Understanding Risk Factors, Clinical Presentation, and Biomarkers in Proven and Suspected Neonatal Sepsis at the Children's Welfare Teaching Hospital, Iraq

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Abstract

Background: Sepsis is recognized as the leading cause of neonatal mortality globally, yet its detection remains challenging due to inconclusive signs and symptoms.

Objective: This study aimed to examine the variations in complete blood count (CBC), D-dimer, ferritin, interleukin-10, urea, and electrolyte levels among neonates with sepsis compared to healthy newborns and to identify and characterize the associated symptoms and risk factors.

Methods: An observational study was conducted at the Children's Welfare Teaching Hospital in Baghdad during the period from first of September 2023 to first of March 2024, the research involved a total of 187 neonates, including 137 neonates exhibiting clinical signs of sepsis and positive cultures, proven and suspected (case group) and 50 healthy neonates without medical condition, screened by a pediatric specialist (control group). Data collection encompassed demographic details, risk factors, and symptoms through questionnaires, followed by CBC, electrolyte, urea, D-dimer, IL-10, and ferritin assessments for all participants.

Results: The total number of patients was 137, including both proven and suspected sepsis cases. The study revealed that 67 (48.91%) were male and 70 (51.14%) were female, including both early and late onset sepsis cases. The mode of delivery showed that 102 (76.12%) of 134 patients were via cesarean section (CS) and 32 (23.88%) via normal vaginal delivery (NVD). Significant differences were identified between the case and control groups in red cell distribution width (RDW), white blood cell count (WBC), and mean corpuscular volume (MCV). The lymphocyte percentage was notably lower in the case group. Hyperkalemia emerged as the most prevalent electrolyte imbalance. Additionally, urea levels were significantly elevated in the case group. Ferritin and D-dimer concentrations were markedly higher in the sepsis group, and the median IL-10 levels were significantly raised.

Conclusions: The findings highlighted possible associations between NS, clinical symptoms, and maternal and neonatal risk factors. Noteworthy observations included elevated WBC, MCV, RDW, hyperkalemia, and urea levels, along with reduced lymphocyte percentages, in sepsis cases. Elevated D-dimer and ferritin levels, as well as increased IL-10 concentrations, were also significant markers. These parameters may assist in the prediction and monitoring of neonatal sepsis.

Keywords: D-dimer; Electrolytes; Ferritin; Risk factors; Sepsis

Introduction

Neonatal sepsis (NS) is a clinical syndrome that commonly occurs within the first month of life. It arises when pathogenic microorganisms—such as viruses, bacteria, or fungi—invade normally sterile fluids, including blood or cerebrospinal fluid (CSF), resulting in hemodynamic abnormalities and systemic clinical manifestations (1). Globally, sepsis is one of the leading causes of disease and death across all ages. Numerous studies indicate that sepsis accounts for approximately 203,000 neonatal deaths annually, positioning it as the third leading cause of neonatal mortality. Accurate and effective detection methods are crucial for patient survival (2,3,4). NS manifests with a wide range of nonspecific signs and symptoms. Initial indicators often include fever or

hypothermia, abnormal heart rate, poor feeding with a tendency to vomit post-feeding, and lethargy. Jaundice, characterized by yellowing of the eyes and skin, may also be present. Other symptoms include thrombocytopenia, hypotension, bulging fontanel, and respiratory distress (3,5).

NS is classified into three categories: early-onset sepsis (EOS), late-onset sepsis (LOS), and very late-onset sepsis (VLOS). EOS typically occurs within the first 72 hours of life, although some scientists suggest defining early onset as within the first 24 hours or up to 7 days. LOS occurs after 72 hours of birth, and some studies suggest it may occur after the first seven days of life or between the fourth and thirty days of life, and VLOS refers to cases identified in neonates admitted to the NICU within the first 30 days of life until discharge. Sepsis can be diagnosed as proven sepsis when clinical signs and laboratory findings are

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present, along with the identification of a pathogenic microorganism in cultures obtained from sterile fluids. and it is categorized as suspected sepsis when clinical and laboratory findings are present, but there is no identification of the causative microorganism [6]. The severity and progression of NS are influenced by various factors, including the timing of exposure, the size of the inoculum, host immunity, and the virulence of the infectious agent. Infection can be acquired during pregnancy, via the mother's vaginal tract, or post-delivery from the community or hospital environment and personnel (7). Several risk factors are associated with an increased incidence of neonatal sepsis, potentially elevating mortality rates. These factors include preterm delivery, maternal infections, prolonged rupture of membranes (PROM), type of delivery, and resuscitation at birth (8,9).

