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Faculty of Medicine

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Preslava Radoslavova Gatseva

Nosocomial Infections in Newborns – Incidence, Risk Factors, Etiology, Diagnosis and Outcomes

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Assoc. Prof. Dr. Nikolay Bulgaranov, PhD

Assoc. Prof. Dr. Viktoria Atanasova, PhD

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The scientific jury was appointed by Order № 1823/01.07.2025 of the Rector of MU – Pleven and consists of:

Internal members (MU – Pleven):

Assoc. Prof. Dr. Chaika Kirilova Petrova, PhD

Assoc. Prof. Dr. Nikola Kalinov Popovski, PhD

Assoc. Prof. Dr. Venetsia Tsvetkova Botsova, PhD (alternate member)

External members (MU – Pleven):

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Assoc. Prof. Dr. Lilia Georgieva Vakrilova, DSc

Assoc. Prof. Dr. Ralitsa Wilson Georgieva, PhD

Prof. Dr. Hristo Nikolov Mumdzhiev, PhD (alternate member)

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List of Abbreviations

- **ABUTI** – asymptomatic bacteriemic urinary tract infection

- **ASB** – asymptomatic bacteriuria
- **AUC** – area under the curve
- **BSI** – bloodstream infection
- **CD** – cluster of differentiation
- **CDC** – Centers for Disease Control and Prevention
- **cfu** – colony-forming unit
- **CLABSI** – central line-associated bloodstream infection
- **CoNS** – coagulase-negative staphylococci
- **CRBSI** – catheter-related bloodstream infection
- **CRI** – catheter-related infection
- **CRP** – C-reactive protein
- **CSEP** – clinical sepsis
- **CVC** – central venous catheter
- **EOS** – early-onset sepsis
- **ESM-1** – endocan
- **FIP** – focal intestinal perforation
- **HCAIs** – healthcare-associated infections
- **HRC** – heart rate characteristics
- **IL** – interleukin
- **I:T index / I/T ratio** – immature-to-total neutrophil ratio
- **LCBI** – laboratory-confirmed bloodstream infection
- **LOS** – late-onset sepsis
- **NBSI** – nosocomial bloodstream infection
- **NC-CPAP** – nasal cannula – continuous positive airway pressure

- **NPV** – negative predictive value
- **OUT** – other urinary tract infections
- **PBSI** – primary bloodstream infection
- **PCR** – polymerase chain reaction
- **PCT** – procalcitonin
- **PICC line** – peripherally inserted central venous catheter
- **PMN** – polymorphonuclear cells
- **PNU** – pneumonia
- **PPV** – positive predictive value
- **SBSI** – secondary bloodstream infection
- **SGA** – small for gestational age
- **SIRS** – systemic inflammatory response syndrome
- **SSI** – surgical site infection
- **sTNFR** – soluble TNF receptor
- **SUTI** – symptomatic urinary tract infection
- **TNF** – tumor necrosis factor
- **UTI** – urinary tract infection
- **VLBW** – very low birth weight
- **VRE** – vancomycin-resistant enterococci

Introduction

Nosocomial infections, or hospital-acquired infections (HAIs), are infections contracted in various healthcare facilities where patients receive medical care. They typically develop after the 72nd hour of hospitalization and can present with clinical manifestations of varying severity. The immunological characteristics of newborns, particularly preterm infants, combined with invasive diagnostic and therapeutic procedures, make the neonatal intensive care unit (NICU) a high-risk environment for the development of HAIs.

HAIs are becoming an increasingly significant concern due to the growing survival rates of extremely premature infants and the constant advancements in neonatal care. These infections are among the leading causes of neonatal morbidity and mortality, contributing to prolonged hospital stays and increased healthcare costs. Despite being recognized as a critical issue in neonatology for decades, comprehensive data have only recently been documented in the literature.

Overall incidence rates vary widely, ranging from 8.9 to 62 infections per 1,000 patient-days or affecting approximately 6–25% of the NICU population. This considerable variability stems from differences in prevention practices, the lack of unified definitions, the absence of automated infection registries on a national level in many countries, and the lack of international databases that would enable cross-country comparisons.

Effective prevention, early diagnosis, and timely, adequate treatment of HAIs in newborns remain a major challenge for clinicians. This requires an understanding of current pathogen prevalence, patient-related risk factors, and the role of medications and invasive procedures that predispose infants to these infections. Monitoring the incidence and characteristics of HAIs is critical not only at the institutional level but also on a national scale.

Studies indicate that infection rates are generally higher in developing countries, where gram-negative bacteria tend to predominate as causative agents. Early diagnosis remains problematic, as initial clinical symptoms are often nonspecific and overlap with noninfectious conditions. Commonly used hematologic markers

lack sufficient sensitivity and specificity, and microbiological cultures require significant time, delaying treatment decisions. The increasing prevalence of antibiotic resistance among pathogens further complicates management strategies.

Given the scarcity of local studies on neonatal HAIs in Bulgaria and the variability in infection rates due to differing diagnostic and definitional practices, this dissertation focuses on a comprehensive analysis of the issue at the Neonatology Clinic, University Hospital 'Dr. Georgi Stranski,' Pleven. As one of the leading tertiary NICUs in Northern Bulgaria, the clinic provides an excellent basis for evaluating pathogen prevalence, risk factors, and diagnostic challenges. The difficulties surrounding the early diagnosis of indicator infections emphasize the need for innovation in clinicians' diagnostic approaches.

I. Objectives and Tasks

The primary objective of this study is to analyze the incidence, etiology, epidemiological structure, risk factors, clinical manifestations, diagnostic strategies, and outcomes of neonatal nosocomial infections in the Intensive Care Unit of the Neonatology Clinic at University Hospital 'Dr. Georgi Stranski,' Pleven. Based on the results, recommendations will be developed for improved prevention and early detection strategies.

Specific Aims:

1. To evaluate the incidence, nosological structure, and microbiological profile of neonatal HAIs for the period 01.01.2021 – 30.06.2023.
2. To analyze the demographic and clinical characteristics of the patient population according to sex, gestational age, birth weight, and hospital stay duration.
3. To identify and assess risk factors associated with the development of HAIs in the NICU.
4. To investigate the initial clinical manifestations of neonatal HAIs and their correlation with the type of microbiological pathogen, severity, and clinical outcome.

5. To evaluate the most common laboratory abnormalities associated with neonatal HAI diagnosis.
6. To determine the diagnostic value of selected serum inflammatory biomarkers — procalcitonin (PCT), interleukin-6 (IL-6), interleukin-8 (IL-8), and endocan — for early detection of late-onset neonatal sepsis and compare them with routinely used laboratory methods.
7. To develop a predictive model for identifying high-risk patients to facilitate early diagnosis.

The findings of this study may serve as the basis for future validation research on diagnostic scoring tools (calculators) for early identification of neonates with late-onset, nosocomial sepsis and for the implementation of new diagnostic biomarkers into clinical practice.

II. Patients and Methods

1. Clinical Cohort and Study Stages

1.1. Technical Unit and Observation Period

The study was conducted in the Intensive Care Unit of the Neonatology Clinic at University Hospital 'Dr. Georgi Stranski,' Pleven, between January 1, 2021, and June 30, 2023.

1.2. Logical Unit for Statistical Observation

Included in the study were term and preterm neonates of both sexes, admitted to the Neonatal Intensive Care Unit (NICU) immediately after birth or transferred from another maternity ward within three days after birth, with a hospital stay longer than 72 hours.

Inclusion criteria:

• Stay in the NICU >72 hours

• Birth in the Clinic or transfer from another hospital within the first 3 postnatal days

Exclusion criteria:

• Admission to the NICU after >3 days at home

• Admission to the NICU after >3 days at another pediatric care facility

• Death occurring <72 hours after admission

Tasks 1, 2, 3. The study covers 519 patients hospitalized in the Neonatology Clinic at the Dr. Georgi Stranksy University Hospital for the period 01.01.2021 – 30.06.2023.

For tasks 4 and 5. The study included 60 patients treated in the Neonatology Clinic at the Dr. Georgi Stranksy University Hospital for the period January 2022 – January 2023.

1.3. Study Design

The study was a clinico-epidemiological, single-center, ambispective investigation. It consisted of two stages, which determined the grouping of patients (Figure 1).

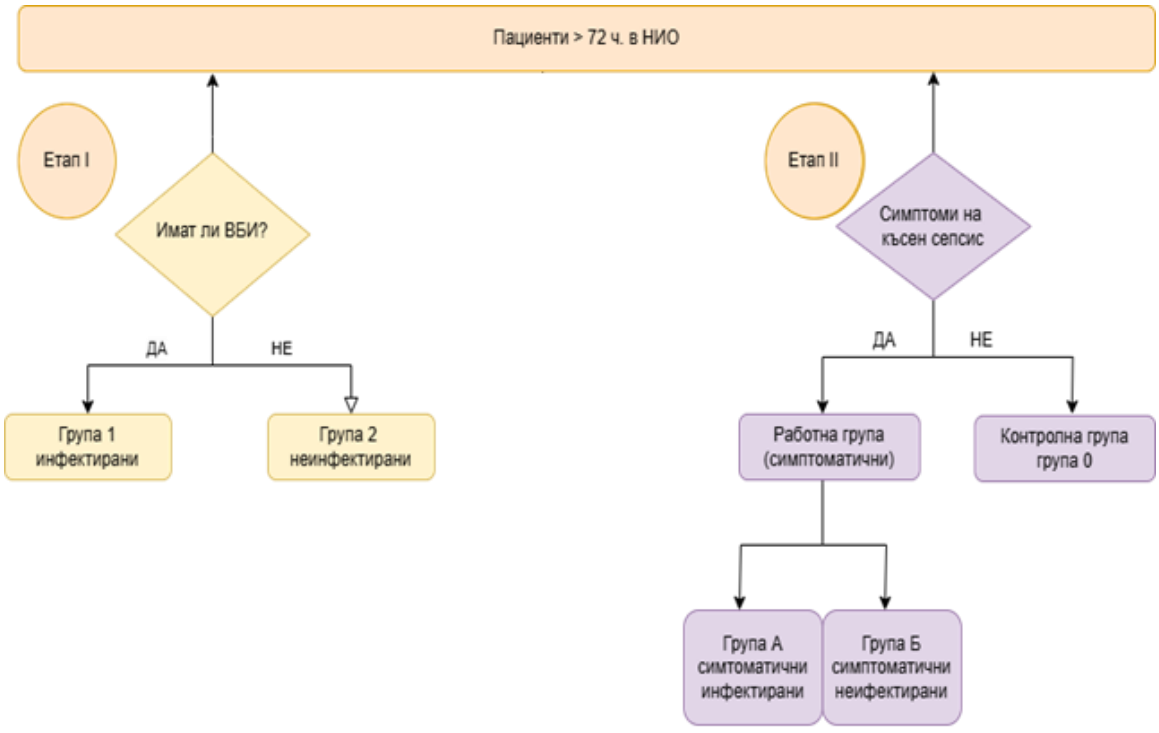

Figure 1. Stages of the research study

Stage I: Observational Study on HAIs in the NICU

For objectives 1, 2, and 3, all patients treated in the NICU and meeting the above criteria during a 30-month period were included. Variables observed included

categorical and quantitative risk factors, clinical and laboratory findings, microbiological results, and outcomes.

Observed Indicators

- **Possible categorical risk factors**:
    - gestational age;
    - birth weight (grams);
    - mode of delivery;
    - concomitant congenital anomalies;
    - diagnosed intraventricular hemorrhage;
    - perinatal asphyxia;
    - presence and type of mechanical ventilation during the three days prior to the onset of HAI;
    - presence of a central venous catheter (CVC, umbilical venous catheter) during the three days prior to the onset of HAI;
    - presence of parenteral nutrition during the three days prior to the onset of HAI;
    - presence of a gastric tube during the three days prior to the onset of HAI;
    - bronchopulmonary dysplasia (BPD);
    - anemia prior to the onset of HAI;
    - prior antibiotic treatment unrelated to the present HAI episode (for congenital infection or a previous HAI episode), including the number of agents used.
- **Possible quantitative risk factors**:
    - total duration of mechanical ventilation prior to the onset of HAI (number of days);
    - total duration of CVC use (number of days);

    - total duration of parenteral nutrition (number of days);
    - time to initiation of enteral feeding (number of days);
    - antibiotic treatment (number of agents used and duration in days).
- Day of infection onset (number of days since hospitalization)
- Clinical symptoms of infection
- Laboratory abnormalities
- Results of microbiological testing of biological material
- Course of the disease
- Outcome of the disease

The subject of the study were so-called **indicator infections**: infections with particularly frequent localizations that comprise the main structure of healthcare-associated infections. These include: lower respiratory tract infections, urinary tract infections, catheter-associated infections, primary sepsis, and surgical site infections. Cases of necrotizing enterocolitis with proven bacterial isolates were also considered.

Patients were grouped according to the main criterion of the presence or absence of indicator healthcare-associated infection:

- **Group 1. "Infected"** – neonates with confirmed indicator HAI.
    - **Subgroup 1A**: neonates with indicator HAI and a proven microbiological pathogen – a positive microbiological result from blood culture, tracheal aspirate, cerebrospinal fluid culture, urine culture, or catheter/ET tube tip culture. Antibiotic treatment was administered during the observed infection episode.
    - **Subgroup 1B**: neonates with indicator HAI but without a proven microbiological pathogen – laboratory abnormalities and clinical symptoms characteristic of systemic infection were present, but microbiological samples remained sterile or were not collected. Antibiotic treatment was administered during the observed infection episode.

- **Group 2. "Non-infected"** – neonates without indicator HAI, i.e., patients without paraclinical abnormalities suggestive of systemic inflammation. No antibiotic treatment was administered after the early neonatal period.

A limitation of the study was the lack of documented cases of proven eye and skin infections. These infections were not included in the analysis due to insufficient available data.

Stage II: Study on Laboratory Markers for HAI Diagnosis

The study was conducted to address **Task 5**, namely: to determine and analyze the levels of early markers for the diagnosis of healthcare-associated infections in neonates—**procalcitonin (PCT), interleukin-6 (IL-6), interleukin-8 (IL-8), and endocan (ESM-1)**; to establish their sensitivity, specificity, diagnostic effectiveness, positive and negative predictive values; and to compare them with routinely used markers of inflammatory activity, such as complete blood count and C-reactive protein (CRP).

The study was funded by the **Medical University of Pleven** as a doctoral project № D3/2022 entitled *"Markers for Early Diagnosis of Healthcare-Associated Infections in Neonates."* Due to limitations in the number of reagents and the one-year timeframe for project completion, a limited number of patients were included (**N = 60**).

Patient Grouping:

Patient selection was based on the presence of symptoms suspicious for systemic infection of nosocomial origin. Since the focus was on so-called *early* infection markers, sampling was performed at the onset of infection symptoms, before its definitive confirmation or exclusion.

Exclusion criteria were severe congenital anomalies and the immediate postoperative period (within 48 hours after surgery).

- **Control group (Group 0):** To analyze reference ranges of the indicators, a control group of asymptomatic, non-infected neonates in stable general condition with a hospital stay of >72 hours was formed. Blood samples were obtained as part of routine control laboratory testing.
- **Study group:** Comprised of patients with newly developed symptoms suspicious for indicator HAIs. Inclusion criteria required at least **three**

clinical and laboratory indicators and at least **one risk factor** suggestive of neonatal infection acquired in the NICU (see Table 1).

Table 1. Clinical symptoms, laboratory abnormalities, and risk factors suggestive of NICU-acquired neonatal infection.

Clinical Symptoms (new-onset)	Laboratory Findings	Risk Factors
Respiratory rhythm disturbances (apnea, dyspnea)	Leukopenia/leukocytosis	Mechanical ventilation
Increased oxygen demand	Thrombocytopenia	Central venous catheter
Need for respiratory support	Hyper-/hypoglycemia	Positive microbiological screening
Skin color changes (pale/gray)	Metabolic acidosis	Parenteral nutrition
Tachycardia/bradycardia		Urethral catheter
Abdominal distension		Gastric tube
Vomiting		Postnatal corticosteroid therapy
Diarrhea		Chronic respiratory/cardiac insufficiency
Decreased motor activity		
Depressed consciousness		
Irritability, seizures		
Bulging fontanelle		
Jaundice		
Weight loss		
Hypo-/hyperthermia		

Table 2. Patient grouping criteria

Grouping Criteria	Group 0 (Control)	Group A (Symptomatic Infected)	Group B (Symptomatic Non-infected)
Microbiological result	-	+/-	-
Clinical signs of indicator HAI	-	+	+

Laboratory markers of systemic infection	-	+	-

The work group of symptomatic patients consists of patients with newly developed symptoms that raise suspicion of an indicative nosocomial infection. Their inclusion in the study takes place at the beginning of a possible episode of NHI. Prospectively, they are divided into symptomatic infected and symptomatic uninfected. The assessment of whether symptomatic patients are infected or not is complex, considering the subsequent hematological tests over the next 5 days, such as elevated CRP, leukocytosis/leukopenia; the results of microbiological samples; the evolution of the clinical condition, treatment, and outcome. For infected children with late neonatal sepsis, we accepted both the diagnosis of clinical sepsis (with negative microbiological results) and septicemia (with positive microbiological results).

Later, the group of symptomatic cases is divided into the following subgroups:

- Group A – symptomatic and infected patients (patients with proven HAI). Diagnoses of clinical sepsis (with negative microbiological results) and sepsis (with positive microbiological results) are accepted. The assessment is comprehensive, taking into account subsequent hematological tests over the next 5 days, such as elevated CRP, leukocytosis/leukopenia; microbiological test results; evolution of clinical condition, treatment, and outcome.

- Group B – symptomatic but uninfected patients (no evidence of sepsis during the next 5 days of stay; control paraclinical tests are within reference ranges and microbiological samples are negative). Another non-infectious condition or disease may be proven.

On the day of suspected infection, 1.5–2 ml of venous blood was collected, and the separated serum was stored at -80˚C until analysis.

The concentrations of IL-6, IL-8, PCT, and ESM-1 were determined by ELISA (Enzyme Linked Immunosorbent Assay) in the laboratory of the Medical University of Pleven, Department of Anatomy, Histology, Cytology, and Biology.

The clinical chart of the patient in the study is presented in Appendix No. 2.

2. Research Methods

2.1. Documentary Method

Patient data were collected from medical records and NICU documentation.

2.2. Laboratory Method

Serum concentrations of PCT, IL-6, IL-8, and Endocan were measured using sandwich ELISA kits: Human IL-6 ELISA (Invitrogen Thermo Fisher Scientific), Human IL-8 ELISA (Invitrogen Thermo Fisher Scientific), Human Procalcitonin ELISA (BioVendor), and Human Endothelial Cell-Specific Molecule 1 ELISA (CUSABIO).

2.3. Statistical Method

Data were processed using IBM SPSS Statistics 27.0.1.0, MedCalc Version 19.6.3, and Microsoft Excel 2021. Statistical significance was set at $p < 0.05$.

III. Results and Discussion

Stage I of the study was used to achieve the objectives of tasks 1, 2, and 3.

1. Results and Discussion on Task 1

To determine the incidence, nosological and microbiological structure of nosocomial infections in newborns in the NICU at University Hospital 'Dr. Georgi Stranski,' Pleven.

1.1. Incidence (Morbidity) and Comparative Analysis of the Main Groups

1.1.1. Study Cohort

Stage I of the study covered 519 newborns treated in the NICU of the Neonatology Clinic, University Hospital 'Dr. Georgi Stranski,' Pleven, during the period 01.01.2021–30.06.2023. Of these, 270 (52%) were male and 249 (48%) female (Figure 2). The mean gestational age of the study cohort was 34.30 ± 4.16 weeks, ranging from 23 to 41.

