] reported the sensitivity, specificity, PPV, and NPV for CRP of 67, 97, 99, and 39% respectively, and for IL-6 of 72, 84, 95, and 42%, respectively, with cut-off values of 4.82 mg/l and 24.65 pg/ml. Elawady et al.[18] reported a sensitivity of 96%, specificity of 100%, PPV of 96.2%, and NPV of 100% for neutrophil CD64, with cut-off values of 45.8% and 46.0% in proven and clinical sepsis groups, respectively. Dilli et al.[19] reported a sensitivity of 88.6% and NPV of 94%. Cetinkaya et al.[20] found sensitivities for CRP, PCT, and serum amyloid A of 72.3, 74.8, and 76.4%, respectively. Abdollahi et al.[21] reported the simultaneous measurement of PCT, IL-6 and high sensitive-CRP (hs-CRP) is more sensitive in the diagnosis of neonate infections. They found that the combination of PCT and IL-6 had a sensitivity of 88%, PCT and hs-CRP had a sensitivity of 82%. Patel et al.[22] evaluated the role of serum PCT as a biomarker of bacterial infection in acute sickle cell vaso-occlusive crisis and they found that PCT value of >2ng/mL is indicative of bacterial infection necessitating early antimicrobial therapy. Presepsin, is another biomarker of sepsis, was also studied in the diagnosis of NS; Poggi et al.[23] reported that its sensitivity, specificity, and AUC were 94%, 100%, and 0.972 respectively. Ozdemir AA et al.[24] evaluated the efficacy of presepsin in the diagnosis of early onset NS by comparing this with CPR and PCT. They found that CRP, PCT, and presepsin had a sensitivity of 83, 67, and 80%, and a specificity of 75, 67, 75%, respectively, with cut-off values for presepsin of 539 pg/mL with an AUC of 0.772. Recently, Bellos et al.[25] reported a meta-analysis about the diagnostic accuracy of presepsin in NS. The findings of this meta-analysis suggest that presepsin may serve as a promising biomarker in NS, given its sensitivity 0.91, specificity 0.97 (AUC 0.99). Our findings indicate that base excess values decreased before the emergence of signs and symptoms of sepsis. We found that the sensitivity, specificity, PPV, and NPV values for base excess were 75, 91, 86, and 84%, respectively, with a cut-off value of −5 mmol/L. We found no differences between the proven and clinical sepsis and the gram-positive and -negative sepsis subgroups. Base excess to predict sepsis in preterm newborns has not been reported. This study is the first to show the base excess levels as evaluative of early diagnosis of NS.

The development of metabolic acidosis during NS has been attributed to progressive tissue ischemia resulting from reduced oxygen delivery. Sepsis causes hemodynamic instability through several processes, resulting in tissue hypoperfusion. Some studies have shown that base excess is a prognostic factor in patients who develop sepsis. Acidosis is a powerful marker of poor prognosis in critically ill patients.[6-10,26] However, base excess for diagnosis of sepsis has not been investigated previously.

Several routine blood gas analysis strategies are used in neonatal intensive care units, and, so, an evaluation of blood gases should be made every morning or at round visits. Blood gas analysis is performed using 0.2 ml of blood, which is also used for respiratory function analysis, particularly in ventilated newborns. Several sepsis biomarkers have been used for the early diagnosis of sepsis, but the use of more than two markers simultaneously in the same infant is not possible due to excessive blood loss. Therefore analysis of blood gases for the diagnosis of sepsis may be a useful, simple and cost-effective method.

Our study had several limitations due to its retrospective and observational nature. Additionally, serum lactate levels were not evaluated to confirm the diagnosis of acidosis.

Conclusion. The results of this trial suggest that base excess < −5 mmol/L has 75% predictability for diagnosing NS. Therefore, NS could be predicted through a simple capillary blood gas analysis test. The clinician may be alerted to an earlier evaluation for possible neonatal infection prior to the development of sepsis. Capillary blood gas analysis is a method commonly practiced in neonatal intensive care units. Because the analysis requires a small amount of blood, it is superior to other laboratory tests. If the base excess

value is < −5 mmol/L with no known underlying reason, close follow-up of the infant for NS may be needed for an early diagnosis. Our findings should be confirmed by more comprehensive controlled and prospective trials.

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