The aim of this study was to determine the patterns of complete blood count (CBC), serum electrolytes, D-dimer, ferritin, interleukin-10 (IL-10), and kidney function parameters in neonates with sepsis compared to healthy neonates. Additionally, the study sought to identify risk factors associated with sepsis and characterize its clinical manifestations. The ultimate goal was to explore the potential of these characteristics as reliable biomarkers for the early detection of neonatal sepsis.

Patients and Methods:
This observational study was conducted among neonates suspected of or confirmed to have sepsis at Children's Welfare Teaching Hospital, Medical City, Baghdad, Iraq, from the first of September 2023 to the first of March 2024. The study involved a total of 187 newborns, 137 of them exhibiting clinical signs of sepsis (case group) and 50 healthy neonates without neonatal sepsis (control group). Hematological criteria, along with clinical signs and symptoms, were used to identify sepsis in neonates. Suspected cases were identified based on clinical symptoms and physician diagnosis, while confirmed cases were identified through positive (blood, cerebrospinal fluid) CSF), and urine culture results. The data collected included patient demographics such as age in days, gender, weight in kilograms, gestational status (term or preterm), twin status, and symptoms including fever, lethargy, poor feeding, vomiting, and diarrhea. Maternal data collected included pregnancy-related diseases (such as hypertension, fever, prolonged rupture of membranes (PROM), and urinary tract infection (UTI)), and mode of delivery (normal vaginal delivery (NVD) or cesarean section (C/S)). Data for all patients were collected from the mothers, along with complete physical examinations and laboratory investigations. For the complete blood count (CBC), approximately 1 ml of venous blood was collected from all neonates in an EDTA tube (Hematological analyzer counter, Swelab, Sweden). For other tests, including serum electrolytes (electrolyte analyzer, Exias, Austria), blood urea (ELITech group kits, France), ferritin (Tosoh, Japan), and IL-10 levels (measured using

ELISA, Human IL-10 kit, Transgen Biotech Company, China), approximately 2 ml of venous blood was collected in a gel tube. Centrifugation was used to separate the serum, which was then stored in Eppendorf tubes in a freezer at -20˚C until analysis. Additionally, 2 ml of venous blood was collected in a sodium citrate tube for the D-dimer test (Maglumi D-dimer kit, China).

Inclusion Criteria: The inclusion criteria for the case group were based on clinical findings and laboratory tests conducted by pediatric specialists, confirming (proven sepsis) through positive blood, CSF, or urine cultures, or suggesting (suspected sepsis) the presence of neonatal sepsis. Prior to sample collection, all newborns in the suspected sepsis group were not treated with antibiotics. However, the majority of neonates in the proven sepsis group received antibiotics as part of their treatment. Neonates without clinical symptoms of infection and who tested negative for sepsis markers (CBC, CRP) met the inclusion criteria for the control group.

Exclusion Criteria: Neonates with heart diseases, chromosomal abnormalities, genetic syndromes, or those who had undergone surgery were excluded from both the case and control groups.

Statistical Analysis
The Statistical Analysis System (SAS) version 9.4 (2018) was used to analyze the data and assess differences between the patient and control groups in study parameters. A t-test was used to compare means significantly, while a chi-square test was used to compare the significance between percentages at 0.05 and 0.01 probability levels. A p-value of less than 0.05 was considered statistically significant, while a p-value of less than 0.01 was considered to provide stronger evidence of statistical significance.

Results:
The study involved a total of 187 newborns, with 137 diagnosed with sepsis (cases) and 50 without neonatal sepsis (controls). Among them, 67 (48.91%) were males and 70 (51.14%) were females. Their mean age was 17.47 ± 1.14 days. The mean weight of 126 patients was 2.48 ± 0.07 kg; among these, 54 (42.86%) were preterm, and 72 (57.14%) were term. Regarding multiple births, 134 patients were recorded, with 11 (8.21%) being twins and 123 (91.79%) not ($P \leq 0.01$). Additionally, from 134, 32 (23.88%) were delivered via normal vaginal delivery (NVD), while 102 (76.12%) were delivered by cesarean section (C/S) ($P \leq 0.01$). The characteristics of the neonates studied are illustrated in Table 1.