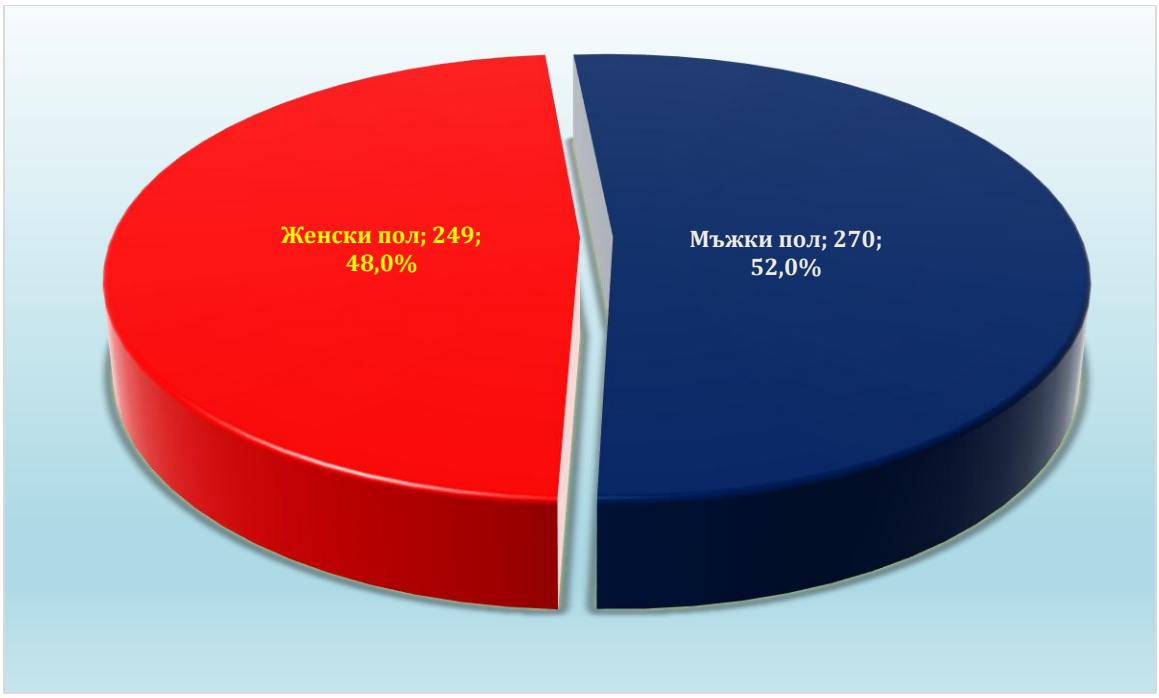

Figure 2. Frequency distribution of the study cohort by sex.

Among male newborns, the largest group (107) was in the 33–37 gestational week range, followed by >37 weeks (79), and the smallest group (28) was <28 weeks. Among female newborns, the largest group (134) was in the 33–37 gestational week range, followed by >37 weeks (52), and the smallest group (18) was <28 weeks (Figure 3).

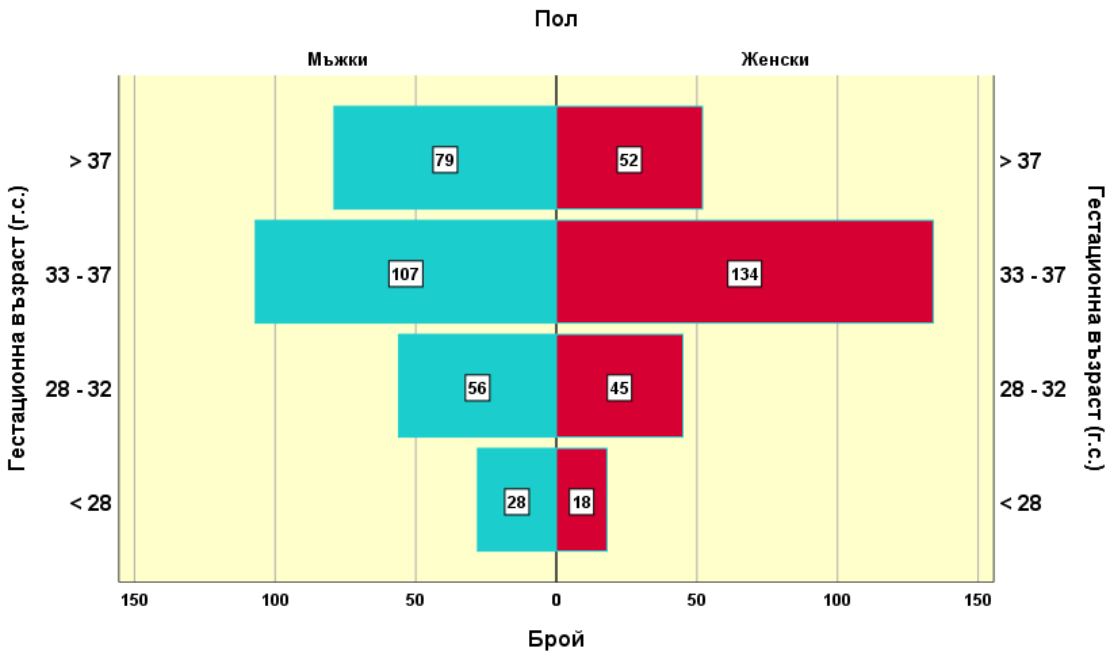

Figure 3. Distribution of the study cohort by sex and gestational week.

1.1.2. Main Groups

The studied cohort in this investigation was divided into two main groups (Figure 4):

• Infected newborns (Group 1) – n=72 (13.9%), of whom microbiologically confirmed infection (Group 1A) – n=46 (8.9%) and microbiologically unconfirmed infection (Group 1B) – n=26 (5.0%).

• Controls – n=447 (86.1%) uninfected newborns (Group 2).

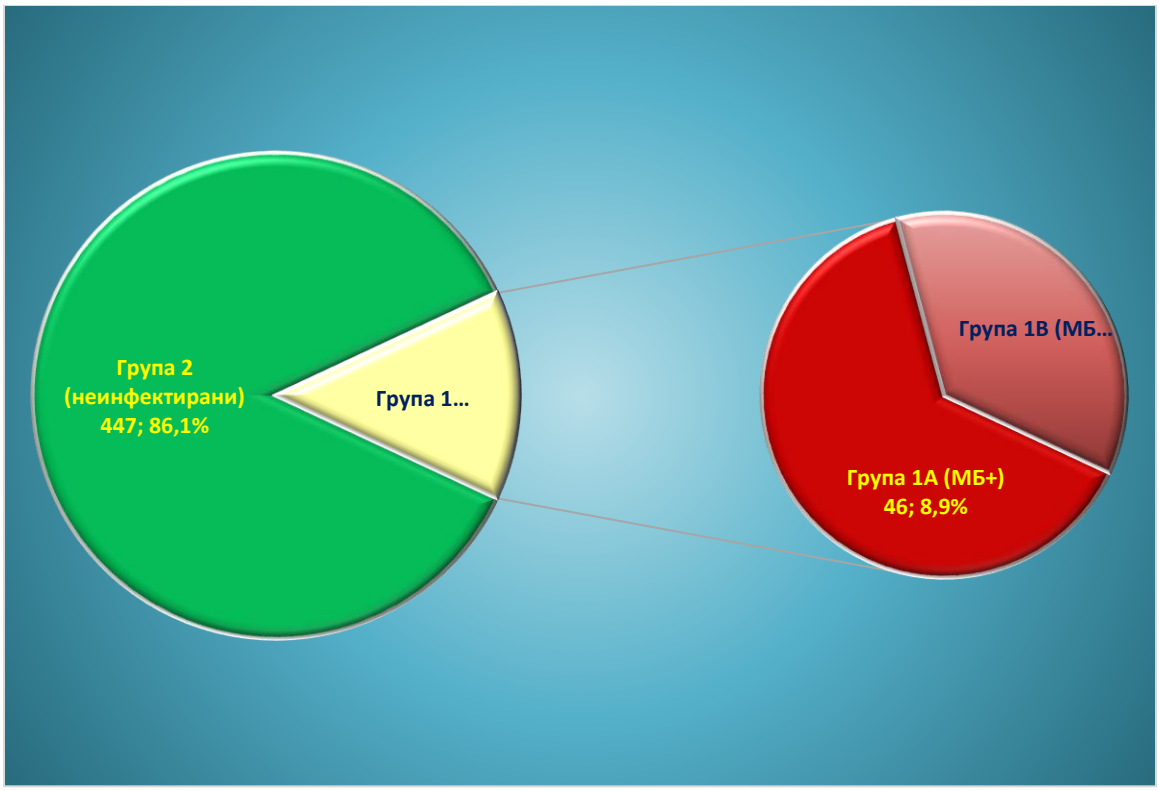

Figure 4. Frequency distribution of the study cohort by main groups.

The average incidence of nosocomial infections during the 2.5-year study period per 100 NICU patients was 13.9%, including cases without isolated bacterial pathogens. This result is comparable with reported studies, showing a higher frequency compared to the study by Rangelova et al. (2020) reporting 9.5%. The difference may be explained by the fact that their mandatory sepsis criterion was a positive blood culture, while our study included clinically manifested infections with typical laboratory abnormalities and treatment, even when a bacterial pathogen was not isolated.

1.1.3. Comparative Analysis

The comparative analysis of patients from the main groups by sex, birth weight, gestational age, and hospital stay showed (Table 3):

- The two groups differed significantly in three indicators: birth weight, gestational age, and hospital stay.
- Infected newborns had significantly lower mean birth weights and gestational ages, and a statistically longer mean hospital stay.
- Sex differences between the groups were statistically insignificant.

Table 3. Differentiation of the two main groups by sex, birth weight, gestational age, and hospital stay.

Indicator	Total (n=519)	Uninfected (n=447)	Infected (n=72)	p-value
Sex (Male/Female)	270 (52%) / 249 (48%)	226 (50.6%) / 221 (49.4%)	44 (61.1%) / 28 (38.9%)	0.100
Birth Weight (g, mean ± SD)	2144.29 ± 818.76	2287.19 ± 743.64	1257.08 ± 700.22	<0.001
Gestational Age (weeks, mean ± SD)	34.30 ± 4.16	35.18 ± 3.39	28.85 ± 4.36	<0.001
Hospital Stay (days, mean ± SD)	16.54 ± 16.62	12.94 ± 11.14	45.71 ± 23.73	<0.001

1.2. Distribution by Nosological Units

In the infected group (n=72), bloodstream infections (sepsis) had the highest relative share (n=39; 54.16%), followed by ventilator-associated pneumonia (n=16; 22.22%), necrotizing enterocolitis (n=9; 12.5%), catheter-associated infections (n=5; 6.94%), and with equal lowest shares were central nervous system infection, endocarditis, and wound infection (n=1; 1.38%).

Table 4. Nosological structure of microbiologically confirmed infections.

Year	Sepsis n (%)	VAP n (%)	CAI* n (%)	NEC n (%)	CNS Infection n (%)	Endocarditis n (%)	Wound Infection n (%)	Total n
2021	10 (43.47)	9 (39.13)	2 (8.69)	1 (4.34)	1 (4.34)	0	0	23
2022	7 (43.75)	3 (18.75)	1 (6.25)	3 (18.75)	0	1 (6.25)	1 (6.25)	16
2023 (6 mo.)	3 (42.85)	2 (28.57)	1 (14.28)	1 (14.28)	0	0	0	7
Total	20	14	4 (8.69)	5	1 (2.17)	1 (2.17)	1 (2.17)	46

(43.47) (30.43) (10.86)

*CAI – catheter-associated infection

**Wound infection – surgical site infection

Table 5. Nosological structure of microbiologically unconfirmed infections.

Year	Sepsis n (%)	VAP n (%)	CAI n (%)	NEC n (%)	Total n
2021	10 (76.92)	1 (7.69)	1 (7.69)	1 (7.69)	13
2022	6 (66.66)	1 (11.11)	0	2 (22.22)	9
2023	3 (75)	0	0	1 (25)	4

Total: 26 (73.07% sepsis, 7.69% VAP, 3.8% CAI, 15.38% NEC).

Among patients with microbiologically confirmed infection for the period January 2021 – June 2023, the following positive results were obtained: **24 blood cultures, 22 tracheal aspirates, 10 catheter tip samples from CVCs, 1 cerebrospinal fluid culture, 3 gastric aspirates, 2 urine cultures, and 2 wound secretions.**

It is important to note that catheter-associated infections in this context refer specifically to infections from central venous catheters placed in the **umbilical vein immediately after birth**. For this purpose, factory-manufactured gastric tubes of appropriate size (ID = 4, 6, 8 mm) were used. Upon removal, the catheter tip was examined for microbiological colonization. During the study period, no other types of central venous catheters were used, nor were percutaneously inserted central catheters (PICC lines), which are widely applied and included in the statistical reporting of CLABSI cases in many developed countries (Bizzarro et al., 2010).

Despite the limited use of central venous catheters, **late-onset neonatal sepsis emerged as the leading healthcare-associated infection in this study (54.16%)**. This result is logical given the mean gestational age (28 weeks) of the infected neonates and their mean birth weight (~1200 g). Previous studies have also reported that bloodstream infections account for the highest proportion of infections in neonatal intensive care units, ranging from 5% to 32% (Sadowska-Krawczenko et al., 2012).

Consistent with the literature, the present study confirmed that **pneumonia** is the second most frequent healthcare-associated infection in the NICU. With an overall prevalence of **22.22%** among infected patients, our data fall within the reported range of **6.8–32.3%** of nosocomial infections cases in NICUs described by Foglia and colleagues (Foglia et al., 2007).

1.3. Causative agents of infections

1.3.1. Descriptive statistics

The frequency analysis presented in Figure 5 established that:

- During the studied period, the following bacterial pathogens were isolated: *Klebsiella pneumoniae, Staphylococcus spp., Escherichia coli, Enterobacter cloacae, Streptococcus spp., Acinetobacter spp.*
- No cases of invasive candidiasis were confirmed.
- *Klebsiella pneumoniae* had the largest relative share (51%), followed by *Staphylococcus spp.* (36.7%) and *E. coli* (28.6%).
- The rarest were *Streptococcus spp.* and *Acinetobacter spp.* (2% each), while *Serratia* was completely absent.

The sum of the percentages exceeds 100, since in some patients more than one pathogen was identified.

Figure 6 shows that the majority (37 or 75.5%) of newborns with isolated infection pathogens had only one such pathogen, followed by those with two (10 or 20.4%) and three (2 or 4.1%).

For the upcoming analysis of the relationship between the studied symptoms, the course of infection, and its outcome depending on the pathogen type, we determined which pathogens have statistical representativeness (Figure 7):

- The figure shows that the largest relative shares and counts are for *Klebsiella pneumoniae, Staphylococcus spp.*, and *Escherichia coli.*
- These three pathogens have the required statistical representativeness and will be included in subsequent analyses.

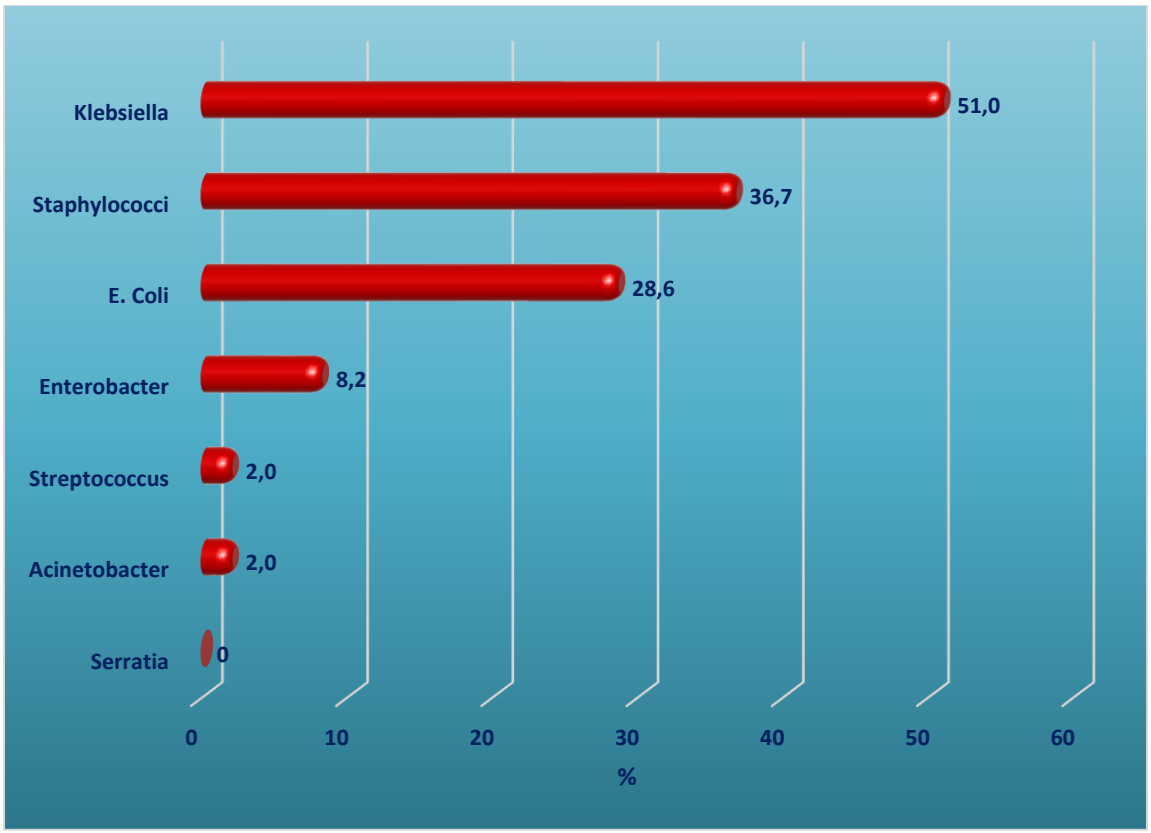

Figure 5. Frequency distribution of infection pathogens in the infected group (the sum of percentages exceeds 100, since in some newborns more than one pathogen was identified).

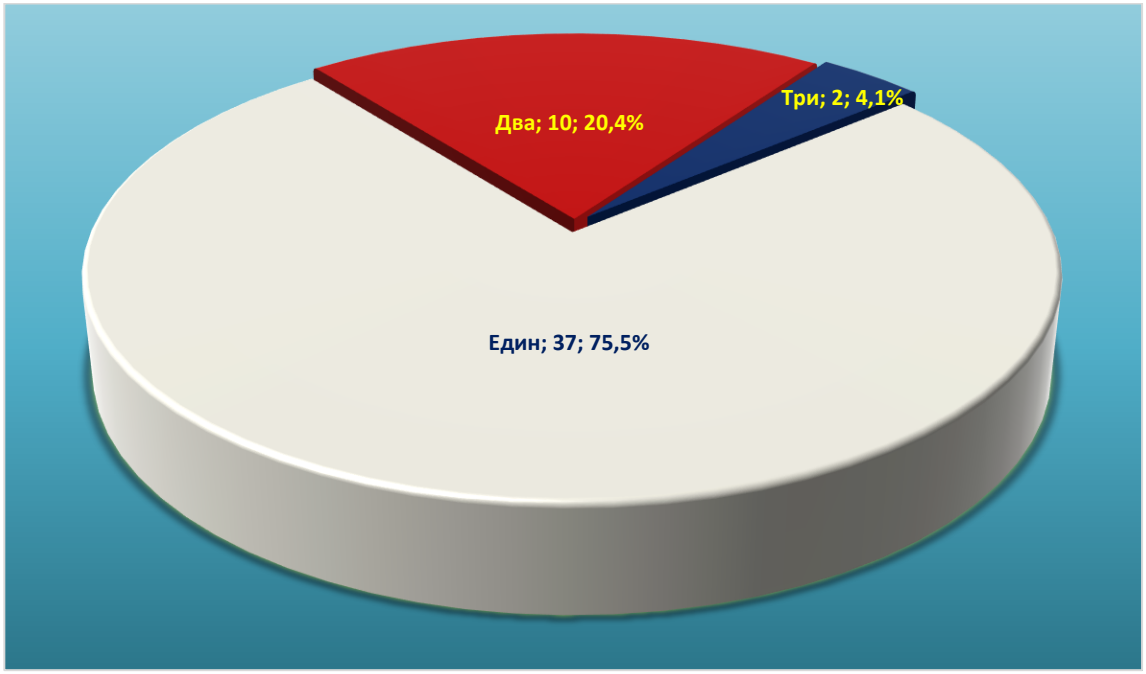

Figure 6. Frequency distribution of infected patients according to the number of pathogens.

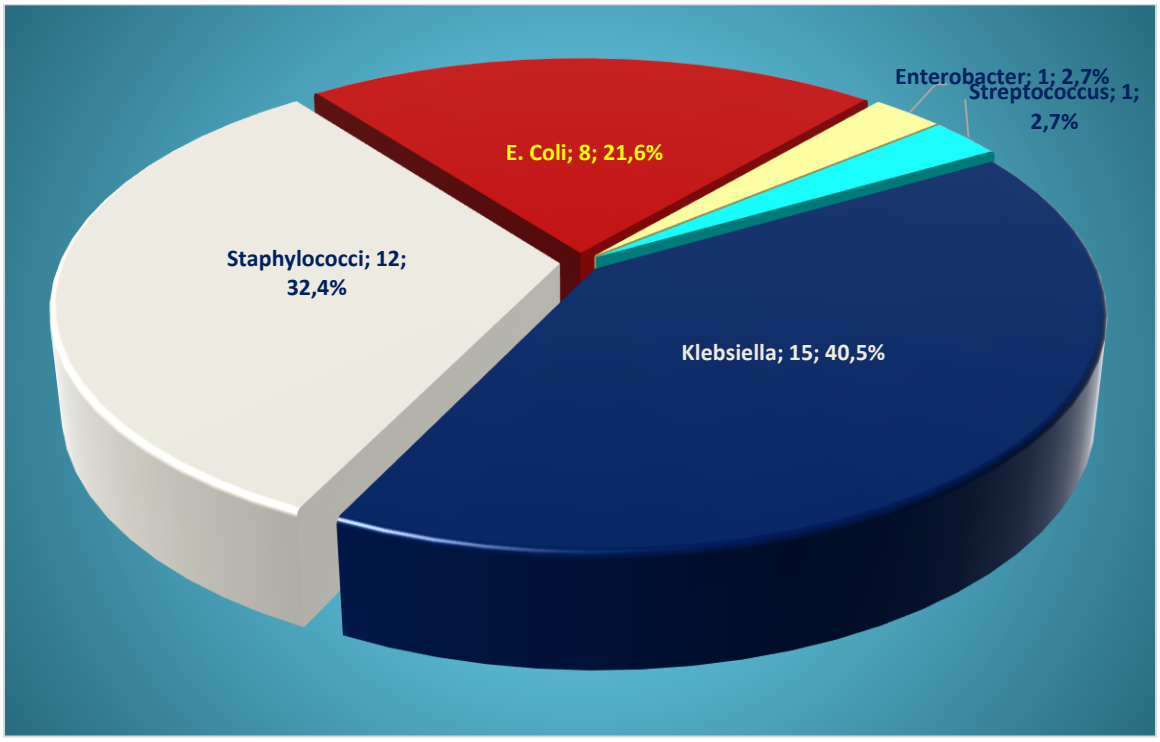

Figure 7. Frequency distribution of infected children with only one pathogen.

