

COMPARATIVE ANALYSIS OF THE IMMUNOLOGICAL STATUS OF SOCCER PLAYERS AT DIFFERENT LEVELS OF COMPETITION

KOMPARATIVNA ANALIZA IMUNOLOŠKOG STATUSA FUDBALERA RAZLIČITIH NIVOA TAKMIČENJA

ŽELJKO SEKULIĆ¹, SLAVKO DRAGOSAVLJEVIĆ², JASMIN BUDIMLIĆ³, NIKOLA ILIĆ^{4,5}

¹University of Banja Luka, Faculty of Physical Education and Sports, Banja Luka, Bosnia and Herzegovina

²University of East Sarajevo, Faculty of Physical Education and Sports, East Sarajevo, Bosnia and Herzegovina

³University of Bihać, Faculty of Education, Department of Physical Education and Sports, Bihać, Bosnia and Herzegovina

⁴Pan-European University Apeiron, Faculty of Sports Sciences, Banja Luka, Bosnia and Herzegovina

⁵Institute of Occupational and Sports Medicine of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina

Correspondence:

Željko Sekulić

University of Banja Luka, Faculty of Physical Education and Sports, Banja Luka, Bosnia and Herzegovina, zeljko.sekulic@ffvs.unibl.org

Abstract: The aim of this study was to examine the immune status of football players at different competition levels in Bosnia and Herzegovina through the analysis of key hematological biomarkers. The sample included 59 football players from the first, third, and fifth leagues, whose immune parameters were analyzed based on medical records and laboratory findings. The measured parameters included absolute counts of leukocytes, lymphocytes, monocytes, neutrophils, eosinophils, and basophils. The results did not show statistically significant differences between players from different leagues, although certain variations in individual biomarkers were observed. These findings suggest that the competition level itself is not a key factor in determining the immune responses of athletes under stable physiological conditions. It is concluded that further research is needed, covering different phases of the training cycle, to better understand the impact of training load and periodization on the immune status of football players.

Keywords: immune status, football, leukocytes, hematological biomarkers, competition level

INTRODUCTION

Variations in the immune profiles of football players of different competitive levels can be partly explained by differences in the intensity and volume of training, which affects the differential distribution of leukocyte subpopulations, including lymphocytes, monocytes, neutrophils, eosinophils and basophils. Understanding the impact of

¹Univerzitet u Banjoj Luci, Fakultet fizičkog vaspitanja i sporta, Banja Luka, Bosna i Hercegovina

²Univerzitet u Istočnom Sarajevu, Fakultet fizičkog vaspitanja i sporta, Istočno Sarajevo, Bosna i Hercegovina

³Univerzitet u Bihaću, Pedagoški fakultet, Odsjek za tjelesni odgoj i sport, Bihać, Bosna i Hercegovina

⁴Panevropski univerzitet Apeiron, Fakultet sportskih nauka, Banja Luka, Bosna i Hercegovina

⁵Zavod za medicinu rada i sporta Republike Srpske, Banja Luka, Bosna i Hercegovina

Korespondencija:

Željko Sekulić

Univerzitet u Banjoj Luci, Fakultet fizičkog vaspitanja i sporta, Banja Luka, Bosna i Hercegovina, zeljko.sekulic@ffvs.unibl.org

Sažetak: Cilj ovog istraživanja bio je da se ispita imunološki status fudbalera različitih takmičarskih nivoa u Bosni i Hercegovini putem analize ključnih hematoloških biomarkera. Uzorak je obuhvatio 59 fudbalera iz prve, treće i pete lige, čiji su imunološki parametri analizirani na osnovu medicinskih kartona i laboratorijskih nalaza. Mjereni su apsolutni brojevi leukocita, limfocita, monocita, neutrofila, eozinofila i bazofila. Rezultati nisu pokazali statistički značajne razlike između fudbalera različitih liga, iako su uočene određene varijacije u pojedinim biomarkerima. Ovi nalazi sugerišu da nivo takmičenja sam po sebi ne predstavlja ključni faktor u određivanju imunoloških odgovora sportista u stabilnim fiziološkim uslovima. Zaključuje se da je potrebno sprovesti dodatna istraživanja koja bi obuhvatila različite faze trenažnog ciklusa kako bi se bolje razumio uticaj trenažnog opterećenja i periodizacije treninga na imunološki status fudbalera.