Table 1: Relationship Between Neonatal Risk Factor Variables and Sepsis

| Variables		Number	Percentage (%)	*p*-value
Gender	Male	67	48.91	0.797
	Female	70	51.09	NS
Twin status	Yes	11	8.21	0.0001
	No	123	91.79	**
NVD-CS	NVD	32	23.88	0.0001
	CS	102	76.12	**
Preterm	Yes	54	42.86	0.108
	No	72	57.14	NS

** (*p*≤0.01), NS: Non-Significant.
**: *p*≤0.01 Statistically significant difference
NS: Statistically Non-Significant difference

A statistically significant correlation (*p*≤ 0.01) was observed between symptoms and neonatal sepsis (NS) as shown in figure (1) The most common symptoms were lethargy, observed in 100 (79.37%) neonates, followed by poor feeding in 94 (74.6%) neonates, and vomiting in 25 (19.84%) neonates. Diarrhea was less prominent, noted in only 6 (5%) neonates, while fever occurred in 18 (14.29%) neonates.

NS showed significant correlations with maternal risk factor variables. The total number of mothers was 53. Among these mothers, 9 (16.98%) experienced high fever during labor, 10 (18.87%) had hypertension during labor, 5 (9.43%) had premature rupture of membranes (PROM), and 15 (28.30%) had urinary tract infections (UTIs) (*p* < 0.01).
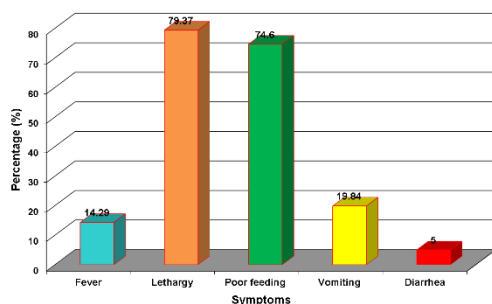
Figure 1: Relationship between Neonatal Symptoms and Neonatal Sepsis

The findings of the complete blood count (CBC) are presented in Table 2. Neonates with sepsis had significantly higher WBC counts (*p*≤0.05(compared to the control group. Conversely, the lymphocyte percentage was significantly lower (*p* ≤ 0.05) in the patient group. The MCV levels were also significantly higher (*p*≤ 0.05) in the patient group. Additionally, the RDW was significantly higher (p ≤ 0.01) in neonates with sepsis compared to the control group. Although the lymphocyte counts and RDW% showed a slight increase in the patients compared to the control group, this difference was not statistically significant. Similarly, RBC, HGB, and PLT counts exhibited a slight decrease in the case group compared to the control group, but these findings were also statistically non-significant.

Table 2: Comparison of CBC Values Between the Patient and Control Groups

Parameters	Mean ±SE		T-test (*p*-value)	
	Patients	Control		
WBC (x10^3)	14.46 ±0.78	9.21 ±0.59	4.76 (0.0312)	*
Lymphocyte (No)	4.79 ±0.25	4.05 ±0.37	1.53 (0.338)	NS
Lymphocyte (%)	36.30 ±1.31	45.32 ±4.17	8.38 (0.0352)	*
RCB (x10^6)	3.90 ±0.08	4.25 ±0.14	0.490 (0.151)	NS(
HGB (g/dl)	13.14 ±0.29	13.72 ±0.59	1.82 (0.533)	NS
MCV	99.98 ±0.75	94.26 ±3.41	5.05 (0.0268)	*
RDWa	77.93 ±1.39	58.25 ±3.81	8.79 (0.0001)	**
RDW (%)	16.18 ±0.14	15.37 ±0.50	0.920 (0.0859)	NS
PLT	292.93 ±17.19	295.83 ±32.31	106.32 (0.956)	NS

* (*p*≤0.05), ** (*p*≤0.01) , NS: Non-Significant.
**: *p*≤0.01 Statistically significant difference
*: *p*≤ 0.05 Statistically significant difference
NS: Statistically Non-Significant difference

Newborns with sepsis showed electrolyte abnormalities, with median sodium and chloride concentrations of 138.09 ± 1.02 mmol/L and 104.11 ± 2.16 mmol/L, respectively. The mean concentrations of potassium and calcium were 10.01 ± 3.06 mmol/L and 6.01 ± 1.95 mg/dL, respectively. Hyperkalemia was the most common electrolyte disorder (*p* ≤ 0.01), followed by hypercalcemia and hypocalcemia. However, no statistically significant correlation was found between the increase and decrease of calcium levels, although there may be a clinical association between these conditions and the occurrence of sepsis compared to the control group with normal electrolyte levels, as presented in Table 3.