Additionally, cases of staphylococcal infections (with one or more isolated pathogens, including staphylococci) were examined in terms of species diversity. Coagulase-positive *Staphylococcus aureus* was isolated in only one case from the tip of a central venous catheter (CVC), representing just 4.7% of all staphylococcal isolates in this group; while coagulase-negative staphylococci were isolated in 95.3% of cases with *Staphylococcus spp.* Among all infected children with isolated staphylococci, the diagnosis was always established in combination with data indicating systemic inflammatory activity.

Klebsiella pneumoniae emerged as the leading bacterial cause of hospital-acquired infections, surpassing Gram-positive staphylococci. A surprising fact is that no cases of invasive candidiasis were registered, which may be related to the relatively short use of the umbilical venous catheter and the non-use of other central venous catheters (average duration of 2.65 days, presented in Table 11).

2. Results and Discussion on Task 2

Objective: To identify and evaluate the risk factors for the occurrence of healthcare-associated infections (HAIs) in newborns in the NICU.

2.1. Comparative Analysis of the Main Groups by Potential Risk Factors

As a first step, a comparative analysis between the two main groups (infected vs. non-infected) was performed using both categorical and quantitative variables selected as potential risk factors for HAI. The results show that the groups differ significantly across all categorical variables except mode of delivery and congenital anomalies. Infected neonates had significantly higher relative shares in lower birthweight categories (≤1499 g), lower gestational age groups (≤32 weeks), diagnosed intraventricular hemorrhage (IVH), birth asphyxia (moderate or severe), mechanical ventilation, presence of central venous catheter (CVC), parenteral nutrition and gastric tube within the last 3 days, bronchopulmonary dysplasia (BPD), prior antibiotic treatment with ≥2 drugs, and anemia prior to the infection episode. Conversely, non-infected neonates had significantly higher shares in higher birthweight categories (≥1500 g) and older gestational age groups (>32 weeks), and more frequently lacked IVH, birth asphyxia, recent ventilation/CVC/parenteral nutrition/gastric tube, BPD, anemia, and had either no prior antibiotics or only one drug.

Table 6. Comparative analysis of categorical risk factors.

Indicator	Category	Non-infected n (%)	Infected n (%)	p-value	Notes
Birth weight (g)	<750	0 (0.0)	6 (8.3)	<0.001	
Birth weight (g)	750–999	14 (3.1)	28 (38.9)	<0.001	
Birth weight (g)	1000–1499	52 (11.6)	23 (31.9)	<0.001	
Birth weight (g)	1500–2499	244 (54.6)	11 (15.3)	<0.001	
Birth weight (g)	≥2500	137 (30.6)	4 (5.6)	<0.001	
Gestational age (weeks)	<28	9 (2.0)	37 (51.4)	<0.001	
Gestational age (weeks)	28–32	79 (17.7)	22 (30.6)	0.010	
Gestational age (weeks)	33–37	233 (52.1)	8 (11.1)	<0.001	

Gestational age (weeks)	>37	126 (28.2)	5 (6.9)	<0.001
Mode of delivery	Vaginal	200 (44.7)	32 (44.4)	NS
Mode of delivery	Operative	247 (55.3)	40 (55.6)	NS
Congenital anomalies	No	419 (93.7)	69 (95.8)	0.602
Congenital anomalies	Yes	28 (6.3)	3 (4.2)	0.602
Diagnosed IVH	No	440 (98.4)	61 (84.7)	<0.001
Diagnosed IVH	Yes	7 (1.6)	11 (15.3)	<0.001
Birth asphyxia	None	353 (79.0)	17 (23.6)	<0.001
Birth asphyxia	Moderate	73 (16.3)	30 (41.7)	<0.001
Birth asphyxia	Severe	21 (4.7)	25 (34.7)	<0.001
Ventilation (last 3 days)	None	302 (67.6)	19 (26.4)	<0.001
Ventilation (last 3 days)	Non-invasive	99 (22.1)	37 (51.4)	<0.001
Ventilation (last 3 days)	Invasive	46 (10.3)	16 (22.2)	0.004
CVC in last 3 days	No	411 (92.2)	45 (62.5)	<0.001
CVC in last 3 days	Yes	35 (7.8)	27 (37.5)	<0.001
Parenteral nutrition (last 3 days)	No	31 (6.9)	0 (0.0)	0.014
Parenteral nutrition (last 3 days)	Yes	416 (93.1)	72 (100.0)	0.014
Gastric tube (last 3 days)	No	252 (56.4)	6 (8.3)	<0.001
Gastric tube (last 3 days)	Yes	195 (43.6)	66 (91.7)	<0.001
Bronchopulmonary	No	438 (98.0)	31 (43.1)	<0.001

dysplasia (BPD)				
Bronchopulmonary dysplasia (BPD)	Yes	9 (2.0)	41 (56.9)	<0.001
Prior antibiotic therapy	None	64 (14.3)	1 (1.4)	0.002
Prior antibiotic therapy	One drug	179 (40.0)	1 (1.4)	<0.001
Prior antibiotic therapy	Two drugs	181 (40.5)	51 (70.8)	<0.001
Prior antibiotic therapy	> Two drugs	23 (5.1)	19 (26.4)	<0.001
Anemia prior to episode	No	387 (86.6)	28 (38.9)	<0.001
Anemia prior to episode	Yes	60 (13.4)	44 (61.1)	<0.001

Table 7. Comparative analysis of quantitative risk factors.

Indicator	Non-infected (n=447) Mean ± SD	Infected (n=72) Mean ± SD	p-value
Duration of mechanical ventilation (days)	1.26 ± 2.92	13.72 ± 12.03	<0.001
CVC duration (days)	0.36 ± 1.27	2.65 ± 3.22	<0.001
Total duration of parenteral nutrition (days)	5.02 ± 4.20	19.31 ± 11.01	<0.001
Day of enteral feeding initiation	1.22 ± 0.51	2.04 ± 1.11	<0.001
Duration of antibiotic therapy (days)	5.36 ± 3.15	10.94 ± 9.00	<0.001

Narrative Discussion of Key Risk Factors

The results confirm that HAI frequency is inversely proportional to birth weight and gestational age—i.e., the degree of prematurity (Downey et al., 2010). Birth

weight <1499 g is validated as a risk factor, with a predominance in the 750–999 g group. The <750 g category has few cases (n=6), yet all of them developed HAI. For infants born ≥1500 g, this variable should not be considered a risk factor.

Evidence regarding the role of mode of delivery is limited and, in our cohort, it did not emerge as a significant risk factor. It is plausible that the birth environment (e.g., extra-hospital delivery) rather than the delivery mechanism per se may influence the risk of late-onset neonatal sepsis (Ogundare et al., 2019).

Among the wide spectrum of comorbidities that may increase the risk of nosocomial infection are congenital anomalies. In our data, no correlation was found between HAI and diagnosed congenital anomalies (p=0.602). Nonetheless, isolated reports suggest that specific anomalies (e.g., cleft palate—with feeding challenges, gastric tube use, aspiration risk; cyanotic heart defects requiring surgery; neural tube defects) could predispose to HAI (Chughtai et al., 2024; Couto et al., 2006).

Table 8. Types and number of identified congenital anomalies.

Type of congenital anomaly	Number of patients
Cleft palate	6
Multiple malformative stigmata*	5
Down syndrome	3
Congenital hydrocephalus	3
MPD / PFO	2
MCD	2
PDA in term infant	2
Clubfoot	2
AVSD, congenital cataract, congenital hydronephrosis, congenital hydrocephalus	1
Unspecified congenital diaphragmatic hernia	1
Critical complex cardiopathy	1
Cystic liver lesion	1
Agenesis of corpus callosum, craniosynostosis	1

* Normal karyotype without evidence of systemic numerical or structural chromosomal aberrations.

Birth asphyxia—capturing delayed acute cardio-pulmonary adaptation at birth (Apgar-based grading: moderate/severe)—emerged as a significant risk factor. Notably, even moderate asphyxia without intubation correlated with higher HAI frequency.

Intraventricular hemorrhage (IVH) is recognized as an independent risk factor in the literature (Kung et al., 2016). In the present study, IVH was significantly more frequent among infected patients (15.3%) versus non-infected (1.6%, $p<0.001$) and was invariably diagnosed before the HAI episode (grade not specified).

Although anemia is not routinely listed as an HAI risk factor in NICU settings, indirect immunologic effects of anemia of prematurity—together with low weight gain and possible nutrient deficiencies—may increase vulnerability (Cibulskis et al., 2021). Here, anemia was present in 61.1% of infected vs. 13.4% of non-infected neonates ($p<0.001$).

BPD has been identified as a risk factor for ICU-acquired infections in neonates. A meta-analysis reported BPD as an independent risk factor for VAP with an odds ratio of 2.21 (Tan et al., 2014). In our cohort, BPD was strongly associated with HAI overall (non-infected vs. infected with BPD = 2% vs. 56.9%; $p<0.001$), likely due to both direct respiratory vulnerability and indirect effects of prolonged hospitalization and ventilation.

Regarding care-related factors, we examined the impact of invasive procedures and devices (CVC, gastric tube, ventilation, parenteral nutrition). All showed significant differences between groups. The presence of a CVC is a well-established risk factor for late-onset sepsis (Araújo & Guimarães, 2020; Geffers et al., 2010; Bekhof et al., 2013a). Infected cases with positive CVC-tip cultures had a longer mean catheter use (6.2 days). These findings underscore minimizing CVC exposure and removing umbilical catheters as early as clinically feasible.

Parenteral nutrition—especially when prolonged—was a significant risk factor (mean duration non-infected vs. infected: 5.02 vs. 19.31 days). Published data similarly link longer parenteral nutrition to increased LOS risk (el Manouni el

Hassani et al., 2019; Tröger et al., 2014; Shah et al., 2014). We note as a limitation that composition and lipid duration were not analyzed.

Although less invasive than vascular access, gastric tube placement warrants strict technique and disinfection, given the observed association with HAI risk in our analysis.

Early enteral feeding within 2–3 days of life is associated with lower HAI levels without increasing NEC rates (Flidel-Rimon, 2004). In our cohort, all infants were initially fed with formula, precluding analysis of human milk's protective effects—reported elsewhere to offer anti-infective benefits and modulate gut permeability (Manzoni et al., 2013).

Multiple studies connect mechanical ventilation with increased HAI risk. Prolonged ventilation (≥7 days) has been linked to ESBL-producing bacterial infections in neonatal units (Huang et al., 2007). While the literature often focuses on invasive ventilation, the specific role of non-invasive ventilation (NIV) is less defined. NIV is a standard in RDS care and superior to invasive ventilation in preventing death or BPD (Mahmoud et al., 2022). In our study, both NIV and invasive ventilation in the last 3 days were significantly more common in infected patients (NIV 22.1% vs. 51.4%, $p<0.001$; invasive 10.3% vs. 22.2%, $p=0.004$). Overall duration of ventilation (both types combined) was also a risk factor (1.26 vs. 13.72 days, $p<0.001$).

2.2. Results and Discussion — Logistic Regression Analysis

To identify the factors influencing the occurrence of healthcare-associated infections (HAIs) and to evaluate their quantitative impact, a multiple binary logistic regression analysis was performed. The following indicators were tested as potential risk factors: sex, birth weight, gestational age, mode of delivery, congenital anomalies, intraventricular hemorrhage (IVH), birth asphyxia, duration of mechanical ventilation (days), type of ventilation in the last 3 days, central venous catheter (CVC) duration (days), CVC use in the last 3 days, parenteral nutrition (PN) use in the last 3 days, total PN duration (days), day of enteral feeding initiation, gastric tube use in the last 3 days, bronchopulmonary dysplasia (BPD), prior antibiotic therapy, duration of antibiotic treatment (days), probiotic prophylaxis, type of probiotic, and anemia prior to the episode.

Quantitative Variables

The requirement for using quantitative variables in binary logistic regression analysis is that they follow a normal distribution. Figures 8–14 present histograms of seven quantitative variables: birth weight, gestational age, duration of mechanical ventilation (days), CVC duration (days), total PN duration (days), day of enteral feeding initiation, and duration of antibiotic treatment (days). None followed a Gaussian distribution.

Therefore, ROC curve analysis was applied to determine threshold values that could distinguish healthy from infected patients. Figures 15–21 show that all seven indicators had significant threshold values, selected according to the Youden index = [maximum (sensitivity + specificity − 1)].

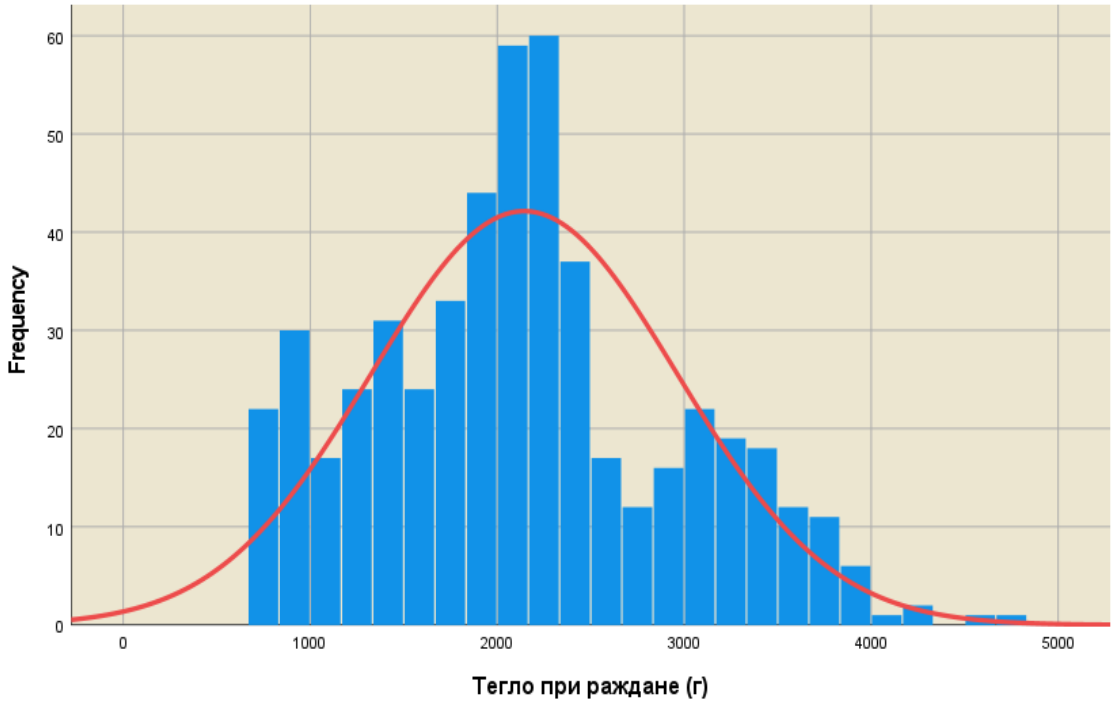

Figure 8. Frequency distribution of birth weight ($p < 0.001$).

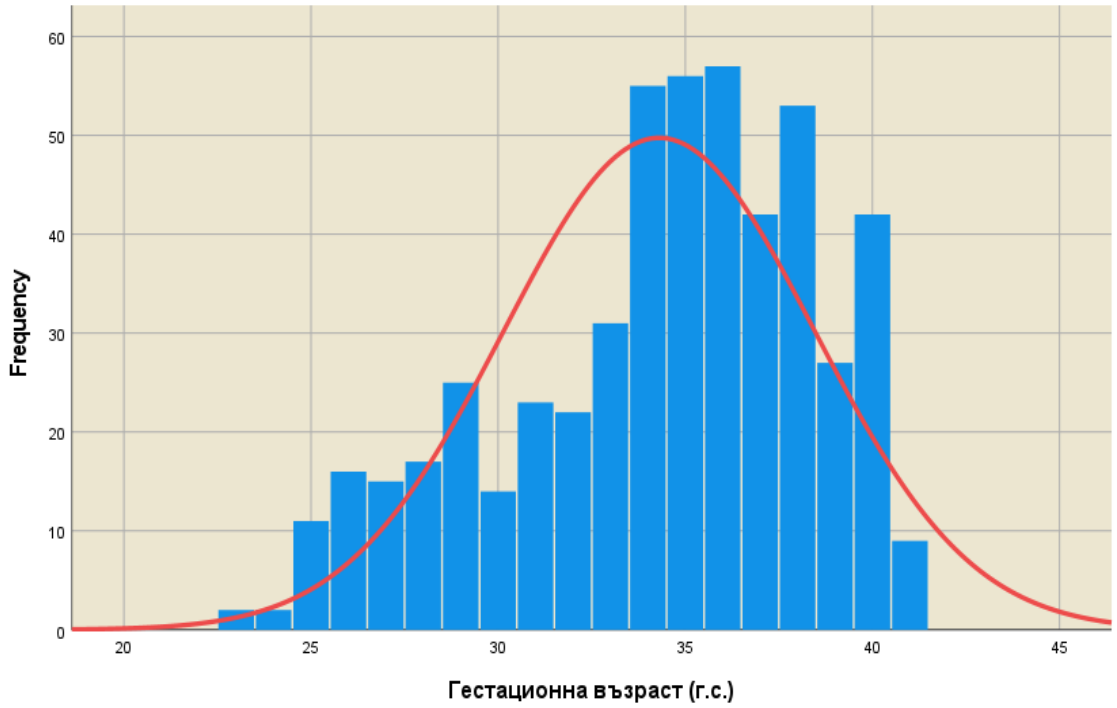

Figure 9. Frequency distribution of gestational age ($p < 0.001$).

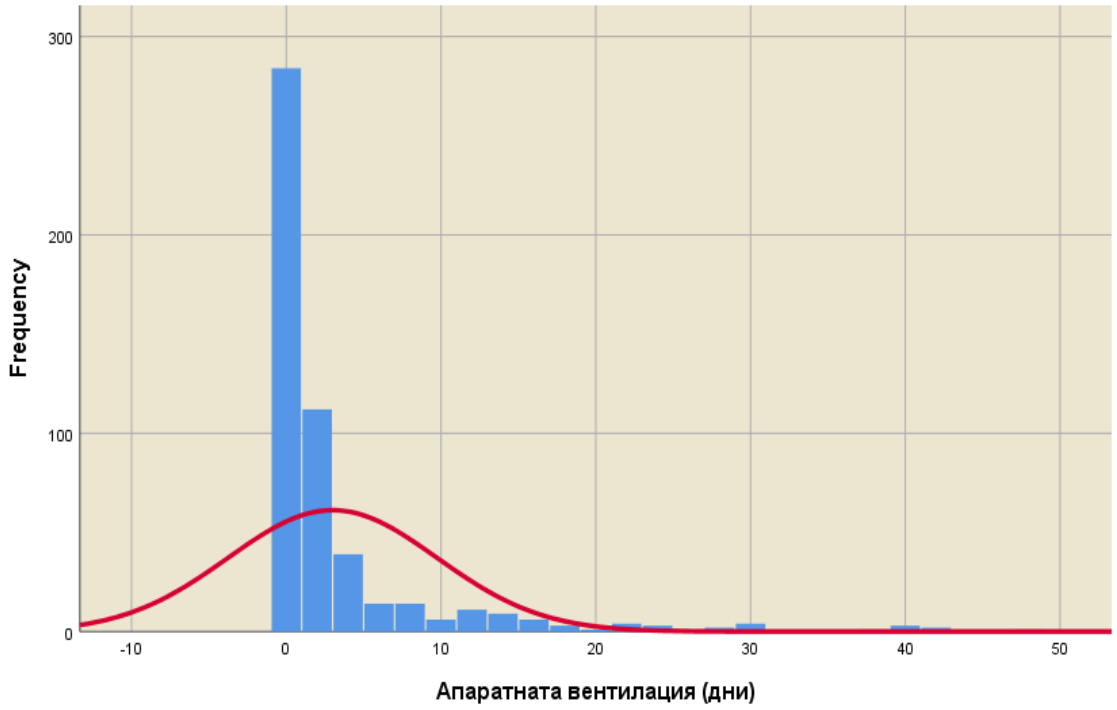

Figure 10. Frequency distribution of mechanical ventilation duration (days) ($p < 0.001$).

