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Original scientific paper

Evaluation of inflammatory markers in patients with confirmed bacterial infection according to the type of isolated pathogen

Jelena Micik[1]*, Evgenija Mihajloska[2], Aleksandar Dimkovski[2], Georgina Gorgieva Atanasova[3], Aleksandar Dimovski[2], Ljubica Shuturkova[2], Zoran Sterjev[2], Aleksandra Grozdanova[2]

[1]*Acibadem Sistina, Str. Skupi 5a, 1000 Skopje, R. North Macedonia*
[2]*Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, R. North Macedonia*
[3]*Institute of Public Health of the Republic of North Macedonia, 50 Divizija no.6, 1000 Skopje, R. North Macedonia*

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Abstract

Frequent bacterial infections in intensive care demand routine microbiological screening and antimicrobial management to effectively address potential Gram-positive and Gram-negative pathogens causing post-surgical infections.

This study aimed to assess whether serum levels of leukocytes, C-reactive protein, and procalcitonin — biomarkers commonly used in daily clinical practice to predict postoperative outcomes — differ between infections caused by Gram-positive or Gram-negative bacteria in adult patients admitted to a cardiac surgical intensive care unit (CICU), in order to support treatment decisions.

Laboratory data were retrospectively collected from 564 adult patients who underwent cardiac surgery and were admitted to the cardiac surgical intensive care unit of the Cardiac Surgery Department at Acibadem Sistina Clinical Hospital. Data included serum leukocyte levels recorded on the day a positive microbiological result was obtained and over the subsequent four days. C-reactive protein and procalcitonin levels were measured starting from the day of pathogen isolation and continued for 48 and 72 hours, respectively. The study population was divided into two groups: Group 1, consisting of patients with isolated Gram-positive microorganisms, and Group 2, consisting of patients with isolated Gram-negative microorganisms.

Both groups showed a progressive reduction in leukocyte levels, with no significant differences in clinical presentation and no statistical differences in C-reactive protein and procalcitonin levels between Gram-positive and Gram-negative infections.

Our findings support the conclusion that monitoring the levels of inflammatory markers, regardless of whether the microorganisms are Gram-positive or Gram-negative, is essential for guiding treatment de-escalation.

Key words: antimicrobial stewardship program, Gram-negative bacteria, Gram-positive bacteria, inflammatory biomarkers

Introduction

Frequent bacterial and fungal infections in the intensive care unit (ICU) necessitate implementation of comprehensive microbiological screening in daily clinical practice (Rawson et al., 2023). Targeted (definitive) therapy, based on precise diagnosis using microbiological diagnostic tests, is a crucial option for pathogen-oriented antimicrobial treatment in the ICU. It represents an

*email: jelena.micic@acibademsistina.mk

invaluable opportunity for the effective antimicrobial management of patients (Cortegiani et al., 2023). This process includes comprehensive screening to achieve an accurate diagnosis by identifying the pathogenic agent and determining its sensitivity to antibiotics. These steps effectively reduce the duration of empiric therapy for ICU patients by enabling rapid determination of targeted therapy, thereby shortening ICU stays, reducing overall hospital stays, and lowering associated treatment costs (Mohayya et al., 2023; Tiseo et al., 2022).

Prolonged hospital stays, particularly in the intensive care unit (ICU), pose a significant risk for the development of nosocomial infections, which represent a substantial public health burden, as evidenced by retrospective reports from the World Health Organization (WHO, 2022). The use of biomarkers to guide antimicrobial therapy in critically ill patients is a key component of antimicrobial stewardship (AMS) (Kyriazopoulou and Giamarellos-Bourboulis, 2022). By definition, a biomarker is a "characteristic that is objectively measurable and evaluated as an indicator of normal biological processes, pathogenic processes, or an assessment of a pharmacological response to a therapeutic intervention" (Biomarkers and surrogate endpoints, 2001; Sechidis et al., 2018).