Ključne riječi: imunološki status, fudbal, leukociti, hematološki biomarkeri, nivo takmičenja

Uvod

Varijacije u imunološkim profilima fudbalera različitih takmičarskih nivoa mogu se dijelom objasniti razlikama u intenzitetu i obimu treninga, što utiče na diferencijalnu raspodjelu leukocitnih subpopulacija, uključujući limfocite, monocite, neutrofile, eozinofile i bazofile. Razumijevanje uticaja treninga na imunološke biomar-

training on immune biomarkers is crucial for understanding potential risks to the immune function of athletes at different levels of competition. Hematological parameters, including the number of leukocytes, are important indicators of immune status and response to physical stress. Research shows that elite soccer players often experience acute changes in leukocyte populations after intense training or matches. In particular, an increase in the number of neutrophils after exertion is noted, which suggests an inflammatory response to physical stress (Fernández-Lázaro et al., 2022). Studies on hematological biomarkers indicate that training volume in elite athletes affects variations in leukocyte profiles, whereby increased neutrophilia is often associated with transient immune suppression known as the “open window” effect (Fernández-Lázaro et al., 2022). In contrast, athletes of lower competitive ranks usually show a more stable leukocyte profile with smaller fluctuations (Díaz et al., 2022). For example, studies suggest that amateur soccer players have higher values of lymphocytes and lower values of neutrophils compared to elite players, which may indicate a more preserved immune function despite increased physical effort (Díaz et al., 2022). These findings are consistent with data indicating that elite athletes have a higher prevalence of negative immune responses, including relative lymphopenia, which may increase the risk of post-competition infections (Fernández-Lázaro et al., 2022; Díaz et al., 2022). In addition to neutrophils and lymphocytes, monocytes and eosinophils also play an important role in the immune response of athletes. Monocytes reflect the inflammatory status after exertion, and in elite soccer players they can vary depending on the level of recovery and load, whereby increased values can indicate a state of chronic inflammation due to overtraining (Fernández-Lázaro et al., 2022). Eosinophils, although less investigated in the context of sports performance, are associated with inflammatory processes and may be relevant in athletes exposed to allergic reactions or specific environmental factors (Díaz et al., 2022). The role of basophils in the immune responses of athletes remains insufficiently investigated, but their increase may indicate hypersensitive reactions associated with intense physical exertion (Fernández-Lázaro et al., 2022). In elite soccer players, increased training load can induce stressful conditions that potentially worsen allergic reactions and affect training adaptation. Continuous hematological monitoring, including the analysis of leukocyte subpopulations, is a key strategy for monitoring the immune status and preventing the negative effects of overtraining in athletes. Regular monitoring of changes in biomarkers allows coaches and sports doctors to optimize workload and ad-