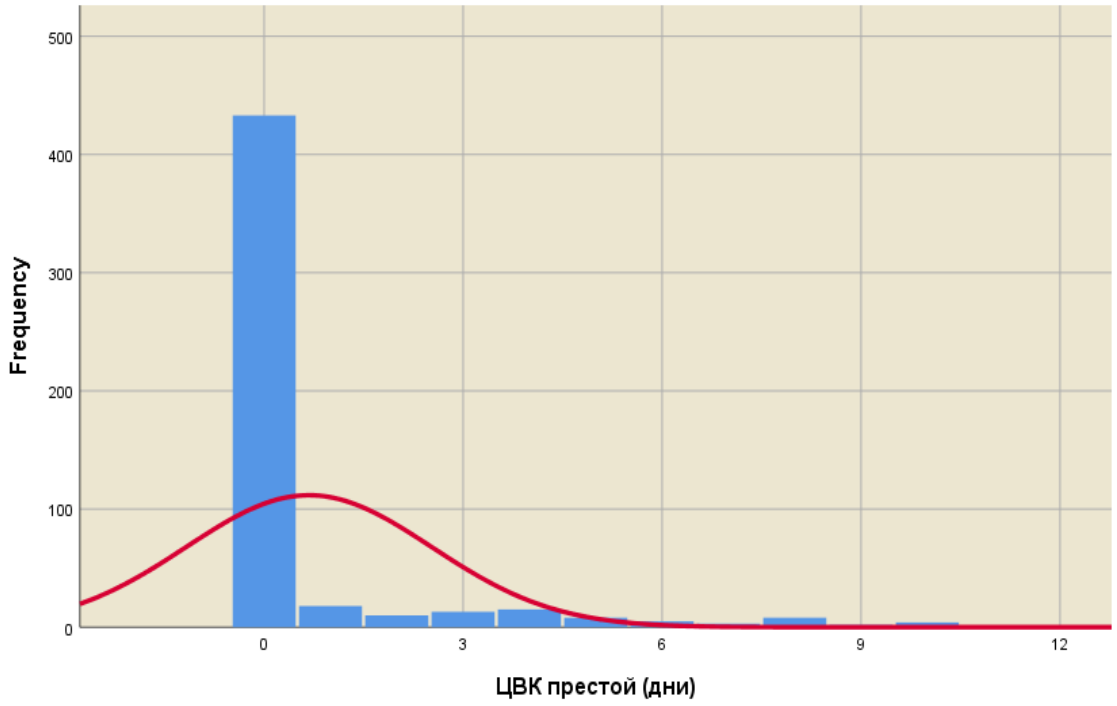

Figure 11. Frequency distribution of CVC duration (days) ($p < 0.001$).

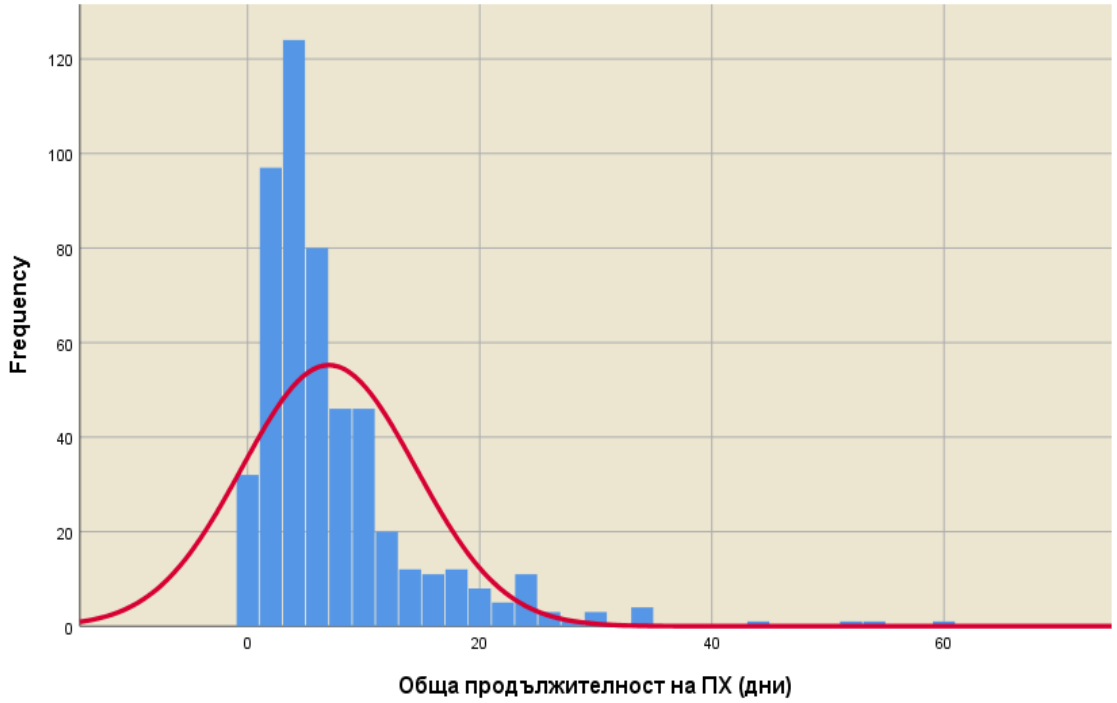

Figure 12. Frequency distribution of total PN duration (days) ($p < 0.001$).

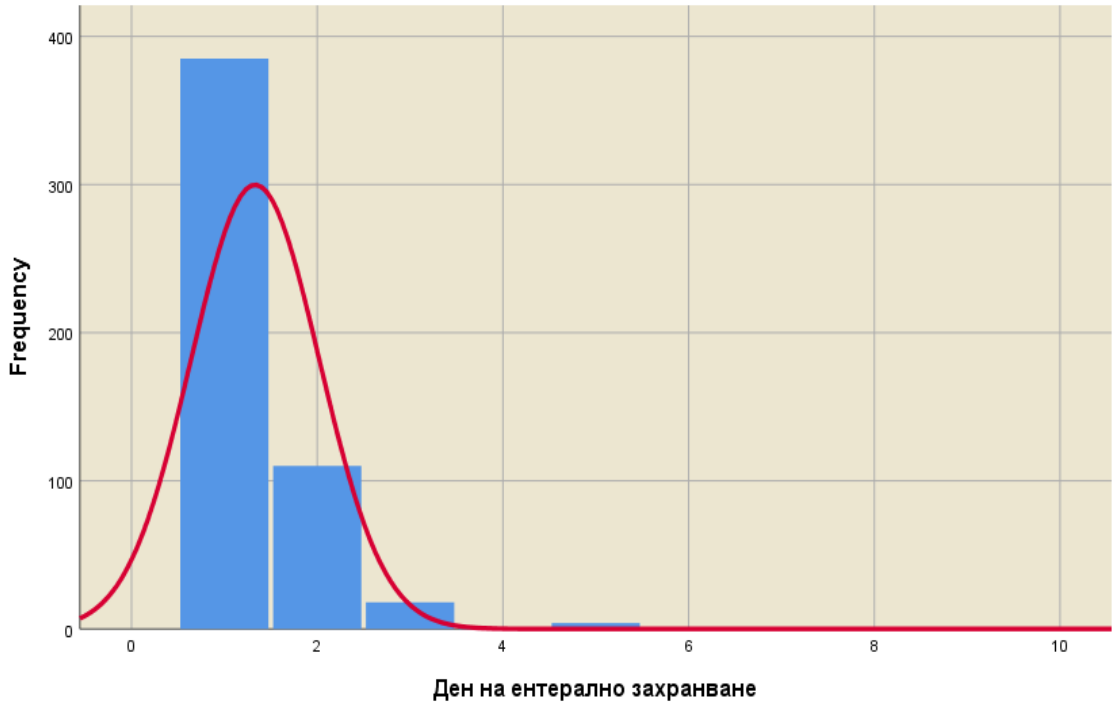

Figure 13. Frequency distribution of day of enteral feeding initiation ($p < 0.001$).

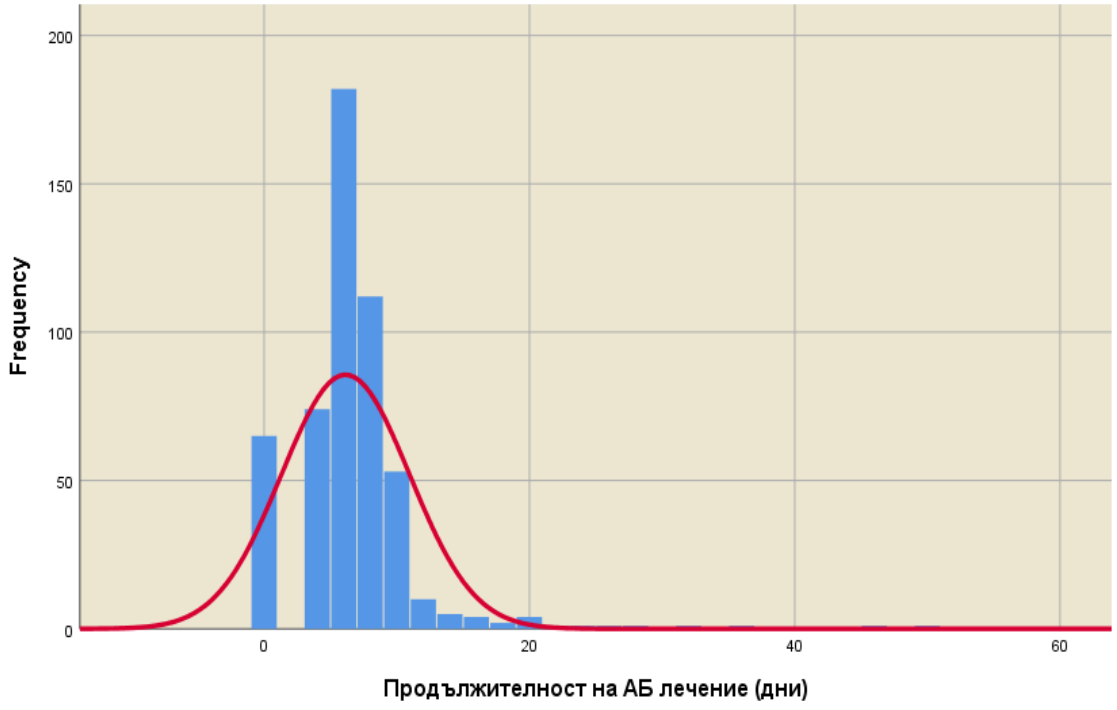

Figure 14. Frequency distribution of antibiotic treatment duration (days) ($p < 0.001$).

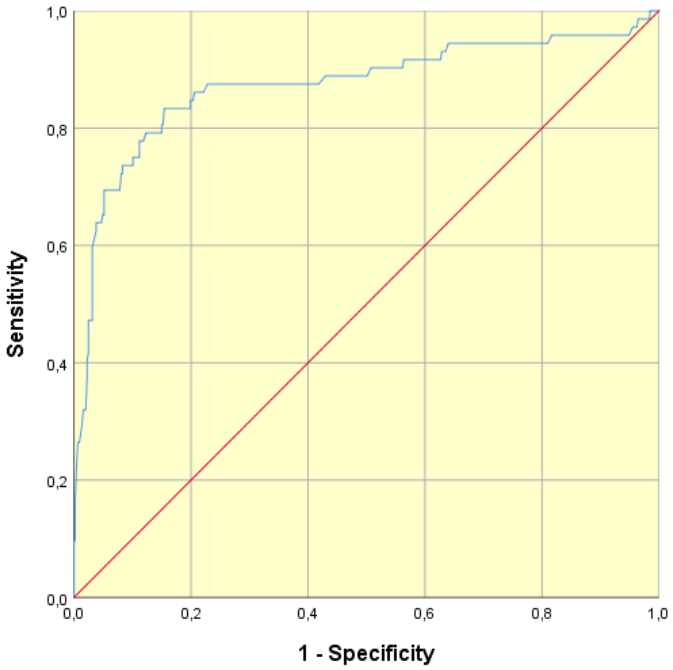

Figure 15. ROC curve of birth weight for distinguishing healthy from infected neonates (AUC = 0.873, $p < 0.001$).

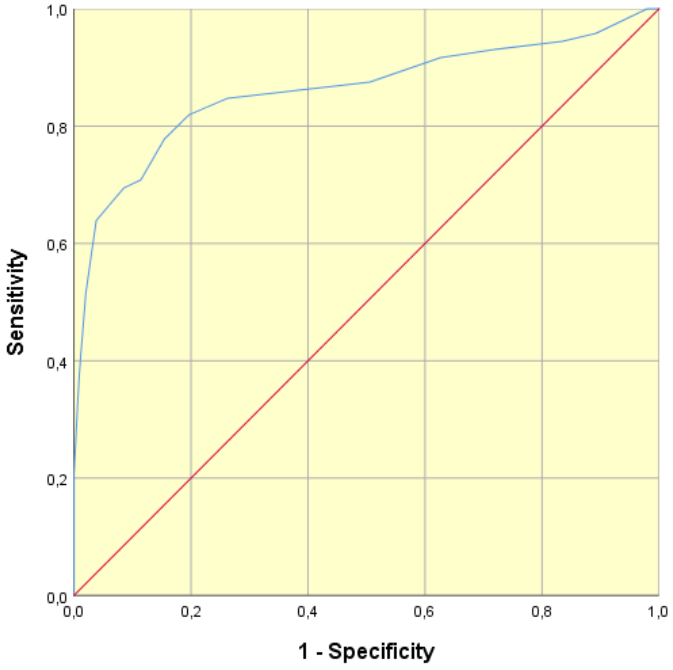

Figure 16. ROC curve of gestational age for distinguishing healthy from infected neonates (AUC = 0.860, $p < 0.001$).

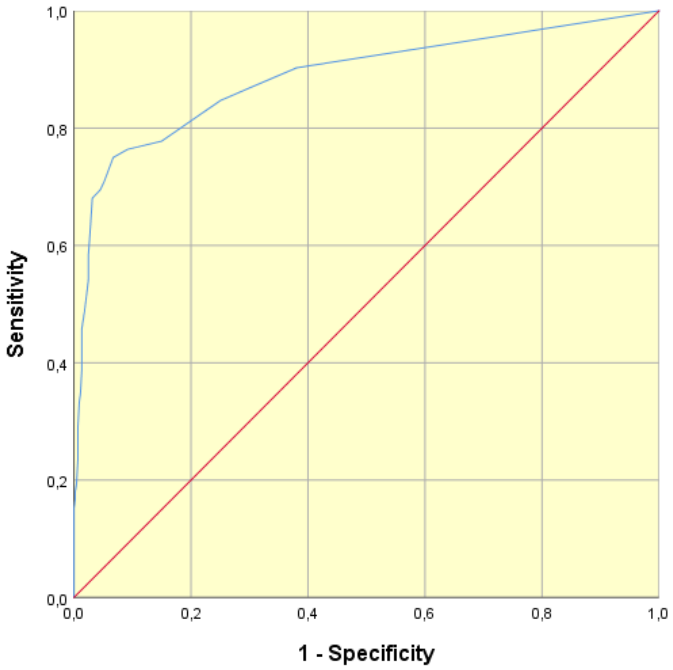

Figure 17. ROC curve of mechanical ventilation duration for distinguishing healthy from infected neonates (AUC = 0.887, $p < 0.001$).

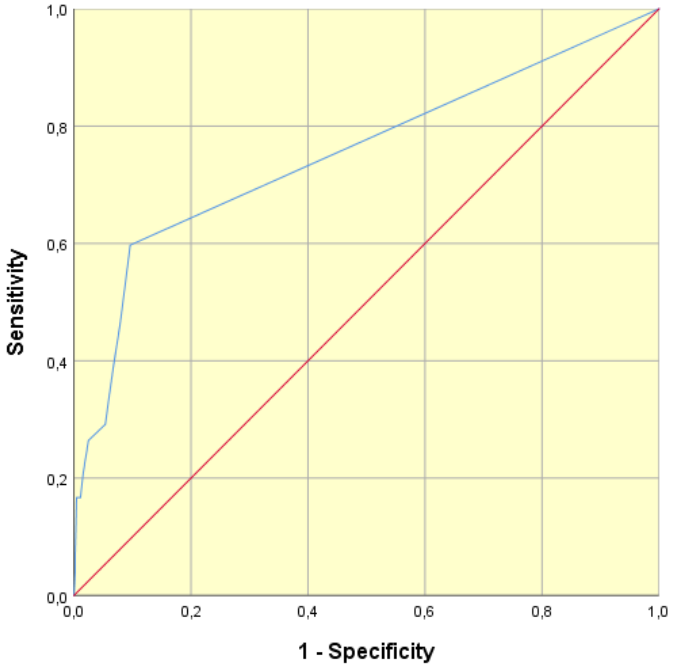

Figure 18. ROC curve of CVC duration for distinguishing healthy from infected neonates (AUC = 0.753, $p < 0.001$).

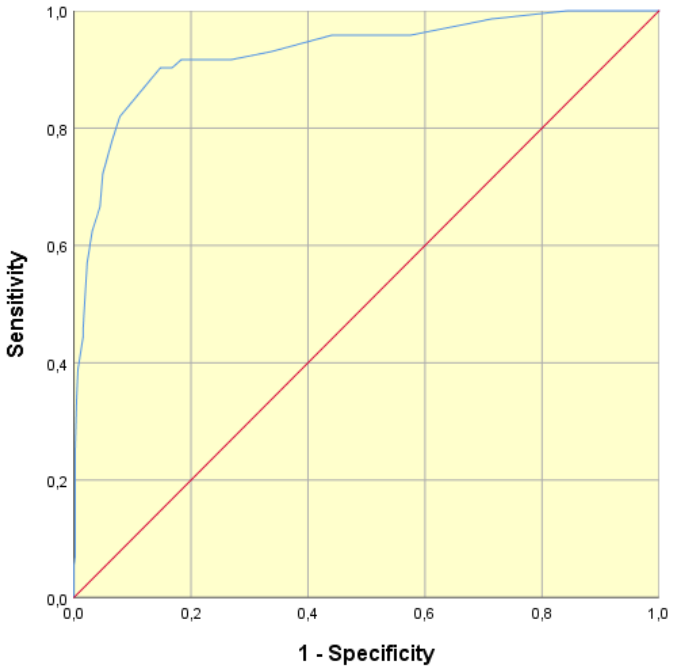

Figure 19. ROC curve of total PN duration for distinguishing healthy from infected neonates (AUC = 0.929, $p < 0.001$).

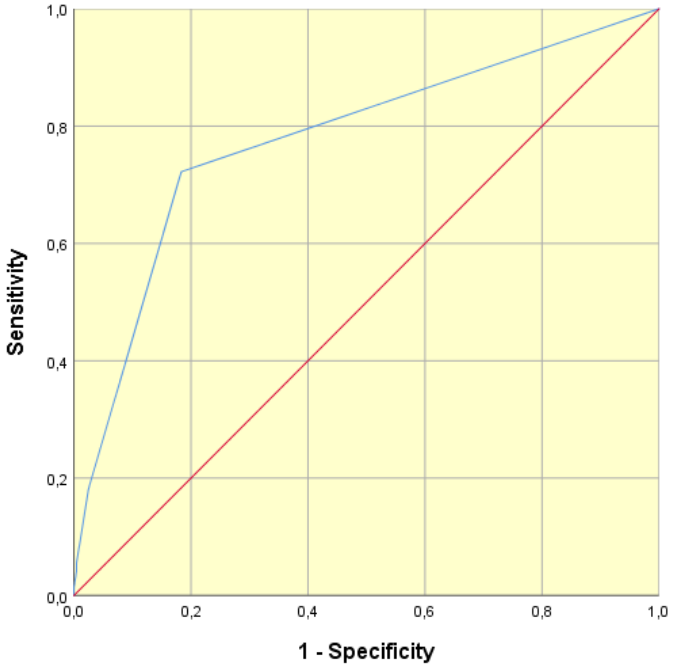

Figure 20. ROC curve of day of enteral feeding initiation for distinguishing healthy from infected neonates (AUC = 0.777, $p < 0.001$).