ICU clinicians who are part of the AMS play a crucial role in determining whether critically ill patients truly require antimicrobials. If antimicrobials are necessary, initial microbiological screening helps guide the selection of the most appropriate drug by determining the correct dose, method, and duration of therapy. Numerous observational studies and randomized clinical trials in adult populations have investigated biomarker-guided antimicrobial treatment. These studies place significant emphasis on biomarkers such as procalcitonin (PCT), c-reactive protein (CRP), and presepsin, which are integral to AMS and guide the antimicrobial therapeutic regimen in ICU patients. Current evidence indicates that procalcitonin is the most extensively studied biomarker to date, with its utility supported by the largest number of randomized clinical trials. This strong evidence base underlines PCT's predictive power, making it an essential element in clinical algorithms aimed at guiding the early de-escalation of antibiotic therapy, thereby promoting more targeted treatment and reducing unnecessary antibiotic exposure (Kyriazopoulou and Giamarellos-Bourboulis, 2022; Moniz et al., 2021; Nora et al., 2017).. .

To be part of AMS, biomarkers should offer certain advantages. One of the key features of predictive biomarkers is their ability to be easily interpreted as indicators of biological and pharmacological responses to the therapeutic regimen (Zhang et al., 2019). Additionally, biomarkers used for detecting sepsis in the ICU must have a high degree of sensitivity and specificity to accurately classify the diagnosis and rule it out when necessary. The most effective approach for early infection identification in ICU patients involves using a combination of two or more biomarkers to enhance sensitivity and specificity in early diagnosis. Moreover, biomarkers should have the ability to predict negative outcomes and assess the host's response to the administered therapeutic regimen, thereby informing decisions to either escalate or de-escalate treatment. These principles form the foundation of the "biomarker-guided treatment" strategy (Sankar and Webster, 2013; Zhang et al., 2019).

The Medicines Management Committee at Acibadem Sistina Clinical Hospital developed an AMS program to address the problem of irrational antibiotic use and misuse. This AMS program involves several critical activities including active involvement of a multidisciplinary team in assessing antimicrobial treatment and monitoring the clinical outcome of antibiotic therapy (Micik et al., 2023). In line with this approach, this study aimed to investigate whether serum leukocyte counts (Leu), and levels CRP and PCT-biomarkers commonly used in clinical practice to predict postoperative outcomes-differ between Gram-positive and Gram-negative bacterial infections in adult patients in a cardiac surgical intensive care unit (CICU), in order to help guide treatment decisions."

Material and methods

Study design

This was retrospective single-centre cohort study designed to examine whether the serum counts of Leu, and levels of CRP and PCT, differ based on the type of pathogenic organism (Gram-positive or Gram-negative bacteria) in adult patients with confirmed bacterial infections.

The study population included total of 564 adult patients who underwent cardiac surgery and were admitted to the cardiac surgical intensive care unit (CICU) at Acibadem Sistina Clinical Hospital, in a period between 2020 and 2023. Patients were divided into two groups: 274 patients (48.58%) with isolated Gram-positive microorganisms (Group 1) and 290 patients (51.42%) with isolated Gram-negative microorganisms (Group 2). As part of the antimicrobial stewardship program, the comprehensive microbiological screening with several microbiological tests was performed on these patients to identify potential Gram-positive and Gram-negative microorganism. These tests involved screening for methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum beta-lactamases (ESBL), and carbapenem-resistant *Enterobacteriaceae* (CRE) using various samples, such as throat swabs, nasal swabs, skin smears from the axillary region, skin swabs from the inguinal region, urine cultures, and sputum samples. The study included only patients with isolated Gram-positive or Gram-negative microorganisms. Patients with fungal infections or co-infections involving both Gram-positive and Gram-negative microorganisms were excluded.

Patient data and laboratory results were obtained from CEREBRAL+ electronic system, which supports daily

clinical operations at "Acibadem Sistina" Clinical Hospital. Data collection included blood sample analyses for Leu counts, and CRP and PCT levels, biomarkers that aligned with the hospital's established guideline for antimicrobial stewardship program, which guide antimicrobial therapy regimens for ICU patients.

Statistical analyzis

Statistical analysis was performed using Statistica 7.1 for Windows and SPSS Statistics 23.0. Differences between these groups in the variables Leu (days 1, 2, 3, 4, 5), CRP (days 1, 2, 3), and PCT (days 1, 2, 3) were assessed using repeated measures ANOVA (F/p), / Post Hoc / Bonferroni test. Significance was determined at $p < 0.05$.