kere ključno je za sagledavanje potencijalnih rizika po imunološku funkciju sportista na različitim nivoima takmičenja. Hematološki parametri, uključujući broj leukocita, predstavljaju važne indikatore imunološkog statusa i odgovora na fizičko opterećenje. Istraživanja pokazuju da elitni fudbaleri često doživljavaju akutne promjene u leukocitnim populacijama nakon intenzivnih treninga ili utakmica. Posebno se bilježi povećanje broja neutrofila nakon napora, što sugerise upalni odgovor na fizički stres (Fernández-Lázaro i sar., 2022). Studije o hematološkim biomarkerima ukazuju da volumen treninga kod elitnih sportista utiče na varijacije u leukocitnim profilima, pri čemu je povećana neutrofilija često povezana sa prolaznom imunološkom supresijom poznatom kao “open window” efekat (Fernández-Lázaro i sar., 2022). Nasuprot tome, sportisti nižih takmičarskih rangova obično pokazuju stabilniji leukocitni profil s manjim fluktuacijama (Díaz i sar., 2022). Na primjer, studije sugerisu da amaterski fudbaleri imaju više vrijednosti limfocita i niže vrijednosti neutrofila u poređenju sa elitnim igračima, što može ukazivati na očuvaniju imunološku funkciju uprkos povećanom fizičkom naporu (Díaz i sar., 2022). Ovi nalazi su u skladu s podacima koji ukazuju da elitni sportisti imaju veću prevalenciju negativnih imunoloških odgovora, uključujući relativnu limfopeniju, što može povećati rizik od infekcija nakon takmičenja (Fernández-Lázaro i sar., 2022; Díaz i sar., 2022). Pored neutrofila i limfocita, monociti i eozinofili takođe igraju važnu ulogu u imunološkom odgovoru sportista. Monociti odražavaju inflamatorni status nakon napora, a kod elitnih fudbalera mogu varirati u zavisnosti od nivoa oporavka i opterećenja, pri čemu povećane vrijednosti mogu ukazivati na stanje hronične upale usljed pretreniranosti (Fernández-Lázaro i sar., 2022). Eozinofili, iako manje istraženi u kontekstu sportskog učinka, povezani su sa inflamatornim procesima i mogu biti relevantni kod sportista izloženih alergijskim reakcijama ili specifičnim ekološkim faktorima (Díaz i sar., 2022). Uloga bazofila u imunološkim odgovorima sportista ostaje nedovoljno istražena, ali njihovo povećanje može ukazivati na hipersenzitivne reakcije povezane sa intenzivnim fizičkim opterećenjem (Fernández-Lázaro i sar., 2022). Kod elitnih fudbalera, povećano trenažno opterećenje može indukovati stresna stanja koja potencijalno pogoršavaju alergijske reakcije i utiču na trenažnu adaptaciju. Kontinuirani hematološki monitoring, uključujući analizu leukocitnih subpopulacija, predstavlja ključnu strategiju za praćenje imunološkog statusa i prevenciju negativnih efekata pretreniranosti kod sportista. Redovno praćenje promjena u biomarkerima omogućava

just recovery strategies, thereby reducing the risk of immune dysfunctions and overtraining (Soriano et al., 2021). The differences in the immune profiles of football players of different competitive levels emphasize the complexity of immune responses induced by training and competition. Increased intensity of training in elite athletes is associated with more pronounced changes in leukocyte status, which may indicate stress-induced immune suppression, while lower league soccer players show more stable immune profiles.

The goal of this research is to determine the differences in immunological biomarkers of football players from the first, third and fifth leagues in Bosnia and Herzegovina, which will enable a better understanding of the influence of the level of competition on the immune status of athletes.

METHODS

Study design

This study used a transference design to examine the immune status of soccer players competing at three different levels in Bosnia and Herzegovina. The data was collected from the medical records of athletes who had a blood analysis performed at the Institute of Sports Medicine of the Republic of Srpska during the competition break in 2024. The study included football players from the first, third and fifth leagues, which enabled a comparative assessment of immune parameters between different levels of competition. The choice of immune biomarkers is based on routine blood tests that were carried out as part of mandatory health examinations of athletes. By using the existing medical records, an objective and standardized assessment of the immune status was ensured without additional testing. Given the transferal nature of the study, the findings allow insight into the immune function of soccer players at one point in time, providing a comparison between levels of competition, but not tracking longitudinal changes or determining causality. The study was conducted in accordance with ethical standards for medical research on humans and in the spirit of the principles of the Declaration of Helsinki. Prior to data collection, institutional consent was obtained, which ensured compliance with the principles of confidentiality and data protection. The measurement of immune parameters was carried out during the winter break, immediately before the start of preparations for the spring season of 2024, in order to ensure stable physiological conditions without acute training stress. Sampling was carried out in January, which corresponds to the period between the two half-seasons (au-

va trenerima i sportskim ljekarima optimizaciju opterećenja i prilagođavanje strategija oporavka, čime se smanjuje rizik od imunoloških disfunkcija i pretreniranosti (Soriano i sar., 2021). Razlike u imunološkim profilima fudbalera različitih takmičarskih nivoa naglašavaju kompleksnost imunoloških odgovora izazvanih treningom i takmičenjem. Povećani intenzitet treninga kod elitnih sportista povezan je s izraženijim promjenama u leukocitnom statusu, što može ukazivati na stresom indukovanu imunološku supresiju, dok fudbaleri nižih liga pokazuju stabilnije imunološke profile.