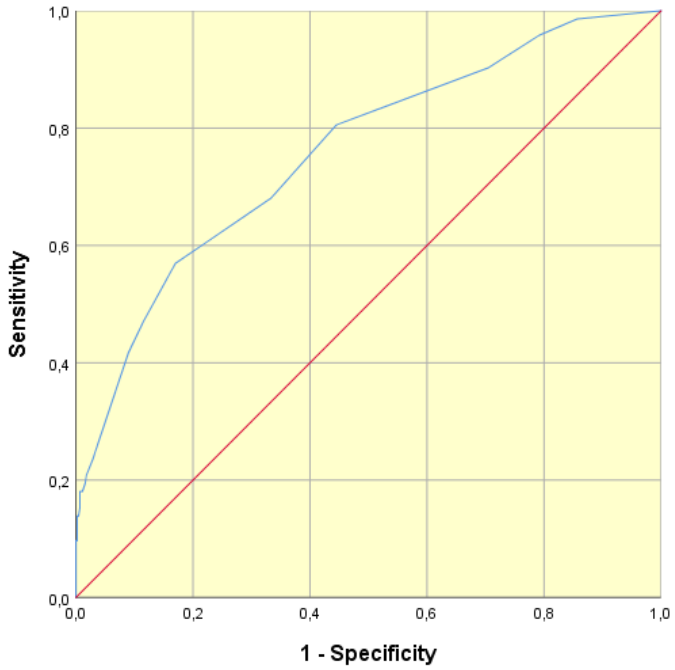

Figure 21. ROC curve of antibiotic treatment duration for distinguishing healthy from infected neonates (AUC = 0.758, $p < 0.001$).

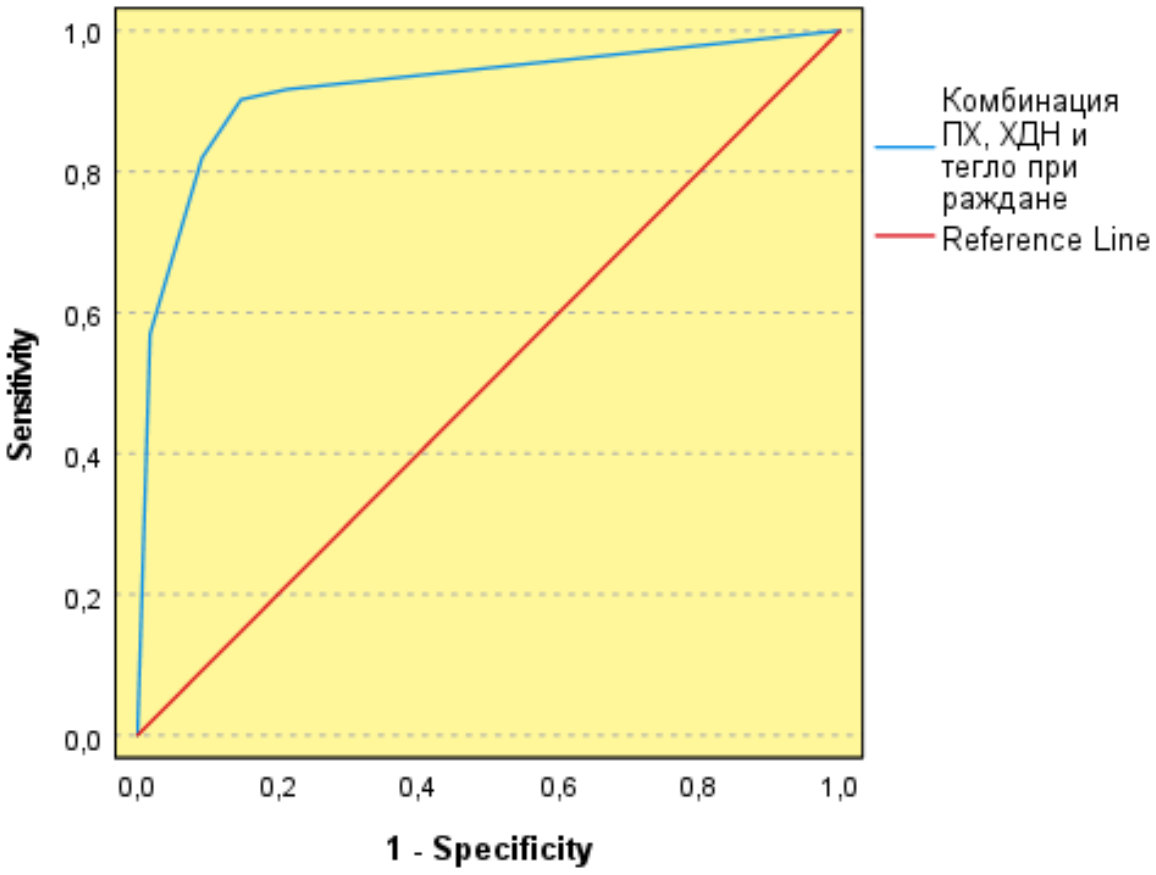

Figure 22. ROC curve of the combination PN days ≥ 9.5 + BPD + birth weight ≤ 1530 g (AUC = 0.918, $p < 0.001$).

Table 9. Threshold values of indicators and validation criteria.

Indicator	Threshold	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Birth weight (g)	≤ 1530	83	85	47	97
Gestational age (weeks)	≤ 31.5	78	85	45	96
Mechanical ventilation (days)	≥ 4.5	75	93	64	96
CVC duration (days)	≥ 0.5	60	90	50	93
Total PN duration (days)	≥ 9.5	90	85	50	98
Day of enteral feeding initiation	≥ 1.5	72	82	39	95
Antibiotic treatment duration (days)	≥ 7.5	57	83	35	92
Combination*	≥ 0.163	90	85	50	98

* Combination includes: PN days ≥ 9.5, BPD, birth weight ≤ 1530 g.

Table 10. Odds ratios (OR) and 95% CI for significant risk factors of HAIs.

Indicator	Comparison	OR	95% CI (Lower–Upper)	p-value	Notes
Total PN duration	≥ 9.5 vs < 9.5 days	53.60	23.56 – 121.99	<0.001	OR adj = 11.77 (3.83–36.22)
BPD	Yes vs No	64.37	28.69 – 144.43	<0.001	OR adj = 8.12 (2.37–27.83)
Birth weight	≤ 1530 vs > 1530 g	61.69	27.48 – 138.45	<0.001	OR adj = 3.10 (1.02–9.47), p = 0.047
Mechanical ventilation	≥ 4.5 vs < 4.5 days	41.70	21.78 – 79.84	<0.001	
Birth asphyxia	Moderate vs No	8.53	4.47 – 16.28	<0.001	
Birth asphyxia	Severe vs No	24.72	11.59 – 52.71	<0.001	
Gastric tube (last 3 days)	Yes vs No	14.22	6.04 – 33.47	<0.001	
CVC duration	≥ 0.5 vs < 0.5 days	13.93	7.91 – 24.54	<0.001	
Day of enteral feeding	≥ 1.5 vs < 1.5	11.57	6.55 – 20.44	<0.001	
IVH	Yes vs No	11.34	4.23 – 30.35	<0.001	
Gestational age	≤ 31.5 vs > 31.5 weeks	11.23	5.51 – 22.90	<0.001	
Anemia prior to episode	Yes vs No	10.14	5.87 – 17.50	<0.001	
CVC (last 3 days)	Yes vs No	7.05	3.91 – 12.70	<0.001	

Antibiotic duration	≥ 7.5 vs < 7.5 days	6.46	3.81 – 10.94	<0.001
Ventilation type	Non-invasive vs No	5.94	3.27 – 10.80	<0.001
Ventilation type	Invasive vs No	5.53	2.65 – 11.52	<0.001

Regression Model

To account for the combined influence of significant indicators and eliminate confounding factors, a multiple regression equation was derived using the backward conditional method. Three indicators remained in the final model: total PN duration ≥ 9.5 days, presence of BPD, and birth weight ≤ 1530 g. All maintained their direction of influence and statistical significance, though birth weight had borderline significance ($p < 0.1$). The strongest risk factor was PN duration ≥ 9.5 days (OR ≈ 15), followed by BPD (OR = 9.15) and birth weight ≤ 1530 g (OR = 2.27). The model's accuracy was 92.5%.

The final predictive equation was:

$$Z = 2.685 \times (\text{PN} \geq 9.5 \text{ days}) + 2.214 \times (\text{BPD}) + 0.818 \times (\text{Birth weight} \leq 1530 \text{ g}) - 4.103$$

Where binary variables take a value of 1 if the condition is met, and 0 otherwise.

For this model, the AUC = 0.918 ($p < 0.001$), with some of the highest validation criteria (Table 9).

3. Results and Discussion on Task 3

Objective:

To evaluate the initial clinical symptoms of healthcare-associated infections (HAIs) and their correlation with the type of microbiological pathogen, disease severity, and clinical outcome. Additionally, to assess the most common laboratory abnormalities at diagnosis.

3.1. Symptoms and Indicators in the Infected Neonates Group

3.1.1. Descriptive Statistics

Based on literature review and clinical experience, **11 clinical symptoms** were selected to assess their significance for infection diagnosis. Data from duty physician examinations at the onset of each nosocomial infection (nHAI) episode were used.

Frequency analysis revealed (Figure 23):

- The most frequent symptom was **poor thriving/weight loss** (69.4%),
- followed by **need for respiratory support or increased oxygen demand** (65.3%),
- and **apnea/dyspnea** (61.1%).
- The least frequent were **depressed consciousness** (13.9%) and **irritability/seizures** (11.1%).

Figure 23. Frequency distribution of symptoms in the infected neonates group.

Late-onset sepsis (LOS), the leading clinical diagnosis among HAIs in this study, manifests with subtle and nonspecific symptoms, making early diagnosis

challenging. Commonly reported symptoms include increased need for respiratory support, prolonged capillary refill time, and grayish skin tone. Other signs may include temperature instability, apnea, tachycardia, dyspnea, feeding difficulties, and irritability, though these are considered less specific (Bekhof et al., 2013b).

Some studies have found that traditionally associated symptoms such as temperature instability and apnea may be too nonspecific for reliable LOS diagnosis in preterm neonates (Bekhof et al., 2013b). This underscores the complexity of diagnosing LOS based solely on clinical signs. Efforts have been made to develop nomograms or artificial neural networks predicting sepsis probability in neonates based on clinical assessment alone, even without laboratory tests (Bekhof et al., 2013b). Although requiring external validation in larger cohorts, such models may aid in antibiotic decision-making when blood cultures are unavailable.

Our findings show predominance of certain early clinical symptoms in >50% of infections:

- poor thriving/weight loss,
- need for respiratory support or increased oxygen demand,
- apnea/dyspnea,
- and reduced motor activity.

Respiratory system manifestations were also dominant in Bekhof's study, although **skin color changes** ranked higher there, while in our cohort they were observed less frequently (33.8%).

For comparison, in a cohort of 497 neonates in Ljubljana, Slovenia (But et al., 2023), no specific clinical pattern of sepsis was established. In fact, frequent absence of clinical signs was observed, despite higher prevalence of signs like temperature instability, tachycardia, tachypnea, prolonged capillary refill, irritability, lethargy, and jaundice. None exceeded 50% prevalence except irritability (53%).

3.2. Correlation of Symptoms with Disease Severity, Outcome, and Hospital Stay

To determine which symptoms are most concerning, correlations were tested between the clinical symptoms and indicators of disease severity, outcome, and hospital stay duration.

- *Figure 24* shows that the majority of infected neonates had **moderate disease severity** (63.9%), followed by those with **shock** (26.4%), and the fewest had **mild disease** (9.7%).

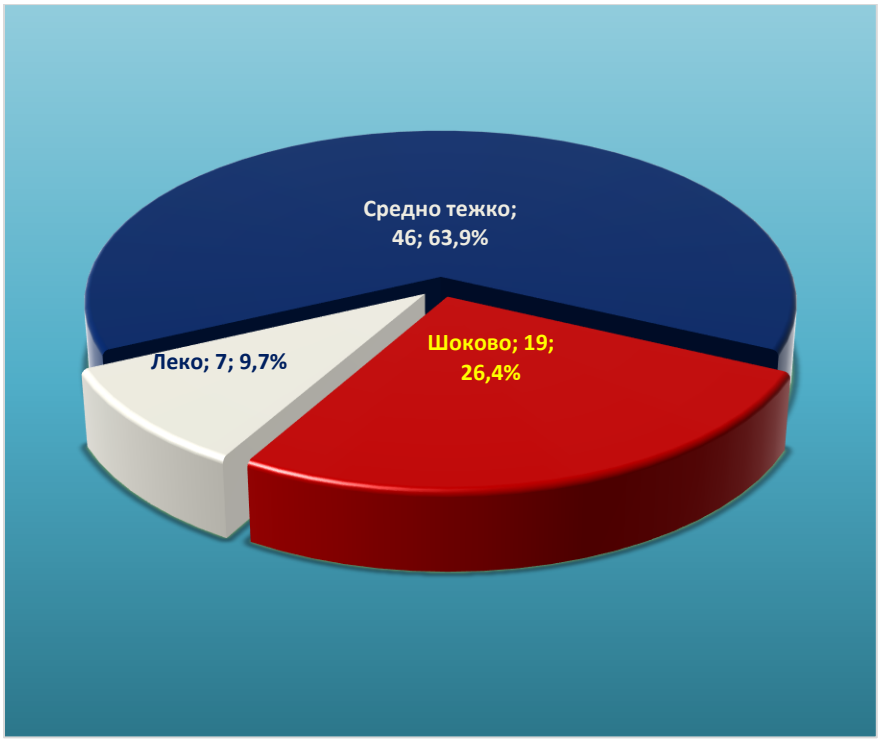

Figure 24. Distribution of infected neonates by disease severity.

- *Figure 25* shows outcomes: the majority **recovered** (76.4%), followed by those with **complications/disabilities** (15.3%), while **7 neonates died** (8.3%).

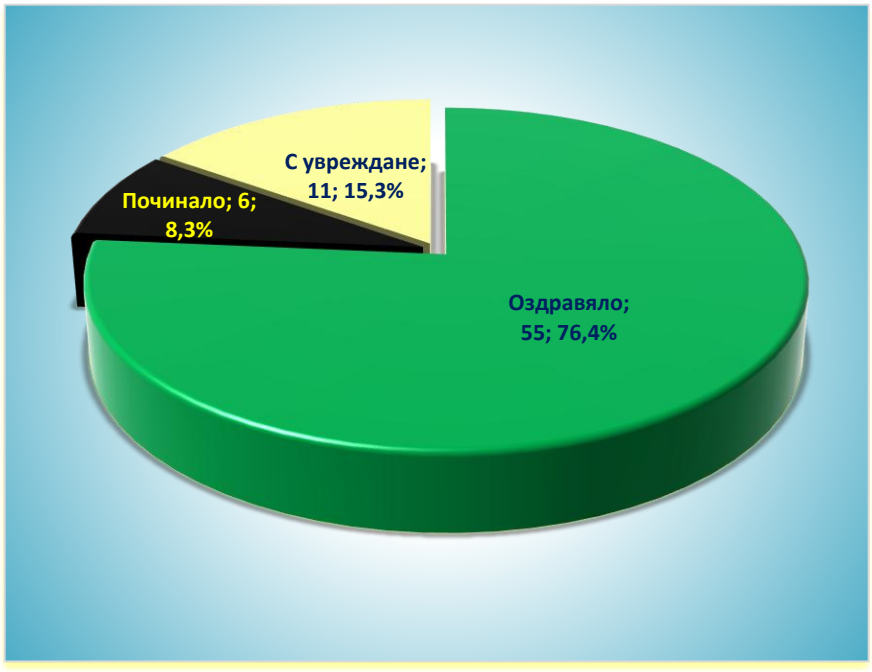

Figure 25. Distribution of infected neonates by outcome.

- *Figure 26* and a one-sample Kolmogorov–Smirnov nonparametric test confirmed that hospital stay duration in infected neonates had a **normal distribution** with mean **45.71 ± 23.73 days** (range 6–113).

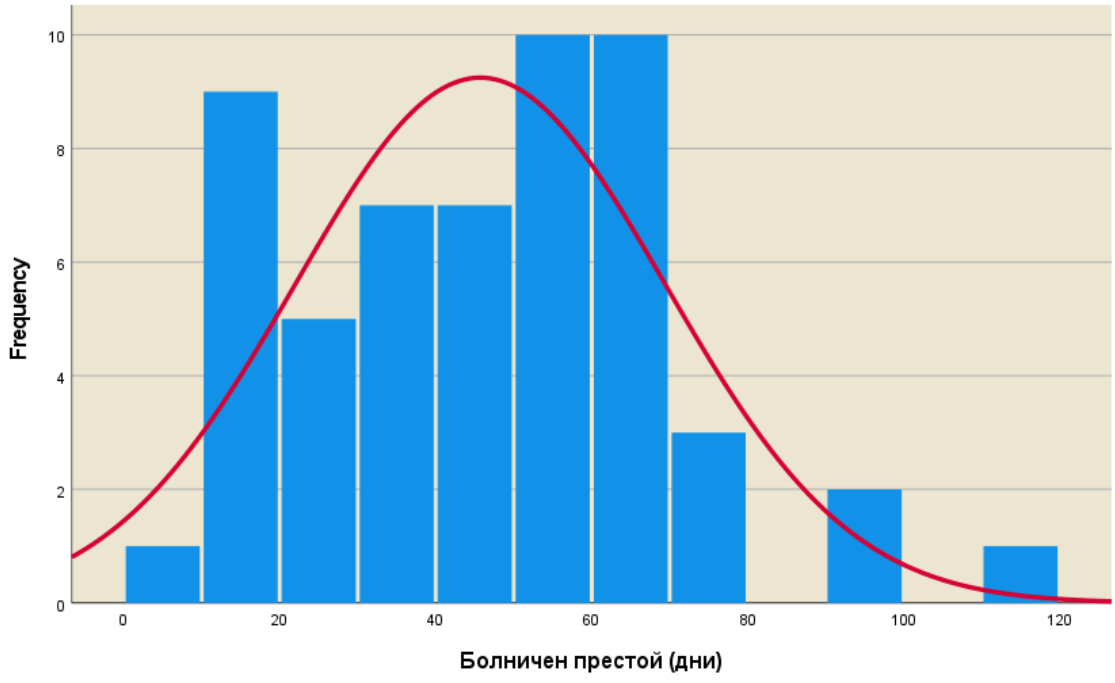

Figure 26. Distribution of hospital stay duration among infected neonates.

Results and Discussion on Task 3

Figures 24–26 illustrated the distribution of infected neonates by disease severity, outcome, and hospital stay.

Table 11 shows that seven of the eleven investigated symptoms were significantly associated with disease severity, namely: apnea/dyspnea; need for respiratory support or increased oxygen demand; skin color changes (pale/grayish skin); abdominal distension; reduced motor activity; depressed consciousness; and poor thriving/weight loss.

Apnea/dyspnea, skin color changes, and poor thriving/weight loss were significantly more frequent among neonates with shock compared to mild cases, while the moderate group did not differ statistically from the other two. Need for respiratory support or increased oxygen demand and reduced motor activity demonstrated a statistically significant upward trend with increasing severity. Abdominal distension was absent in mild cases, significantly differentiating them from moderate and shock groups, which did not differ significantly from each other. Depressed consciousness was significantly more frequent in shock compared to moderate, but not compared to mild, which did not differ statistically from the others. For the remaining symptoms (tachycardia, vomiting, irritability/seizures, and hypo-/hyperthermia), group differences were statistically insignificant.

Table 11. Association of symptoms with disease severity.

Symptom	Mild n (%)	Moderate n (%)	Shock n (%)	p (0–1)	p (0–2)	p (1–2)
Apnea/dyspnea	2 (28.6)	27 (58.7)	15 (78.9)	0.140	0.019	0.124
Need for resp. support	1 (14.3)	28 (60.9)	18 (94.7)	0.022	<0.001	0.007
Skin color changes	0 (0.0)	15 (33.3)	9 (47.4)	0.073	0.027	0.291
Tachycardia	0 (0.0)	14 (31.1)	5 (26.3)	0.087	0.139	0.703
Abdominal distension	0 (0.0)	19 (41.3)	8 (42.1)	0.036	0.043	0.953
Vomiting	1 (14.3)	19 (41.3)	4 (21.1)	0.174	0.702	0.124
Reduced motor activity	0 (0.0)	22 (47.8)	15 (78.9)	0.018	<0.001	0.022
Depressed consciousness	0 (0.0)	4 (8.7)	6 (31.6)	0.421	0.096	0.021
Irritability/seizures	0 (0.0)	6 (13.0)	2 (10.5)	0.316	0.382	0.728
Poor thriving/weight loss	2 (28.6)	31 (67.4)	17 (89.5)	0.051	0.002	0.067
Hypo-/hyperthermia	0 (0.0)	10 (21.7)	4 (21.1)	0.175	0.196	0.958

*Identical superscript letters across rows indicate lack of significant difference; different letters indicate significance ($p < 0.05$).

Table 12. Association of symptoms with clinical outcome.