Ethics committee of Acibadem Sistina Clinical Hospital agreed that the findings in this report were based on normal clinical practice and therefore were suitable for dissemination.

Results and discussion

The Leu counts measured on days 1, 2, 3, 4, and 5 for each group are summarized in the Table 1 and Fig. 1 presented below.

Table 1. Serum Leukocyte count from day 1 (day of positive microbiological result) to day 5 for both patient groups

Patient group	Leukocytes day	Leukocyte counts ($x10^9$/L) LS mean
1	day 1	14.18
1	day 2	12.68
1	day 3	11.73
1	day 4	10.34
1	day 5	9.34
2	day 1	14.97
2	day 2	13.22
2	day 3	11.53
2	day 4	10.23
2	day 5	9.18

LS Mean (Least Squares Mean): F (4, 2248) = 2.78, p=0.03
Group 1 (Gram-positive)
Group 2 (Gram-negative)

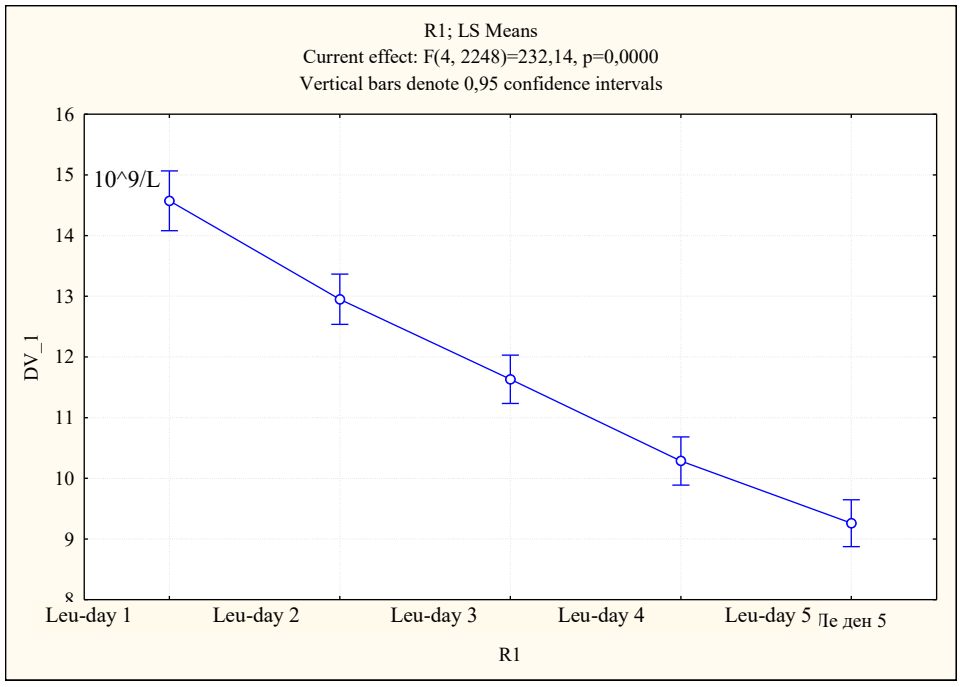

Fig. 1. LS Means of leukocyte count from day 1 to day 5 in both groups of patients

The results showed continuous decrease in leukocyte marker kinetics over 5 days (Fig. 1). Both patient groups exhibited a progressive reduction in this inflammatory biomarker, with no significant differences in post-surgical clinical presentation for infections caused by Gram-positive or Gram-negative bacteria (*p=0.62*). This indicates that both groups exhibited a similar response, with leukocyte counts decreasing after implementation of all principles of the antimicrobial stewardship program. These findings align with a previously published studies conducted in the ICU, where no notable difference was observed in leukocyte biomarker levels among patients with confirmed bacterial infections caused by Gram-positive and Gram-negative pathogens (Nešković et al., 2023; Pereverzeva et al., 2022). In our study a significant difference was observed in leukocytes counts between day 1 and 5 in both groups of patients. This difference was presented in detail through Post-hoc / Bonferroni test analysis for LS Means of leukocytes in both groups of patients (Table 2). The highest value 14.97 10^9/L of LS Means was registered in group 2 / day 1, and the lowest value 9.18 10^9/L of LS Means in group 2 / day 5. Previous research has similarly examined leukocyte counts in the context of survival outcomes, particularly exploring the association between leukocyte trends in the first three days of ICU admission and 28-day mortality rates. In Kaplan-Meier survival analysis, this study demonstrated that a decrease in leukocyte count during the first three days was linked to longer survival, highlighting the importance of leukocyte kinetics as a key prognostic indicator in ICU patients (Li et al., 2021).