Table 3: Comparison of Electrolyte levels in patients and control groups

Group	Means ±SE				
	S. Electrolyte/ Ph	S. Electrolyte. Ca (mg/dl)	S. Electrolyte. Cl (mmol/l)	S. Electrolyte. K (mmol/l)	S. Electrolyte. Na (mmol/l)
Patients	7.34 ±0.13	6.01 ±1.95	104.11 ±2.16	10.01 ±3.06	138.09 ±1.02
Control	7.39 ±0.02	4.72 ±0.08	102.04 ±0.67	4.37 ±0.15	140.34 ±0.89
T-test	0.562 NS	1.674 NS	7.957 NS	2.574 **	7.021 NS
p-value	0.902	0.152	0.895	0.0083	0.904

** (*P*≤0.01), NS: Non-Significant.
**: *p*≤0.01 Statistically significant difference
NS: Statistically Non-Significant difference

As shown in Table 4, the sepsis group of neonates had significantly higher ferritin and D-dimer levels than the control group (*p* < 0.01).

Table-4: Comparison of D-dimer and ferritin levels between patient and control groups

Group	Mean ±SE of D-dimer (FEU/ml)	Mean ±SE of Ferritin (ng/dl)
Patients	1.968 ±0.20	345.42 ±29.41
Control	0.527 ±0.15	95.72 ±29.34
T-test	0.812 **	123.05 **
p-value	0.0008	0.0064
** ($p \leq 0.01$).		

**: $p \leq 0.01$ Statistically significant difference

The mean blood urea level was 29.01 ± 2.59 ($p \leq 0.05$). The urea levels were significantly higher in the case group compared to the control group.

The median concentration of IL-10 was elevated in septic newborns 52.81 ±7.47 compared to non-septic newborns 25.98 ±4.22, and this difference was statistically significant ($p < 0.05$).

Discussion:

NS is a significant global health concern. A study at the Central Teaching Pediatric Hospital in Baghdad identified sepsis as one of the three leading causes of admission to the Neonatal Intensive Care Unit (NICU) [10]. In our study, we observed no association between gender and sepsis, which aligns with the findings of Almaroof et al. [11]. However, Salama and Tharwat found that NS is more common in males than in females [12]. We also noted that sepsis is more prevalent in twin babies, consistent with previous research [13].

Utomo et al. showed that infants delivered by cesarean section (C/S) had a higher risk of early-onset sepsis (EOS) compared to those born vaginally. This may be due to C/S performed for non-medical reasons or the risk of lacerations during C/S [14].

This study results indicated that prematurity had no significant effect on the development of NS. Although most infants in this study were term births, a considerable proportion were preterm. While prematurity was not statistically significant, it may have clinical relevance. Nurrosyida et al. found prematurity to be a significant risk factor for NS, although not an independent one (15). Conversely, Alsaqee and other studies indicated that preterm infants significantly contribute to NS (16). The development of sepsis was notably impacted by prolonged rupture of membranes (PROM). Shifera et al. found that the risk of sepsis was nearly five times higher in newborns whose mothers had PROM (17). This is in line with this study, which demonstrates a substantial correlation between PROM and sepsis.

This study also showed that maternal UTIs significantly increased the risk of NS. Infants born to mothers with UTIs were more likely to develop sepsis, consistent with studies by Shifera et al. and Bayih et al. Untreated UTIs can lead to colonization of the birth canal with infectious microbes, raising the risk of sepsis during delivery. NS was associated with maternal intrapartum fever as a risk factor. This study demonstrated that neonates born to mothers with fever during labor were more likely to develop sepsis, consistent with other studies (17,18).