Symptom	Recovered n (%)	Deceased n (%)	With disability n (%)	p (0–1)	p (0–2)	p (1–2)
Apnea/dyspnea	31 (56.4)	4 (66.7)	9 (81.8)	0.631	0.118	0.496
Need for resp. support	32 (58.2)	6 (100.0)	9 (81.8)	0.047	0.144	0.280
Skin color changes	16 (29.6)	4 (66.7)	4 (36.4)	0.070	0.658	0.246
Tachycardia	14 (25.9)	3 (50.0)	2 (18.2)	0.218	0.592	0.182
Abdominal distension	19 (34.5)	3 (50.0)	5 (45.5)	0.456	0.492	0.863
Vomiting	20 (36.4)	0 (0.0)	4 (36.4)	0.074	1.000	0.101
Reduced motor activity	23 (41.8)	6 (100.0)	8 (72.7)	0.007	0.063	0.171
Depressed consciousness	3 (5.5)	4 (66.7)	3 (27.3)	<0.001	0.023	0.126
Irritability/seizures	6 (10.9)	0 (0.0)	2 (18.2)	0.398	0.502	0.280
Poor thriving/weight loss	35 (63.6)	6 (100.0)	9 (81.8)	0.074	0.246	0.280
Hypo-/hyperthermia	10 (18.2)	2 (33.3)	2 (18.2)	0.381	1.000	0.496

Three of the eleven symptoms were significantly associated with clinical outcome: need for respiratory support or increased oxygen demand, reduced motor activity, and depressed consciousness. The other symptoms showed no significant association.

Table 13. Association of symptoms with hospital stay duration.

Symptom	Absent (n) Mean ± SD	Present (n) Mean ± SD	p-value
Apnea/dyspnea	20; 34.25 ± 20.05	35; 52.26 ± 23.41	0.012
Need for resp. support	21; 38.67 ± 20.30	34; 50.06 ± 24.91	0.084
Skin color changes	35; 48.80 ± 24.04	19; 38.79 ± 22.31	0.140
Tachycardia	38; 47.08 ± 26.07	16; 41.00 ± 16.92	0.315
Abdominal distension	34; 46.91 ± 21.24	21; 43.76 ± 27.74	0.637
Vomiting	38; 40.39 ± 23.86	17; 57.59 ± 19.15	0.011
Reduced motor activity	25; 55.76 ± 22.00	30; 37.33 ± 22.09	0.003
Depressed consciousness	48; 48.65 ± 23.21	7; 25.57 ± 17.51	0.015
Irritability/seizures	49; 47.06 ± 24.39	6; 34.67 ± 14.35	0.231
Poor thriving/weight loss	20; 46.40 ± 17.97	35; 45.31 ± 26.71	0.872
Hypo-/hyperthermia	43; 48.58 ± 25.37	12; 35.42 ± 12.60	0.018

*Lack of statistical representativeness for groups with small n (marked with *).

Summary

The spectrum of disease severity ranged from moderate signs of infection to critical illness with severe organ dysfunction and potential multi-organ failure. The most severe or shock-like progression and highest lethality were observed in patients with need for respiratory support or increased oxygen demand, and reduced motor activity.

Conclusion

In such patients, clinicians must exercise heightened vigilance, ensuring timely expanded laboratory testing, hemodynamic monitoring, and a more aggressive antibiotic policy.

3.3. Evaluation of the Most Common Laboratory Abnormalities at the Diagnosis of nHAI

The next step in the study of healthcare-associated infections (nHAIs) was the evaluation of diagnostic markers routinely used in the Clinic. The goal was to determine which are the most frequent and most significant laboratory abnormalities at the onset of an nHAI episode.

Variation analysis of the quantitative blood parameters studied demonstrated that C-reactive protein (CRP) had the largest variation (112.8%), followed by white blood cell count (WBC, 68.3%), while the immature-to-total neutrophil ratio (I:T index) had the smallest variation (38.5%) (Table 14).

Table 14. Variation analysis of blood parameters.

Indicator	n	Mean	SD	Median	IQR (25–75)	Min	Max	V (%)
CRP (mg/L)	69	22.16	24.99	11.7	3.85 – 31.45	1.2	100	112.8
WBC ($\times 10^9$/L)	71	18.27	12.48	14.3	9.2 – 25.1	2.9	64.2	68.3
Platelets (G/L)*	72	224.57	131.68	201.5	129.25 – 311.25	33	652	58.6
I:T index*	55	0.39	0.15	0.4	0.29 – 0.49	0.05	0.74	38.5

*These indicators had normal distribution.

Frequency analysis (Figure 27) revealed: the I:T index had the highest proportion of abnormal values (92.7%), followed by CRP (73.9%). The lowest proportions of abnormalities were observed for platelet count (33.3%) and WBC (32.4%).

Thus, in suspected nHAI, reliance may be placed primarily on the I:T index, followed by CRP, while WBC and platelet count are less informative. These

findings are consistent with those of other authors (But et al., 2023). Unlike in older children and adults, the normal range of WBC in neonates is broad and therefore cannot serve as a precise diagnostic tool (Camacho-Gonzalez et al., 2013). Based on literature and our results, the I:T ratio may prove the most sensitive indicator of neonatal sepsis among hematologic indices (Celik et al., 2022). Serum CRP concentration remains one of the most important features for diagnosing sepsis, as values are significantly higher in infected neonates, although large variation exists—even nHAI cases with very low CRP values (1.2 mg/L) were observed.

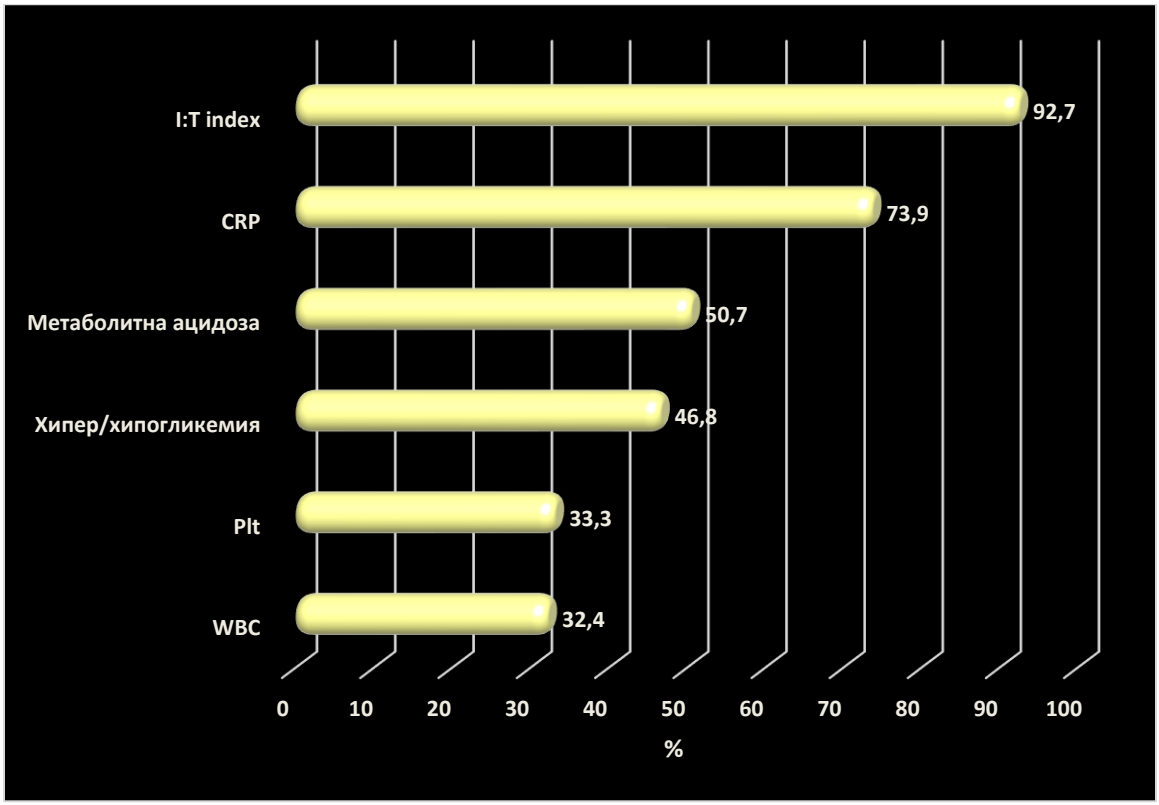

Figure 27. Frequency distribution of abnormal blood values and presence of hyper-/hypoglycemia and metabolic acidosis in the infected neonates group.

3.4. Analysis of the Relationship Between Infection Course, Outcome, and Pathogen Type

According to literature, Gram-negative sepsis manifests with faster symptom onset and more specific clinical markers, indicating potentially more severe progression (Gowda et al., 2017). Mortality is much higher for Gram-negative compared to Gram-positive infections (Makhoul et al., 2005). Staphylococci are usually

classified as less virulent pathogens and associated with lower mortality (Bizzarro et al., 2015).

Analysis of the relationship between pathogen type, infection course, and outcome did not reveal significant associations (Tables 15–16).

Table 15. Association between pathogen type and infection course (p=0.240).

Pathogen	Mild n (%)	Moderate n (%)	Shock n (%)	Total n
Klebsiella	1 (33.3)	10 (47.6)	4 (36.4)	15
Staphylococcus	2 (66.7)	8 (38.1)	2 (18.2)	12
E. coli	0 (0.0)	3 (14.3)	5 (45.5)	8

Table 16. Association between pathogen type and outcome (p=0.107).

Pathogen	Recovered n (%)	Deceased n (%)	Disabled n (%)	Total n
Klebsiella	9 (40.9)	1 (25.0)	5 (55.6)	15
Staphylococcus	10 (45.5)	0 (0.0)	2 (22.2)	12
E. coli	3 (13.6)	3 (75.0)	2 (22.2)	8

No significant association was found between pathogen type and infection course or outcome. However, mortality was highest in E. coli infections, while Klebsiella infections were more frequently associated with disability.

Table 17. Association between symptoms/lab abnormalities and pathogen type.

Indicator	Klebsiella n (%)	Staphylococcus n (%)	E. coli n (%)	p-value
Apnea/dyspnea	8 (53.3)	6 (50.0)	8 (100.0)	0.049
Need for resp. support	9 (60.0)	6 (50.0)	7 (87.5)	0.272
Skin color changes	6 (40.0)	4 (33.3)	3 (37.5)	1.000
Abnormal platelets	9 (60.0)	1 (8.3)	1 (12.5)	0.010

Significant associations were observed only for apnea/dyspnea and abnormal platelet count. Apnea/dyspnea was significantly more frequent in E. coli

infections, while thrombocytopenia was significantly more frequent in Klebsiella infections.

These findings suggest that when apnea/dyspnea is present, Escherichia coli is statistically the most likely pathogen, and appropriate empiric antibiotic therapy should be considered. Thrombocytopenia at the onset of a nosocomial infection episode points toward Klebsiella pneumoniae as the probable causative agent. Some authors have also observed thrombocytopenia and coagulation abnormalities in Klebsiella sepsis (De Stoppelaar et al., 2014). Interestingly, thrombocytopenia is a frequent finding in sepsis and is associated with worse outcomes. In a murine model of K. pneumoniae sepsis, severe thrombocytopenia ($<5 \times 10^9$/L) correlated with decreased survival, enhanced bacterial growth, and hemorrhage at the site of primary infection. Recent studies even suggest a protective role of platelets against K. pneumoniae, as activated platelets can enhance monocyte-mediated bacterial killing, possibly through the formation of platelet–monocyte aggregates (Gautam et al., 2023).

4. Results and Discussion on Task 4

Diagnostic Value of Additional Serum Biomarkers of Inflammation

The objective was to determine the diagnostic value of certain additional serum biomarkers of inflammation—procalcitonin (PCT), interleukin-6 (IL-6), interleukin-8 (IL-8), and endocan (ESM-1)—and to compare them with routinely used laboratory methods.

For this purpose, Stage II of the study was conducted, including 60 neonates, of whom 42 (70%) were male and 18 (30%) were female (Figure 28). The mean gestational age was 29.75 ± 3.61 weeks, ranging between 25 and 40 weeks.

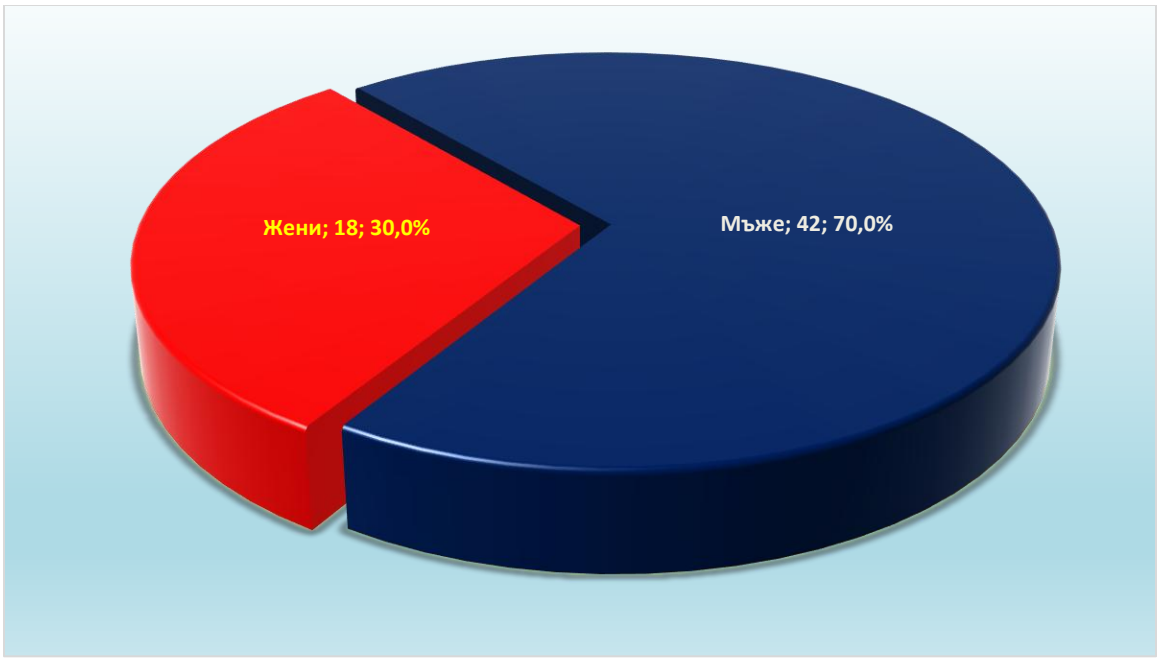

Figure 28. Frequency distribution of the study cohort by sex.

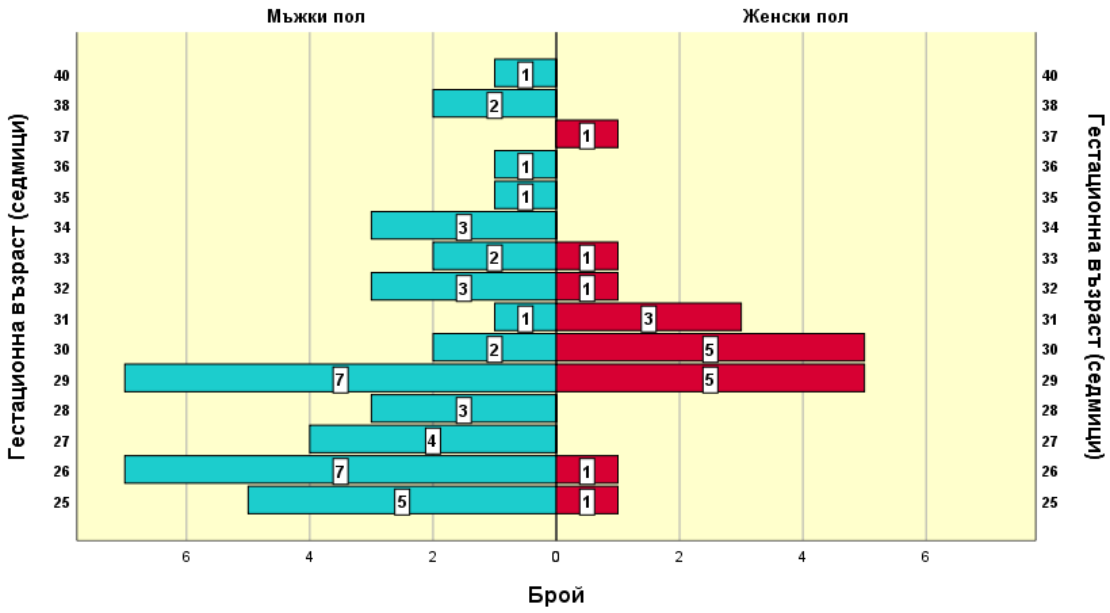

Figure 29. Distribution of the study cohort by sex and gestational week.

4.1. Distribution of the Study Cohort by Main Groups

The sample was divided into three main groups (Figure 30):

• Controls (n=19; 31.7%): neonates without symptoms of indicator nosocomial infection.

• Symptomatic group (n=41; 68.3%): neonates with newly appeared symptoms suspicious for indicator HAI.
– Group A (Symptomatic Infected, n=21; 35.0%): neonates with symptoms of indicator HAI, subsequently confirmed paraclinically.
– Group B (Symptomatic Non-infected, n=20; 33.3%): neonates with symptoms of indicator HAI, not confirmed paraclinically.

Figure 30. Frequency distribution of the study cohort by main groups.

4.2. Causative Agents of Infection

Microbiological samples were positive in 66% of infected patients (14/21). The most common pathogen responsible for HAI was Gram-negative Klebsiella pneumoniae (7/14; 50%), followed by Gram-positive Staphylococcus spp. (3/14; 21%). Less frequent pathogens included Escherichia coli, Serratia spp., and Enterobacter spp.

4.3. Comparative Analysis of the Main Groups by Biomarker Values

A comparative analysis of patients from the main groups was conducted using the values of PCT, ESM-1, IL-6, IL-8, and the indicators CRP, I:T index, platelet count (PLT), and white blood cell count (WBC). The results (Table 18) showed:
• The three groups differed significantly in four indicators: PCT, IL-6, I:T index, and PLT.

• For PCT and IL-6, Group A had significantly higher mean values compared to the other two groups, whose means did not differ statistically.
• For I:T index, Group A had significantly higher mean values compared to Group B, but not compared to controls.
• For ESM-1, IL-8, and CRP, differences among groups were statistically insignificant.

Table 18. Comparative analysis of biomarker values (mean ± SD).

Biomarker	Group 0 Controls	Group A Infected	Group B Non-infected	p-value	Notes
PCT (ng/mL)	0.43 ± 0.57a	2.27 ± 3.22b	0.44 ± 0.58a	$p<0.01$	a≠b
ESM-1 (pg/mL)	118.21 ± 84.00a	163.52 ± 125.11a	116.00 ± 85.33a	NS	no sig. diff.
IL-6 (pg/mL)	29.81 ± 104.02a	30.72 ± 53.48b	5.07 ± 5.39a	$p<0.05$	a≠b
IL-8 (pg/mL)	45.25 ± 106.80a	141.98 ± 346.59a	27.62 ± 40.20a	NS	no sig. diff.
CRP (mg/L)	3.96 ± 2.78a	21.24 ± 30.13a	4.83 ± 2.52a	NS	no sig. diff.
I:T index	0.29 ± 0.21ac	0.35 ± 0.16bc	0.24 ± 0.11a	$p<0.05$	a≠b
PLT ($\times 10^9$/L)	384.00 ± 136.13a	265.57 ± 167.86bc	321.90 ± 126.00ac	$p<0.05$	a≠b≠c
WBC ($\times 10^9$/L)	12.59 ± 4.01a	16.87 ± 13.34a	13.20 ± 4.54a	NS	no sig. diff.

* Identical superscript letters within a row indicate lack of significant difference; different letters indicate presence of significant difference ($p<0.05$).

4.4. Threshold Values of Infection Biomarkers

To determine threshold values distinguishing Group A (infected) from Groups 0 and B (controls and non-infected), ROC curve analysis was applied for PCT, ESM-1, IL-6, IL-8, CRP, I:T index, and PLT. Figures 31–38 show that significant thresholds were established for PCT, IL-6, I:T index, and PLT. The thresholds were selected according to the Youden index = [maximum (sensitivity + specificity – 1)].

Established thresholds:
• PCT ≥ 0.46 ng/mL
• IL-6 ≥ 4.97 pg/mL
• I:T index ≥ 0.335
• PLT ≤ 161.5 ×10⁹/L

Additionally, normative cut-offs were assessed for PCT (≥0.5) and I:T index (≥0.25), as well as the combination of four variables: PCT ≥0.46 + IL-6 ≥4.97 + I:T index ≥0.335 + PLT ≤161.5.