Cilj ovog istraživanja je da se utvrde razlike u imunološkim biomarkerima fudbalera iz prve, treće i pete lige u Bosni i Hercegovini, čime će se omogućiti bolje razumijevanje uticaja nivoa takmičenja na imunološki status sportista.

METODE

Dizajn studije

Ova studija koristila je transferzalni dizajn kako bi ispitala imunološki status fudbalera koji se takmiče na tri različita nivoa u Bosni i Hercegovini. Podaci su prikupljeni iz medicinskih kartona sportista koji su tokom takmičarske pauze 2024. godine obavili analizu krvi u Zavodu za medicinu sporta Republike Srpske. Studijom su obuhvaćeni fudbaleri iz prve, treće i pete lige, što je omogućilo komparativnu procjenu imunoloških parametara između različitih nivoa takmičenja. Izbor imunoloških biomarkera zasnovan je na rutinskim analizama krvi koje su sprovedene u sklopu obaveznih zdravstvenih pregleda sportista. Korištenjem postojećih medicinskih kartona osigurana je objektivna i standardizovana procjena imunološkog statusa bez dodatnih testiranja. S obzirom na transferzalni karakter studije, nalazi omogućavaju uvid u imunološku funkciju fudbalera u jednoj vremenskoj tački, pružajući poređenje između nivoa takmičenja, ali ne i praćenje longitudinalnih promjena ili utvrđivanje uzročnosti. Studija je sprovedena u skladu s etičkim standardima za medicinska istraživanja na ljudima i u duhu principa Helsinške deklaracije. Prije prikupljanja podataka pribavljena je institucionalna saglasnost, čime je osigurano poštovanje principa povjerljivosti i zaštite podataka. Mjerenje imunoloških parametara sprovedeno je tokom zimske pauze, neposredno prije početka priprema za proljetni dio sezone 2024. godine, u cilju osiguravanja stabilnih fizioloških uslova bez akutnog trenažnog stresa. Uzorkovanje je realizovano u januaru, što vremenski odgovara periodu između dvije polusezone (jesenje i proljet-

tumn and spring). In this way, the potential impact of the acute load characteristic of the competition period or the early stages of preparation was avoided.

Respondents

A total of 59 male soccer players who compete at different levels in Bosnia and Herzegovina participated in the research. The sample is approximately evenly distributed between the three competitive leagues, with 20 players each from the first and third leagues, and 19 players from the fifth leagues. The respondents came from three different football clubs and competition ranks: FK Borac Banja Luka (Premier League of BiH), FK Naprijed Banja Luka (Second League of Republika Srpska), and FK Krupa from Krupa na Vrbasu (Fourth League of Republika Srpska). All subjects were active competitors during the 2024 season and underwent routine medical examinations, including blood analysis, at the Institute of Sports Medicine of the Republic of Srpska. The criteria for inclusion in the research were active playing in one of the three selected leagues, possession of complete medical documentation with relevant immunological parameters and the absence of acute infections or chronic diseases at the time of testing. Players who had a recent illness, injury or used immunomodulatory drugs were excluded from the study in order to reduce the influence of possible factors that could impair the reliability of the results. Prior to data collection, institutional consent was obtained, and the study was conducted in accordance with the ethical guidelines and principles of the Declaration of Helsinki.