The CRP levels measured on day 1, day 2 and day 3 for each group are summarized in the Table 3 and Fig. 2 presented below.

The results of our study showed no significant difference in the CRP levels between the two patient groups (*p=0.76*) Similarly, a study found no differences in CRP levels between Gram-negative and Gram-positive sepsis at 72 hours (Li et al., 2021). However, other studies have reported considerably higher serum CRP levels in surgical patients with Gram-negative infections, suggesting that CRP levels are notably elevated in Gram-negative bacterial infections compared to Gram-positive ones, underscoring a prognostic difference between sepsis caused by these types of bacteria (De La Torre-Prados et al., 2015; Nešković et al., 2023; Tang et al., 2023). The CRP levels in our study exhibited a dynamic trend over the first three days for both groups (Fig. 2), indicating an early inflammatory response, which was subsequently followed by clinical improvement and a decline in CRP levels. In clinical context, such variations in CRP levels are important since they help estimate the progression of the underlying condition. Consistently elevated or rising CRP levels, for example, may signal ongoing infection or complications, while a decline could indicate a favorable response to treatment. In our study a significant difference was observed in CRP levels between day 1 and 3 in both groups of patients. This difference was presented in detail through Post-hoc / Bonferroni test analysis for LS Means of CRP in both groups of patients (Table 4). The highest value 14.26 mg/dL of LS Means was registered in group 1 / CRP- day 2 and the lowest value 10.80 mg/dL of LS Means was registered in group 1 / CRP- day 1.

Table 2. Post-hoc Bonferroni Analysis of Leukocyte count between day 1 and day 5 in both patient groups

		Leukocyte count (×10^9/L) LS Mean									
Patient group	Leukocytes day	{1} 14.18	{2} 12.68	{3} 11.73	{4} 10.34	{5} 9.34	{6} 14.97	{7} 13.22	{8} 11.53	{9} 10.23	{10} 9.18
1	day 1		0.000	0.000	0.000	0.000	1.00	1.00	0.000	0.000	0.000
1	day 2	0.000		0.03	0.000	0.000	0.000	1.00	0.32	0.000	0.000
1	day 3	0.000	0.03		0.000	0.000	0.000	0.02	1.00	0.02	0.000
1	day 4	0.000	0.000	0.000		0.02	0.000	0.000	0.24	1.00	0.30
1	day 5	0.000	0.000	0.000	0.02		0.000	0.000	0.000	1.00	1.00
2	day 1	1.00	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
2	day 2	1.00	1.00	0.02	0.000	0.000	0.000		0.000	0.000	0.000
2	day 3	0.000	0.32	1.00	0.24	0.000	0.000	0.000		0.000	0.000
2	day 4	0.000	0.000	0.02	1.00	1.00	0.000	0.000	0.000		0.006
2	day 5	0.000	0.000	0.000	0.30	1.00	0.000	0.000	0.000	0.006	

LS Mean (Least Squares Mean)
Group 1 (Gram-positive)
Group 2 (Gram-negative)

Table 3. CRP levels from day 1 to day 3 for both patient groups

Patient group	CRP day	CRP levels (mg/dL) LS mean
1	day 1	10.80
1	day 2	14.26
1	day 3	11.82
2	day 1	11.69
2	day 2	14.06
2	day 3	11.79

LS Mean (Least Squares Mean): *F(2, 1124)=1.12, p=0.33*
Group 1 (Gram-positive)
Group 2 (Gram-negative)