Logistic Regression Models

For the four-variable combination, binary logistic regression yielded the following equation:

$$Z = 1.389 \times (\text{PCT} \geq 0.46) + 1.371 \times (\text{IL-6} \geq 4.97) + 1.637 \times (\text{I:T index} \geq 0.335) + 3.240 \times (\text{PLT} \leq 161.5) - 3.232$$

Variables take value 1 if they meet the threshold, 0 otherwise. For example, if PCT ≥0.46, then the multiplier is active, otherwise it is 0.

For this model, AUC = 0.880, $p<0.001$ (Figure 39).

A simplified model with only two variables (PCT ≥0.46 and IL-6 ≥4.97) was also tested:

$$Z = 1.636 \times (\text{PCT} \geq 0.46) + 1.646 \times (\text{IL-6} \geq 4.97) - 2.363$$

For this model, AUC = 0.803, $p<0.001$ (Figure 40). Although less precise and specific, the two-variable model retained high sensitivity (94%) and negative predictive value (95%), making it clinically applicable.

Figures 31–40 show ROC curves for each biomarker and their combinations.

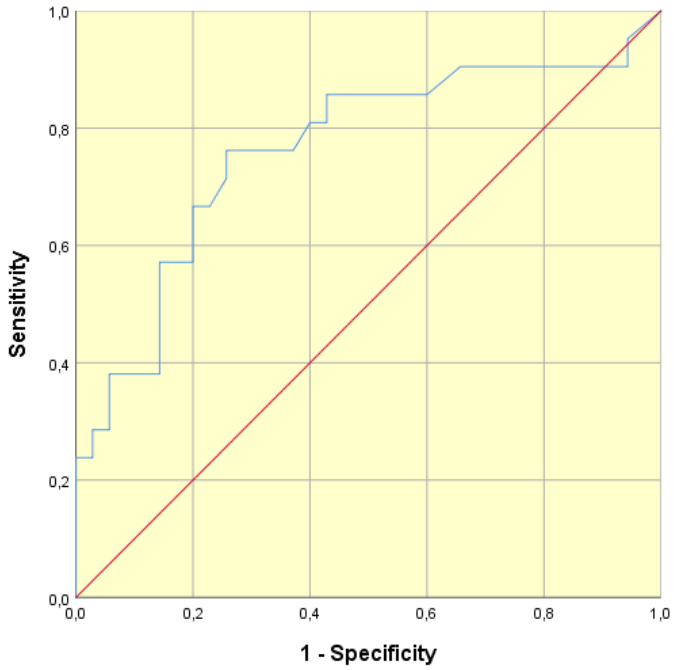

Figure 31. ROC curve of PCT for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.763, p = 0.001).

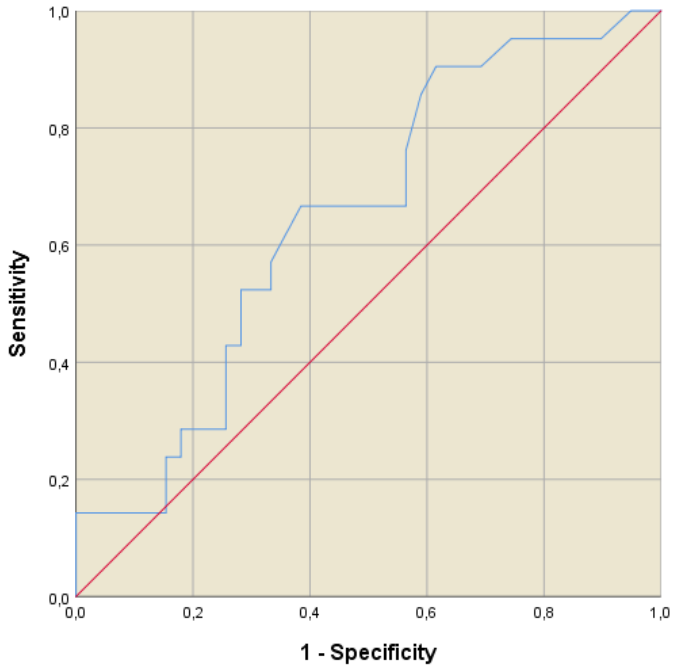

Figure 32. ROC curve of ESM-1 for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.648, p = 0.061).

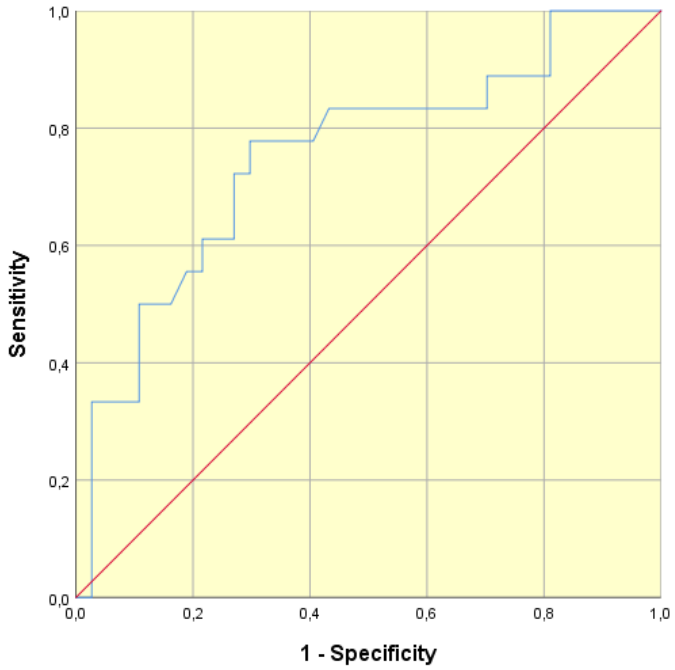

Figure. 33. ROC curve of IL-6 for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.752, p = 0.003).

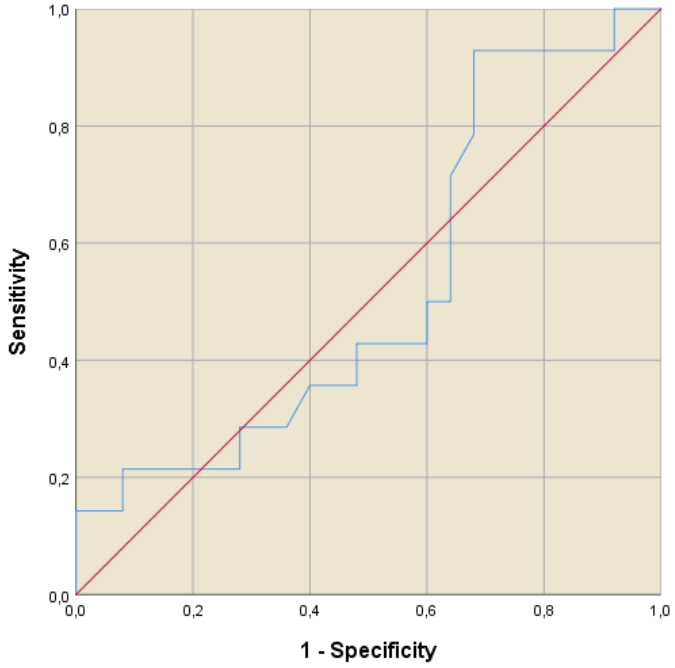

Figure 34. ROC curve of IL-8 for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.523, p = 0.815).

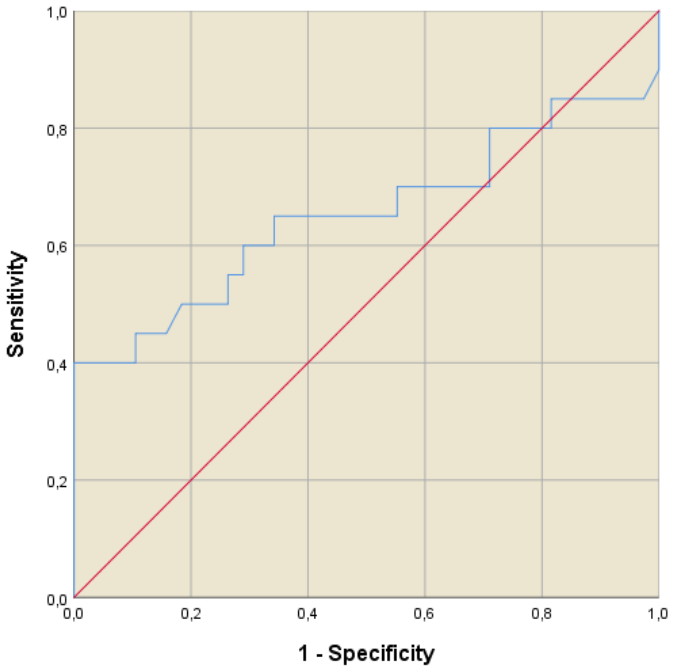

Figure 35. ROC curve of CRP for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.653, p = 0.058).

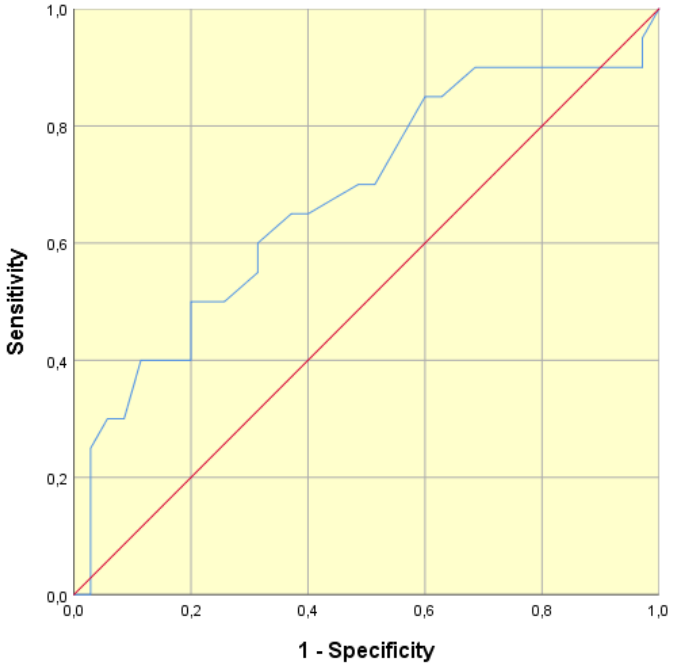

Figure 36. ROC curve of the I:T index for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.677, p = 0.030).

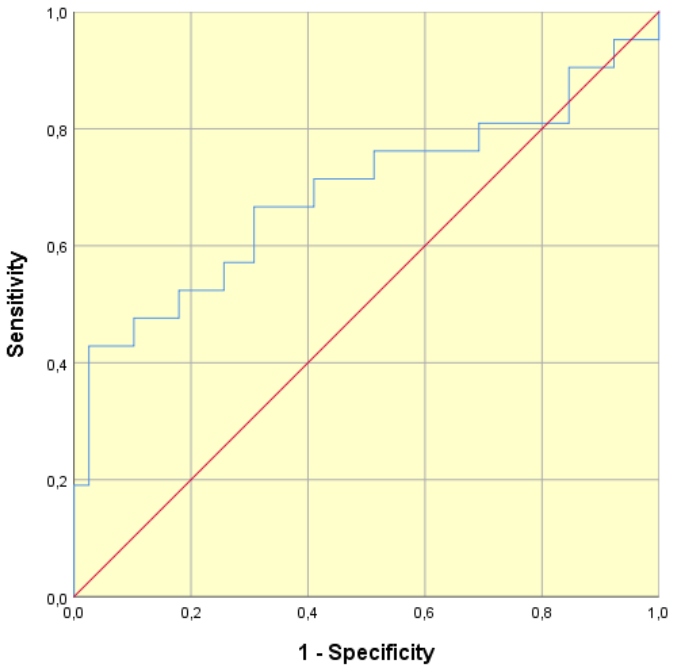

Figure 37. ROC curve of platelets for determining the threshold value for distinguishing infected from uninfected study participants (area under the curve 0.690, p = 0.016).

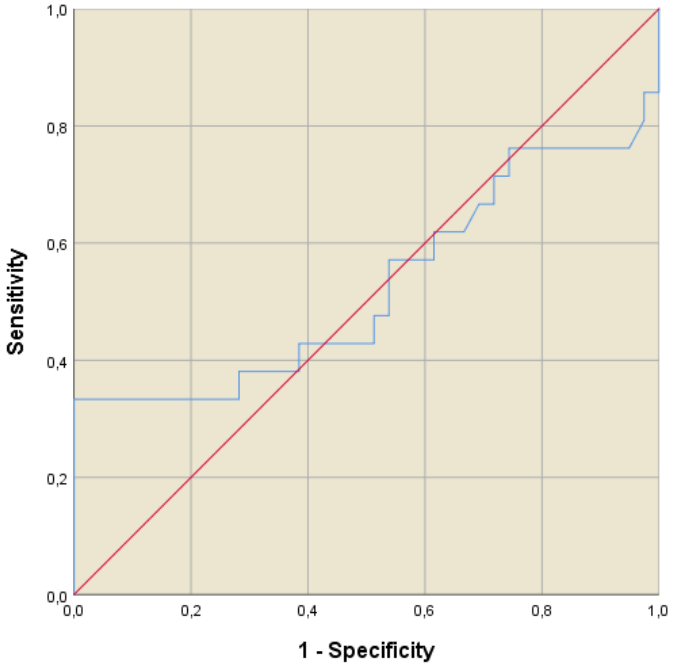

Figure 38. ROC curve of leukocytes for determining the threshold value for distinguishing infected from uninfected study participants (area under the curve 0.526, p = 0.739).

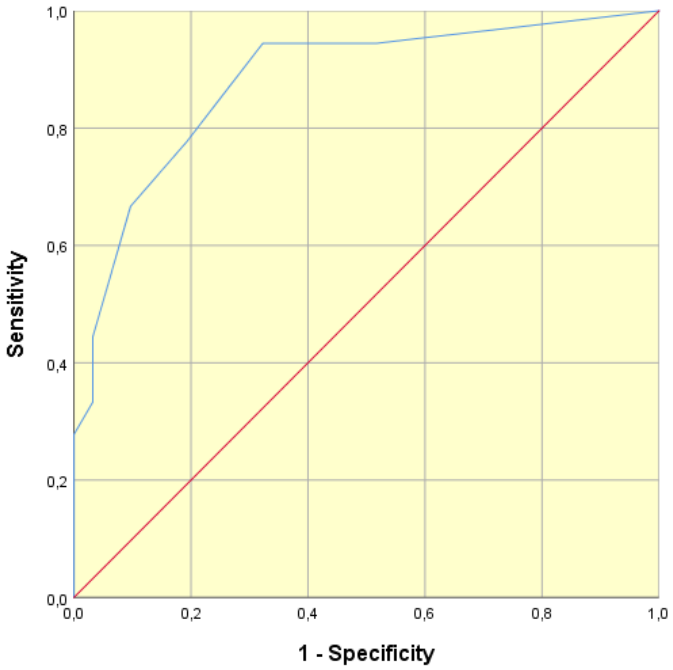

Figure 39. ROC curve of the combination PCT ≥0.46 + IL-6 ≥4.97 + I:T index ≥0.335 + Plt ≤161.5 for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.880, p <0.001).

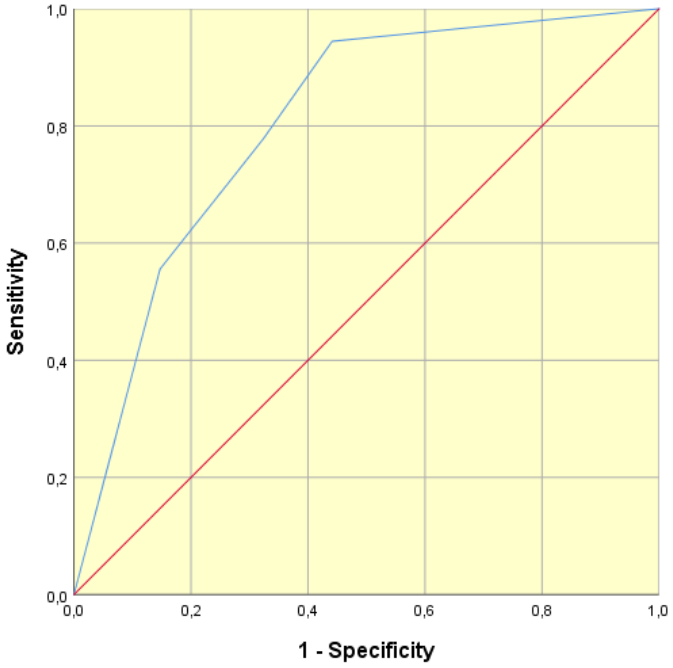

Figure 40. ROC curve of the combination PCT ≥0.46 + IL-6 ≥4.97 for determining the threshold value for distinguishing group 1 from groups 0 and 2 (area under the curve 0.803, p <0.001).

Validation Criteria for Biomarker Thresholds

For the established thresholds, validation criteria were assessed (Table 19):

• Best sensitivity (94%) and negative predictive value (95%) were achieved with the binary logistic regression models using four and two variables.

• Best specificity (97%) and positive predictive value (90%) were observed for platelet count.

• Best accuracy (78%) was demonstrated for platelet count and for the four-variable combination.

These results suggest that the choice of biomarker or biomarker combination may depend on the specific diagnostic goal—maximizing one of the validation criteria.

Table 19. Threshold values of markers (PCT, IL-6, I:T index, PLT, and combinations) and validation criteria.

Indicator	Threshold	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
PCT	≥0.46*	76	74	64	84	75
PCT	≥0.5	71	74	63	81	73
IL-6	≥4.97*	78	70	56	87	73
I:T index	≥0.335*	50	80	59	74	69
I:T index	≥0.25	20	97	80	68	69
Platelets (G/L)	≤161.5	43	97	90	76	78
PCT + IL-6 + I:T index + PLT	≥0.276*	94	68	63	95	78
PCT + IL-6	≥0.206*	94	56	53	95	69

*Threshold values were calculated from the present study.

According to our results, patients with indicator HAIs showed significantly higher frequency of leukopenia/leukocytosis and thrombocytopenia. Platelet count demonstrated the best specificity (97%), positive predictive value (90%), and accuracy (78%). A possible correlation can be suggested with the prevailing

Gram-negative bacteria causing nosocomial infections in the studied NICU. Some reports indicate differences in platelet response between infections with Gram-negative and Gram-positive microorganisms (Akarsu et al., 2005).

Unexpectedly, the I:T index also performed very well, with specificity of 97% and positive predictive value of 80% (threshold ≥0.25), although it is usually considered a 'late' marker of inflammation. This relatively strong performance may also reflect the expertise of the clinical laboratory team.

C-reactive protein (CRP) is an acute-phase protein often interpreted alongside complete blood count. Typically, it is not elevated in the early stage of infection. As hypothesized, in this analysis it did not reach statistically significant levels. Its peak is reached after 24 hours, meaning sensitivity is lowest in the early stage but improves at 24–48 hours after symptom onset. Specificity and positive predictive value range from 93 to 100%, which is why CRP is considered a 'specific' but 'late' marker for neonatal infection (Shah & Padbury, 2014).

Significant research is currently focused on serum biomarkers of inflammation to develop highly sensitive 'early' methods for detecting infection. These include PCT, IL-6, IL-8, and ESM-1, whose concentrations rise in the first hours of the inflammatory response. While showing promise, results from prior studies remain inconsistent, and standardization of methodology is still lacking (Gude et al., 2022).

Procalcitonin (PCT) is now more widely used in neonatal infection diagnostics. It is produced by monocytes and hepatocytes in response to systemic inflammation, and may be more sensitive than CRP in bacterial infections (Eschborn & Weitkamp, 2019). However, PCT synthesis can also be induced nonspecifically after major surgery, trauma, or in the early neonatal period, which is why such neonates were excluded from the study (Atanasova et al., 2002). In neonatal sepsis, PCT levels rise within 4 hours of endotoxin exposure and peak at 6–8 hours, earlier than CRP. Its half-life is 25–30 hours and levels are independent of gestational age. PCT is considered a highly specific marker for diagnosis and monitoring of bacterial infections and sepsis, and reflects infection severity. It is also a marker of therapeutic success, as plasma levels decline 24 hours after initiation of treatment (Atanasova et al., 2002). Diagnostic profiles suggest sensitivity and specificity of 87–100% for systemic bacterial infections and necrotizing enterocolitis. In a prospective observational cohort of 53 neonates with

suspected late-onset sepsis, PCT showed sensitivity of 88%, specificity of 71.4%, and NPV of 87% (Bustos & Araneda, 2012).

In Bulgaria, only a few neonatal centers currently test PCT levels routinely, which explains the limited research in this area. Most studies focus on early-onset sepsis rather than late-onset, and laboratory methods differ. For example, Georgieva et al. (2001) studied 87 neonates with early and late sepsis using semi-quantitative immunochromatography, showing sensitivity of 63% and specificity of 100% at >2 ng/mL.