A history of maternal hypertension during pregnancy was identified as a risk factor for NS. Neonates born to hypertensive mothers had a higher likelihood of developing sepsis, aligning with Okoye et al. study. This increased risk may be due to fetal hypoxia in pregnancies complicated by hypertension [19]. Based on the findings of this study, factors such as twin status, mode of delivery (particularly C/S), maternal conditions including PROM, UTI, fever, and hypertension are all associated with the occurrence of neonatal sepsis. These factors can be considered as key risk factors for predicting both the onset and the severity of the condition.

Lethargy, poor feeding, fever, vomiting, and diarrhea were significant clinical symptoms associated with NS. These findings are consistent with Hayes et al. (20).

The values of RDW, WBC, and MCV were significantly higher in the sepsis group compared to the control group, consistent with Regassa et al. and Mahmoud et al. findings. Leukocytosis was a notable factor in NS diagnosis, although Worku et al. reported that leukopenia was more frequently associated with NS (21, 22, 23). Lymphocyte counts in newborns with sepsis were significantly lower than those in the control group, reflecting natural immune responses, which is characterized by elevated neutrophil counts and decreased lymphocyte counts. These findings are in agreement with those of Al Nady et al. (24).

Studies have suggested that increased RDW values can serve as independent prognostic markers in septic shock or severe sepsis. These findings, consistent with Salim et al. (25) showed higher RDW in septic patients, indicating oxidative stress and inflammation during sepsis (26).

Hyperkalemia was the most common electrolyte disorder in septic newborns, followed by hypercalcemia and hypocalcemia. This aligns with Ahmad et al.'s study (27). Hyperkalemia may result from various causes, including hemolysis during sample collection and metabolic acidosis in sepsis-affected infants.

The urea level was significantly higher in the sepsis group, which is consistent with Li et al. and Iqtidar et al. (28, 29). Urea is an independent risk factor for NS and correlates with infection markers like PCT and CRP

The study group had significant higher serum D-dimer levels in the sepsis group, consistent with Al-Biltagi et al.'s results (30). Elevated D-dimer levels in patients with late onset sepsis (LOS) may be due to gram-negative bacterial infections and invasive procedures (30,31).

Higher serum ferritin levels were associated with increased organ failure and were found to be predictive of mortality in severe sepsis. This aligns with Himanshu et al. and Sarkar et al.'s findings (32, 33). Elevated ferritin levels are associated with various inflammatory disorders, including sepsis and multiorgan dysfunction syndrome (MODS) . Serum ferritin can indicate disease severity and predict prognosis in severe illness.

IL-10 is crucial for early sepsis identification in neonates, generated by various immune response cells. This findings, consistent with Tosson et al. ,and Leal et al. showed elevated blood IL-10 levels in septic neonates compared to controls (34,35).

Limitations:
The relatively small sample size may limit the generalizability of the findings, as a larger sample could provide more comprehensive data. Additionally, variations in treatment protocols and management techniques throughout institutions may have contributed to differences in results. Despite efforts to standardize diagnostic criteria and therapeutic modalities, these factors could still have affected the results.

Conclusion:
The study findings suggest that clinical manifestations of neonatal sepsis (NS) are correlated with the condition. The occurrence of NS appears to be associated with both maternal and neonatal risk factors. CBC parameters such as WBC, MCV, and RDW were found to be higher in the sepsis group compared to the control group, while lymphocyte percentages were lower in the patient group than in the controls. A correlation between hyperkalemia and sepsis was observed. Elevated levels of urea, ferritin, D-dimer, and IL-10 were also noted in the patient group compared with the control group. These parameters and risk factors could potentially be used to predict the risk and severity of sepsis.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in (Children's Welfare Teaching Hospital/ Medical city /Baghdad) according to the code number (44414) on (21/ 11/ 2023).
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Authors' contributions:
Study conception & design: (Rasha Majid Abdul Amir Alhumairi). Literature search: (Saja Hussam Mohammed Ali). Data acquisition: (Saja Hussam Mohammed Ali). Data analysis & interpretation: (Aamer Jabbar Ali Al Sudani & Saja Hussam Mohammed Ali). Manuscript preparation: (Saja Hussam Mohammed Ali). Manuscript editing & review: (Saja Hussam Mohammed Ali & Rasha Majid Abdul Amir Alhumairi).