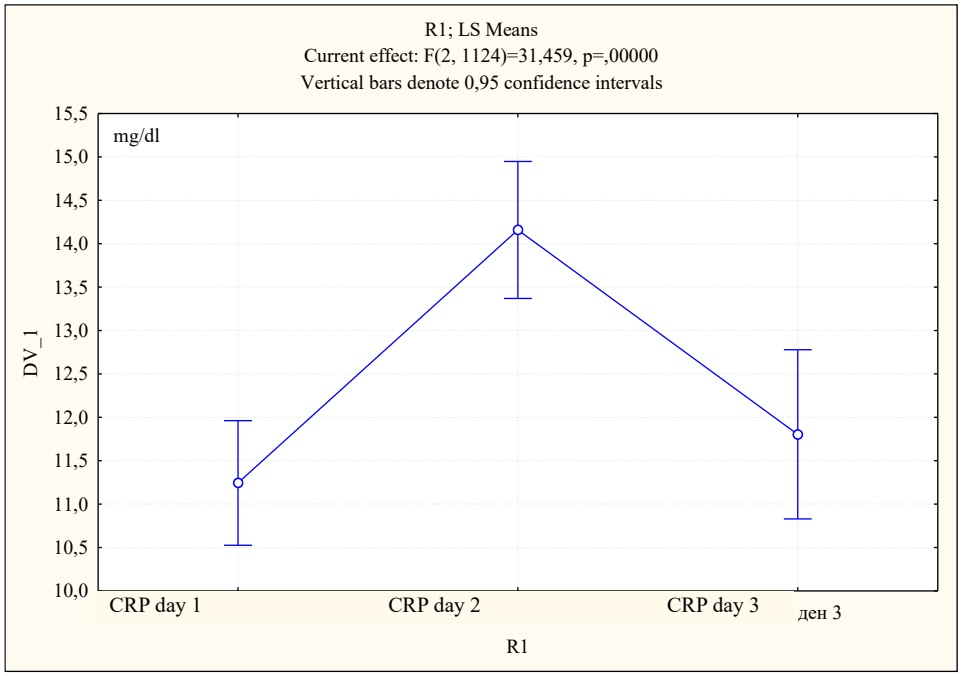

Fig. 2. LS Means of CRP levels from day 1 to day 3 in both patient groups.

Table 4. Post-hoc Bonferroni Analysis of CRP levels between day 1 and day 3 in both patient groups

		CRP levels (mg/dL) LS Mean					
Patient group	CRP day	{1} 10.80	{2} 14.26	{3} 11.82	{4} 11.69	{5} 14.06	{6} 11.79
1	day 1		0.000	1.00	1.00	0.002	1.00
1	day 2	0.000		0.000	0.04	1.00	0.06
1	day 3	1.00	0.000		1.00	0.13	1.00
2	day 1	1.00	0.04	1.00		0.000	1.00
2	day 2	0.002	1.00	0.13	0.000		0.000
2	day 3	1.00	0.06	1.00	1.00	0.000	

LS Mean (Least Squares Mean)
Group 1 (Gram-positive)
Group 2 (Gram-negative)

Table 5. PCT levels from day 1 to day 3 for both patient groups

Patient group	PCT day	PCT levels (ng/mL) LS Means
1	day 1	0.92
1	day 2	0.87
1	day 3	0.77
2	day 1	1.47
2	day 2	1.09
2	day 3	0.88

LS Means (Least Squares Mean): *F(2, 1124)=2.87, p=0.06*
Group 1 (Gram-positive)
Group 2 (Gram-negative)