In our study, with a threshold of >0.46 ng/mL, PCT demonstrated sensitivity of 76%, specificity of 74%, and NPV of 84%, which are comparable to literature values. This highlights the need to include PCT in the standard sepsis screening panel in Bulgaria.

Among cytokine biomarkers, IL-6 plays a key role in the early immune response. A review of studies from 1990 to 2020 showed IL-6 is the most frequently studied interleukin in neonates (Eichberger & Resch, 2022). Reported thresholds vary, with higher thresholds increasing specificity at the cost of sensitivity. Our mean (30.72) and threshold (≥27.5) values are similar to those reported by Adib et al., with sensitivity 78%, specificity 95%, PPV 100%, and NPV 87% at 30 pg/mL. In our study, Group A patients had significantly higher IL-6 levels than the other groups, consistent with prior literature.

For IL-8, results were less satisfactory. Although mean IL-8 levels were elevated in infected neonates (141.98 pg/mL), large variability limited diagnostic accuracy. Comparisons among groups were statistically insignificant. Literature suggests IL-8 rises early in infection, but in our cohort it lacked discriminative power.

Similarly, ESM-1 (endocan) did not perform well. Although it has been suggested as a promising biomarker in late neonatal sepsis, our results showed low levels without significant group differences or threshold values. Literature remains scarce, but some studies found ESM-1 levels elevated in sepsis with specificity and sensitivity around 94% (Buyuktiryaki et al., 2019). Our findings, however, do not confirm this.

In conclusion, introduction of PCT and IL-6 into routine diagnostics could improve timely detection of neonatal infections, optimize therapy, and reduce complications of HAIs. The I:T index and platelet count also provide valuable diagnostic information, particularly when interpreted alongside PCT and IL-6. Our

results suggest that ESM-1 and IL-8 are not reliable markers for late neonatal sepsis. Larger studies are needed to validate these findings and explore additional early biomarkers.

5. Results and Discussion on Task 5

Objective: To develop a prognostic model for identifying high-risk patients, supporting the early diagnosis of infections.

To date, the best combination of markers for predicting late neonatal sepsis with the highest level of validation criteria has not been identified. Well known to many clinicians is the neonatal calculator for early sepsis (Kaiser Neonatal Sepsis Calculator), which combines maternal risk factors and the baby's clinical presentation (Kuzniewicz et al., 2016). However, a similar diagnostic tool for late neonatal sepsis has not yet been approved. Some proposals have been made, such as the sepsis prediction scale by Sofouli et al. (2023), a retrospective study in Greece on 120 newborns with suspected sepsis combining eight clinical and laboratory parameters (temperature instability, decreased feeding volume, platelet count <150,000/mm³, glucose changes, CRP >1 mg/dL, worsening circulation and breathing). The model was then prospectively validated in 145 neonates, but results were again inconclusive, as many septic infants scored very low.

Based on the equations derived in Task 2 and Task 4 from stages I and II of our study, two prognostic models for infection risk were developed, combined in the tool NeoInfectCalc. For convenient and practical use, the calculator is available at: https://neoinfectcalc.mvision.info/

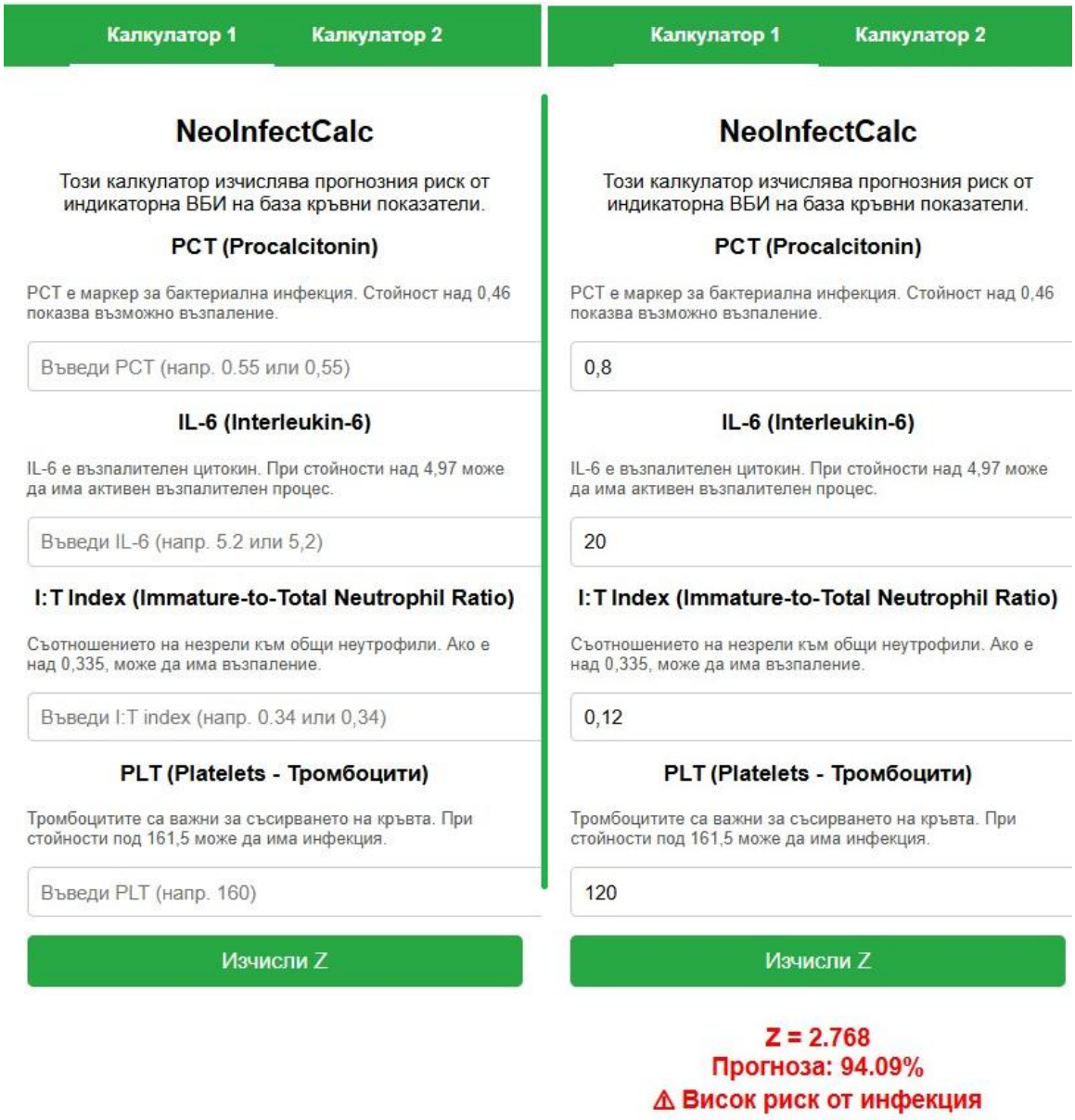

Figure 41. Use of NeoInfectCalc to calculate predicted risk of HAI based on blood indicators.

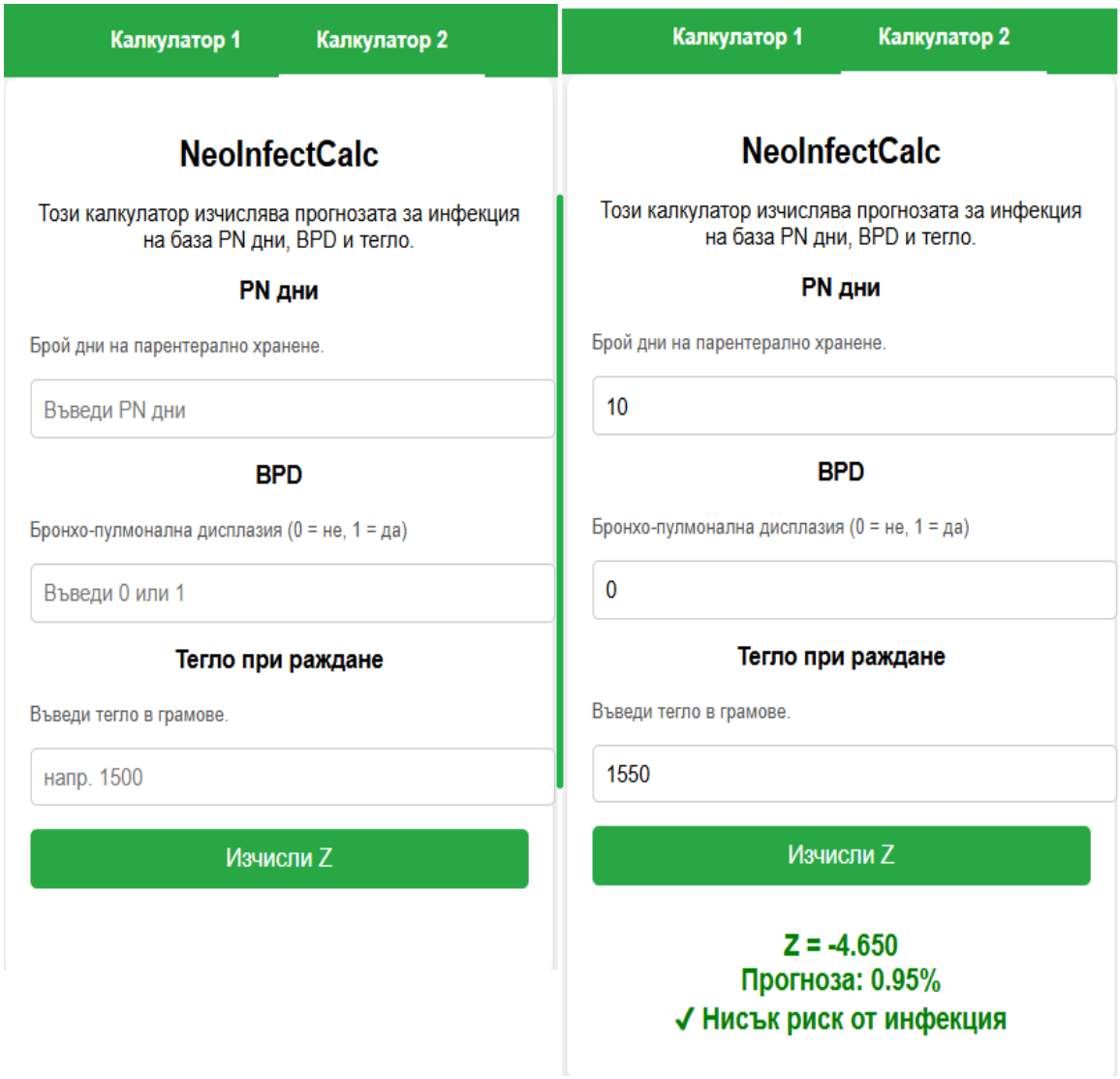

Figure 42. Use of NeoInfectCalc to calculate predicted risk of HAI based on risk factors. (*PN – parenteral nutrition; *BPD – bronchopulmonary dysplasia).

Model Based on Risk Factors

The first model includes risk factors: duration of parenteral nutrition (days), presence of bronchopulmonary dysplasia (BPD), and birth weight. From the equation in Task 2:

$$Z = 2.685 \times (\text{PN days} \geq 9.5) + 2.214 \times (\text{BPD present}) + 0.818 \times (\text{Birth weight} \leq 1530\ \text{g}) - 4.103$$

Where:

• PN days – number of days on parenteral nutrition.

• BPD – bronchopulmonary dysplasia (0 = absent, 1 = present).
• Birth weight – in grams.
• Z – logistic function, converted to percentage by the sigmoid function.
• P (%) – predicted risk of infection: P = 1 / (1 + e^(-Z)) × 100

Example for Patient A:
• PN days = 10 (≥9.5 → Yes → 1) → 2.685 × 1 = 2.685
• BPD = 1 → 2.214 × 1 = 2.214
• Birth weight = 1500 g (≤1530 → Yes → 1) → 0.818 × 1 = 0.818
Subtract 4.103

Z = 2.685 + 2.214 + 0.818 – 4.103 = 1.614

P = 1 / (1 + e^(-1.614)) × 100 ≈ 83.4%

Result: Predicted risk of infection = 83.4%

Model Based on Diagnostic Markers

The second model includes diagnostic markers: PCT, IL-6, I:T index, and platelet count (PLT). From the equation in Task 4:

Z = 1.389 × (PCT ≥ 0.46) + 1.371 × (IL-6 ≥ 4.97) + 1.637 × (I:T index ≥ 0.335) + 3.240 × (PLT ≤ 161.5) – 3.232

Where:
• PCT – procalcitonin (ng/mL).
• IL-6 – interleukin-6 (pg/mL).
• I:T index – immature-to-total neutrophil ratio.
• PLT – platelet count (×10^9/L).
• Z – logistic function, converted to percentage by the sigmoid function.
• P (%) – predicted risk of infection: P = 1 / (1 + e^(-Z)) × 100

Example for Patient 1:
• PCT = 0.5 (≥0.46 → Yes → 1) → 1.389 × 1 = 1.389
• IL-6 = 2.2 (<4.97 → No → 0) → 0
• I:T index = 0.3 (<0.335 → No → 0) → 0
• PLT = 100 (≤161.5 → Yes → 1) → 3.240 × 1 = 3.240
Subtract 3.232

$Z = 1.389 + 3.240 – 3.232 = 1.397$

$P = 1 / (1 + e^{(-1.397)}) \times 100 \approx 80.2\%$

Result: Predicted risk of infection = 80.2%

Depending on available patient data, either calculator may be used to provide a percentage estimate of risk for developing hospital-acquired infection. NeoInfectCalc may thus serve as a useful clinical tool to guide assessment of systemic infection risk at the initial suspicion of HAI. The model requires further validation in a prospective study on a new cohort of patients to track its practical utility.

IV. Conclusions

1. The average incidence of healthcare-associated infections (HAIs) in the Neonatal Intensive Care Unit of the Neonatology Clinic, UMHAT 'Dr. Georgi Stranski' EAD—representative of a level III neonatal care unit serving Central Northern Bulgaria—was 13.9%, including cases without microbiologically confirmed pathogens.
2. HAIs most commonly affect infants with extreme prematurity and very low birth weight, regardless of sex or delivery mode. Typically, HAIs debut around day 16 after birth.
3. Klebsiella pneumoniae emerged as the leading bacterial pathogen of HAIs, surpassing Gram-positive staphylococci. No invasive candidiasis cases were registered during the observation period.
4. Late-onset neonatal sepsis was established as the main clinical manifestation of HAIs in this study, followed by ventilator-associated pneumonia. The absence of urinary tract infections and the low rate of confirmed CNS infections suggest insufficient urological and CSF culture testing.
5. HAIs in neonates were closely related to several risk factors, most notably birth weight <1499 g, gestational age <32 weeks, and comorbidities such as asphyxia and intraventricular hemorrhage. Pre-infection anemia was a significant risk factor. Prolonged mechanical ventilation, central venous catheterization (umbilical catheter), parenteral nutrition, and placement of a gastric tube within the three days prior to infection were confirmed as significant risk-associated medical procedures. Non-invasive ventilation also emerged as an independent risk factor.

6. Among quantitative risk factors, the strongest effect was observed for 'Total duration of parenteral nutrition ≥9.5 days,' with odds ratio (OR) ≈12, indicating substantial risk increase. Next strongest were 'BPD' and 'Birth weight ≤1530 g' (OR = 8.12 and 3.10, respectively).
7. Respiratory system symptoms such as apnea, dyspnea, and need for respiratory support predominated in the early infection stage and often preceded severe conditions like shock or organ dysfunction. Neonates with these symptoms, especially reduced motor activity, were at highest risk of critical illness and mortality.
8. Among routinely used laboratory markers, the I:T index and CRP proved most important for diagnosing neonatal sepsis, with the I:T index showing high sensitivity. Leukocyte and platelet counts had lower diagnostic value, warranting caution in interpretation. Specific pathogens were associated with specific symptoms: Escherichia coli was most likely in neonates with apnea and dyspnea, while Klebsiella pneumoniae correlated with low platelet counts.
9. Incorporation of PCT and IL-6 into routine practice may allow timely diagnosis of HAIs. The I:T index and platelet count can also be relied upon, especially when interpreted alongside PCT and IL-6. According to our results, endocan (ESM-1) and IL-8 are not reliable markers for late neonatal sepsis.
10. The NeoInfectCalc tool, created based on two prognostic models for infection risk—including risk factors and laboratory markers of inflammation—may serve clinicians as a useful aid in assessing systemic HAI risk at initial suspicion.

V. Contributions

Original Contributions

- A database was created including 519 patients for the period January 2021 – June 2023, of whom 72 were with HAIs and 447 were healthy controls.
- Novel early inflammatory biomarkers (PCT, IL-6, IL-8, Endocan) were studied for the first time in the Neonatology Clinic of UMHAT, Pleven; Endocan was studied for the first time in neonates in Bulgaria.
- Two prognostic models were developed for estimating infection risk, combined into the tool NeoInfectCalc, based on risk factors (birth weight, duration of parenteral nutrition, bronchopulmonary dysplasia) and laboratory inflammatory markers (PCT, IL-6, I:T index, PLT).

Scientific and Theoretical Contributions

- Theoretical knowledge was presented in the field of definitions, risk factors, etiology and diagnostic methods of HAIs in neonates.
- An analysis was performed of numerous neonatal risk factors and medical procedures used in the NICU.

Applied Contributions

- An input document was created for organizing a patient database at risk of developing HAIs, well as a patient database with clinical suspicion of late neonatal sepsis.
- Twenty-one qualitative and quantitative indicators were tested for their impact on the occurrence of HAIs in neonates.
- A quantitative assessment was made of established risk factors for infection development.
- Threshold values of studied indicators were determined for distinguishing healthy from infected neonates.
- A threshold value for IL-6 was established, previously unstandardized in neonates.
- Validation criteria were tested for routinely used diagnostic markers as well as for emerging ones such as PCT and IL-6.

VI. Appendices

Appendix 1. List of Scientific Publications Related to the Dissertation:

- Gatseva, Preslava, Alexander Blazhev, Zarko Yordanov, and Victoria Atanasova. 2023. "Early Diagnostic Markers of Late-Onset Neonatal Sepsis." Pediatric Reports 15(3): 548-559. https://doi.org/10.3390/pediatric15030050
- Preslava Radoslavova Gatseva, Victoria Atanasova Georgieva. 2023. "Outbreak of nosocomial sepsis in NICU by multidrug-resistant Klebsiella pneumoniae: Diagnostic challenges." World Journal of Advanced Research and Reviews 20(01): 015–022. https://doi.org/10.30574/wjarr.2023.20.1.1989
- Gatseva, Preslava, Alexander Blazhev, Zarko Yordanov, and Victoria Atanasova. 2023. "Diagnostic Utility of Endocan and Interleukins for Late-Onset Neonatal Sepsis." Journal of Biomedical and Clinical Research 16: 124-130. https://doi.org/10.2478/jbcr-2023-0016

Conference Participation Abroad:

- Gatseva P. R., Blazhev A. B., Yordanov Z. Y., Atanasova V. G. MARKERS FOR EARLY DIAGNOSIS OF LATE-ONSET SEPSIS IN NEWBORNS. 5th JENS Congress of joint European Neonatal Societies, 19-23.09.2023, Rome, Italy.
- P. Gatseva, V. Atanasova, S. Porov, Z. Yordanov. OUTBREAK OF NOSOCOMIAL SEPSIS IN NICU BY MULTIDRUG-RESISTANT KLEBSIELLA PNEUMONIAE – DIAGNOSTIC CHALLENGES. 12th International Congress of UENPS, 2-4.09.2022, Krakow, Poland.
- Gatseva P., Atanasova V. Nosocomial infections in neonatal intensive care unit – incidence, etiology and risk factors. 14th World Congress of Perinatal Medicine, 11-14.09.2019, Istanbul, Turkey.

Conference Participation in Bulgaria:

- Gatseva P., Blazhev A., Yordanov Z., Atanasova V. MARKERS FOR EARLY DIAGNOSIS OF HEALTHCARE-ASSOCIATED INFECTIONS IN NEONATES. National Neonatology Conference, 21-23.04.2023, Hisarya.
- Gatseva P. R., Blazhev A. B., Yordanov Z. Y., Atanasova V. G. DIAGNOSTIC UTILITY OF ENDOCAN AND INTERLEUKINS FOR LATE-ONSET NEONATAL SEPSIS. XX International Medical Scientific Conference for Students and Young Doctors, 16-20.10.2023, Pleven.
- Preslava R. Gatseva, Viktoria G. Atanasova, Vanya N. Nedkova. New Approaches to Diagnosis of Nosocomial Infections in Neonatal Intensive Care Unit. Jubilee Scientific Conference – 45 years of the University in Pleven, 31.10-02.11.2019, Pleven.

Participation in Scientific Projects:

MARKERS FOR EARLY DIAGNOSIS OF HEALTHCARE-ASSOCIATED INFECTIONS IN NEONATES, Reg. № D/2022.