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فهم عوامل الخطر ,الاعراض السريرية والمعلمات البيولوجية في الإنتان الوليدي المؤكد والمشتبه به في مستشفى حماية الأطفال التعليمي، العراق

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الملخص:

الخلفية :يُعد الانتان من الأسباب الرئيسية لوفاة حديثي الولادة على مستوى العالم، إلا أن تشخيصه لا يزال صعبًا بسبب الأعراض السريرية غير الواضحة.

الهدف :تهدف هذه الدراسة الى تحديد الاختلافات في تعداد الدم الكامل (CBC) ، الدي-دايمر ، الفريتين، الإنترلوكين-10، واليوريا , بالإضافة الى مستوى الالكتروليتات في حديثي الولادة المصابين بالإنتان بالمقارنة مع حديثي الولادة الاصحاء و للتعرف وتحديد الاعراض وعوامل الخطر المرتبطة

المرضى والمنهجية :دراسة ملاحظة تم إجراؤها في مستشفى حماية الأطفال التعليمي في بغداد, خلال الفترة من الاول من سبتمبر 2023 إلى الاول من مارس 2024،شملت الدراسة اجمالي 187 حديث ولادة , بما في ذلك 137 مولودًا حديثًا يظهرون علامات سريرية للإنتان ونتائج زراعة موجبة ، سواء كان الانتان مؤكد او مشتبه به (مجموعة الحالة) و50 مولودًا سليمًا بدون حالات طبية تم فحصهم بواسطة اختصاصي أطفال (مجموعة الضبط). شمل جمع البيانات التفاصيل الديموغرافية , عوامل الخطر و الاعراض من خلال الاستبيانات , تليها تقييم تعداد الدم الكامل CBC, الالكتروليتات , اليوريا , دي-دايمر, و IL-10, و الفريتين لجميع المشاركين .

النتائج :كان إجمالي عدد المرضى 137 مريضًا، بما في ذلك حالات الانتان المؤكدة والمشتبه بها. كشفت الدراسة أن 67 (48.91%) من المرضى كانوا ذكورًا و 70 (51.14%)كانوا إناثًا، بما في ذلك حالات الانتان المبكر والمتأخر.اظهرت طرق الولادات ان، 102 (76.12%) من 134 مريضا , كانت بواسطة الولادة القيصرية (C/S) و 32 (23.88%) عن طريق الولادة الطبيعية(NVD) . تم العثور على اختلافات ذات دلالة إحصائية بين مجموعة الحالة ومجموعة الضبط في عرض توزيع كريات الدم الحمراء (RDW) ، وعدد خلايا الدم البيضاء(WBC) ، ومتوسط حجم كرية الدم الحمراء (MCV). كان هناك انخفاض ملحوظ في نسبة الخلايا اللمفاوية في مجموعة الحالات .كان فرط البوتاسيوم هو أكثر اضطرابات الإلكتروليتات شيوعًا .علاوة على ذلك، كانت مستويات اليوريا مرتفعة بشكل كبير في مجموعة الحالات .كانت تراكيز الفيريتين ودي-دايمر أعلى بشكل ملحوظ في مجموعة الانتان كما كان تركيز الإنترلوكين-10 (IL-10) مرتفعًا بشكل ملحوظ.

الاستنتاجات: أبرزت النتائج وجود ارتباطات محتملة بين الإنتان الوليدي، والمظاهر السريرية، وعوامل الخطر لدى الأمهات والمواليد. من الملاحظات البارزة زيادة مستويات خلايا الدم البيضاء(WBC) ، ومتوسط حجم الخلية(MCV) ، وعرض توزيع كريات الدم الحمراء(RDW) ، وفرط البوتاسيوم، وزيادة مستويات اليوريا, مع انخفاض النسبة المئوية للخلايا اللمفاوية في حالات الانتان . كما كانت مستويات الفيريتين ودي-دايمر مرتفعة, بالإضافة إلى ذلك، كانت تركيزات الإنترلوكين-10 (IL-10) أعلى في المواليد المصابين بالإنتان. تشير هذه المعايير إلى أنها قد تكون مفيدة في التنبؤ بالإنتان ومراقبته.

الكلمات المفتاحية : الالكتروليتات؛ الانتان؛ دي-دايمر؛ عوامل الخطر؛ فيريتين.