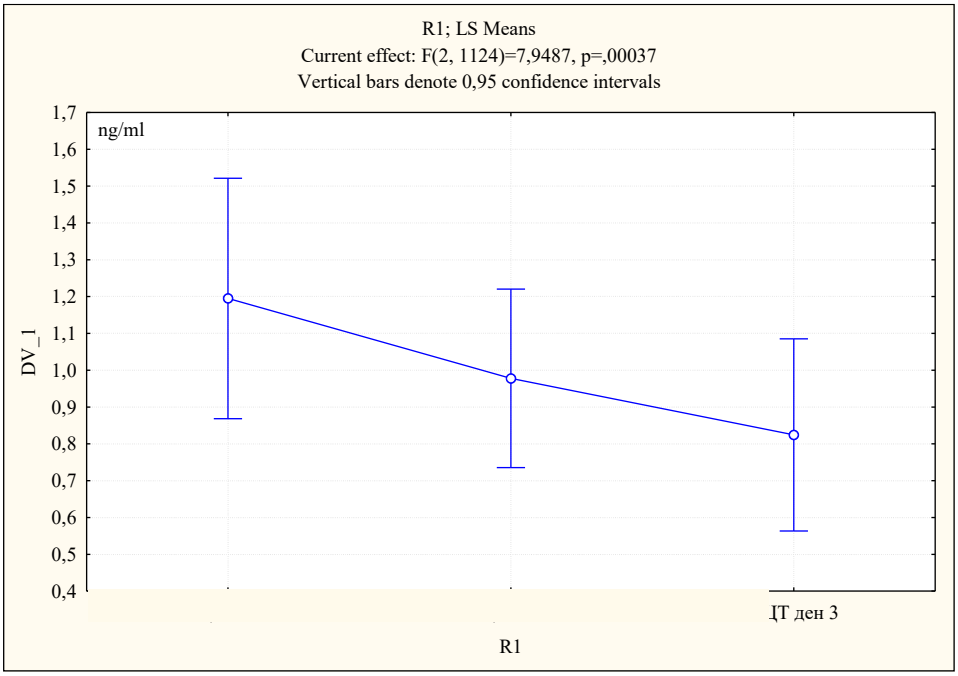

Fig. 3. LS Means of PCT levels from day 1 to day 3 in both patient groups.

The PCT levels measured on day 1, day 2 and day 3 for each group are summarized in the Table 5 and Fig. 3 presented below.

There was no significant difference in PCT levels between the two groups of patients (*p=0.27*). In contrast to our results, other studies reported that patients with a Gram-negative isolate had a significantly higher serum PCT level compared to patients with a Gram-positive isolate, indicating that PCT may serve as a marker of Gram-negative bacteremia in patients with sepsis (Guo et al., 2015; Liu et al., 2017; Nešković et al., 2023). While no statistically significant difference was found between the two patient groups, our study observed that the serum levels of the inflammatory markers PCT and CRP showed a higher concentration range in group 2 (Gram-negative bacteria) compared to group 1 (Gram-positive bacteria), particularly within the first 48 hours of the postoperative period (Table 3 and 4, Fig. 2 and 3) This difference was most notable for PCT, where serum levels tended to increase, especially in patients experiencing postoperative infectious complications. In contrast, CRP is less specific for distinguishing the postoperative inflammatory response and exhibits a slower rate of increase compared to PCT, with a more pronounced peak around 3 days postoperatively. The results shown in Table 6 refer to the Post-hoc / Bonferroni test analysis for LS Means of PCT in both groups of patients. The highest value 1.47 ng/mL of LS Means of PCT was registered in group 2 / PCT day 1, and the lowest value 0.77 ng/mL of LS Means of PCT in group 1 / PCT day 3.

The major limitation of this study is its retrospective design, which can introduce challenges in defining post-

Table 6. Post-hoc Bonferroni Analysis of PCT levels between day 1 and day 3 in both patient groups

		PCT levels (ng/mL) LS Mean					
Patient group	PCT day	{1} 0.92	{2} 0.87	{3} 0.77	{4} 1.47	{5} 1.09	{6} 0.88
1	day 1		1.00	1.00	1.00	1.00	1.00
1	day 2	1.00		1.00	0.55	1.00	1.00
1	day 3	1.00	1.00		0.22	1.00	1.00
2	day 1	1.00	0.55	0.22		0.054	0.000
2	day 2	1.00	1.00	1.00	0.054		1.00
2	day 3	1.00	1.00	1.00	0.000	1.00	

LS Mean (Least Squares Mean)
Group 1 (Gram-positive)
Group 2 (Gram-negative)

cardiac surgery infection accurately due to varied presentations, overlapping symptoms with other conditions, subjective factors, subtle early signs in ICU settings. Additionally, we did not include other patient factors like pre-existing heart, liver, or kidney conditions that might influence CRP and PCT levels, potentially affecting the reliability of these biomarkers. The study also did not specify the type of cardiac surgery performed or include postoperative complications, such as pericarditis or pericardial effusion which could impact these inflammatory markers. A new prospective study that addresses these gaps could offer more accurate insights into leukocyte count, and CRP and PCT levels in post-surgical patients.

Conclusion

In conclusion, while leukocytes, CRP and PCT are widely used biomarkers for predicting postoperative infection outcomes, this study reveals that these markers do not significantly differ between infections caused by Gram-positive and Gram-negative bacteria in cardiac surgical patients. Both groups showed similar trends in biomarker levels. Our findings support the conclusion that monitoring the levels of inflammatory markers, regardless of the microorganisms, Gram-positive or Gram-negative, is essential for guiding treatment de-escalation. This approach is particularly crucial in intensive care settings, where timely adjustments to therapy can significantly impact patient outcomes. The use of biomarkers to guide antimicrobial treatment is a key to the goals of antimicrobial stewardship programs, but further studies are needed to explore alternative diagnostic tools or combinations of markers that may offer more precise differentiation in the clinical management of bacterial infections in the intensive care setting.

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Резиме

Евалуација на воспалителни маркери кај пациенти со потврдена бактериска инфекција според видот на изолираниот патоген

Јелена Мициќ[1]*, Евгенија Михајлоска[2], Александар Димковски[2], Георгина Ѓоргиева Атанасова[3], Александар Димовски[2], Љубица Шутуркова[2], Зоран Стерјев[2], Александра Грозданова[2]

[1]Аџибадем Систина, ул. Скупи 5а, 1000 Скопје, С. Македонија
[2]Фармацевтски факултет, Универзитет „Св. Кирил и Методиј“, Мајка Тереза 47, 1000 Скопје, С. Македонија
[2]Институт за јавно здравје на РС Македонија, ул. 50та дивизија 6, 1000 Скопје, С. Македонија

Клучни зборови: антимикробен програм, Грам-негативни бактерии, Грам-позитивни бактерии, инфламаторни маркери

Поради чести бактериски инфекции во центрите за интензивна нега потребен е рутински микробиолошки мониторинг и соодветна програма за антимикробно управување чија цел е ефективно справување со потенцијалните Грам-позитивни и Грам-негативни патогени што предизвикуваат постхируршки инфекции.

Во таа насока, целта на оваа студија е да се процени дали серумските нивоа на леукоцити, Ц-реактивен протеин и прокалцитонин — биомаркери што рутински се користат во секојдневната клиничка практика за предвидување на постоперативните исходи — се разликуваат кај инфекции предизвикани од Грам-позитивни или Грам-негативни бактерии, кај возрасни пациенти хоспитализирани во кардиохируршката единица за интензивна нега, со цел поддршка во процесот на донесување одлуки за терапија.

Лабораториските податоци беа ретроспективно собрани од 564 возрасни пациенти кои биле подложени на кардиохируршка интервенција и биле примени во кардиохируршката единица за интензивна нега на Одделот за кардиохирургија при Клиничката болница „Аџибадем Систина“. Податоците вклучуваат серумски нивоа на леукоцити измерени на денот на добивање позитивен микробиолошки резултат и последователно во следните четири дена. Нивоата на Ц-реактивен протеин и прокалцитонин беа мерени почнувајќи од денот на изолација на патогенот, па во текот на следните 48, односно 72 часа. Пациентите беа поделени во две групи: Група 1 — пациенти со изолирани Грам-позитивни микроорганизми и Група 2 — пациенти со изолирани Грам-негативни микроорганизми.

И во двете групи беше забележано прогресивно намалување на нивото на леукоцити, без значајни разлики во клиничката презентација и без статистички значајни разлики на нивоата на Ц-реактивен протеин и прокалцитонин помеѓу инфекциите предизвикани од Грам-позитивни и Грам-негативни бактерии.

Овие резултати го поддржуваат заклучокот дека следењето на нивоата на воспалителните биомаркери е од суштинско значење за следење и намалување на терапијата, без оглед на Грам-статусот на микроорганизмите.