Variables

The immune status of the subjects was assessed using blood biomarkers obtained from a routine hematological analysis. The measured variables included absolute values of key immune cell populations: leukocytes (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS) and basophils (BAS). These biomarkers were chosen because they provide insight into the function of the immune system and possible variations in the immune response between different levels of competition. Blood samples were collected by standardized venipuncture, performed by an accredited laboratory technician. All samples were taken in the morning, on an empty stomach, in order to reduce variations caused by the circadian rhythm and external factors that can affect the number of immune cells. The analysis was carried out using a Sysmex XN-330 automatic hematology analyzer, a validated and widely used system for precise measurement of the differential blood count. Throughout the process, estab-

ne). Na taj način je izbjegnuto potencijalni uticaj akutnog opterećenja karakterističnog za takmičarski period ili rane faze priprema.

Ispitanici

U istraživanju je učestvovalo ukupno 59 fudbale-
ra muškog pola koji se takmiče na različitim nivoima u Bosni i Hercegovini. Uzorak je približno ravnomjerno raspoređen između tri takmičarske lige, sa po 20 igrača iz prve i treće lige, i sa 19 igrača iz pete lige. Ispitanici su dolazili iz tri različita fudbalska kluba i takmičarska ranga: FK Borac Banja Luka (Premijer liga BiH), FK Naprijed Banja Luka (Druga liga Republike Srpske), i FK Krupa iz Krupe na Vrbasu (Četvrta liga Republike Srpske). Svi ispitanici su bili aktivni takmičari tokom sezone 2024. godine i prošli su rutinske medicinske preglede, uključujući analizu krvi, u Zavodu za medicinu sporta Republike Srpske. Kriterijumi za uključivanje u istraživanje bili su aktivno igranje u jednoj od tri odabrane lige, posjedovanje kompletne medicinske dokumentacije sa relevantnim imunološkim parametrima i odsustvo akutnih infekcija ili hroničnih oboljenja u trenutku testiranja. Igrači koji su u nedavnom periodu imali bolest, povredu ili koristili imunomodulatorne lijekove bili su isključeni iz studije kako bi se smanjio uticaj mogućih faktora koji bi mogli narušiti pouzdanost rezultata. Prije prikupljanja podataka, pribavljena je institucionalna saglasnost, a studija je sprovedena u skladu s etičkim smjernicama i principima Helsinške deklaracije.

Varijable

Imunološki status ispitanika procijenjen je putem biomarkera iz krvi dobijenih iz rutinske hematološke analize. Mjerene varijable obuhvatale su apsolutne vrijednosti ključnih imunoloških ćelijskih populacija: leukocita (WBC), limfocita (LYM), monocita (MON), neutrofila (NEU), eozinofila (EOS) i bazofila (BAS). Ovi biomarkeri su odabrani jer pružaju uvid u funkciju imunološkog sistema i moguće varijacije imunološkog odgovora između različitih nivoa takmičenja. Uzorci krvi prikupljeni su standardizovanom venepunkcijom, koju je izvodio akreditovani laboratorijski tehničar. Svi uzorci uzeti su u jutarnjim satima, natašte, kako bi se smanjile varijacije uzrokovane cirkadijalnim ritmom i spoljnim faktorima koji mogu uticati na broj imunoloških ćelija. Analiza je sprovedena pomoću Sysmex XN-330 automatskog hematološkog analizatora, validiranog i široko korištenog sistema za precizno mjerenje diferencijalne krvne slike. Tokom cijelog procesa poštovani su uspostavljeni protokoli kontrole kvaliteta kako bi se osigurala

lished quality control protocols were followed to ensure the reliability and accuracy of the results. All blood analyses were performed in an accredited laboratory, strictly adhering to standardized sampling, processing, and storage procedures. The analysis methodology was aligned with international hematology guidelines, ensuring that the measured immunological parameters accurately reflected the physiological status of the subjects.

Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics 20 for Windows software (IBM Corp., Armonk, NY, USA). Descriptive statistical indicators, including mean value, standard deviation, minimum and maximum values, were calculated for all investigated variables, in order to summarize the immune status of football players of different competitive levels. The normality of data distribution was tested using the Shapiro-Wilk test. Homogeneity of variances was assessed using Levene's test. One-way analysis of variance (ANOVA) was used to determine differences in immune parameters between the three competitive leagues. The threshold of statistical significance was set at $p < 0.05$. All analysis were conducted in accordance with standard statistical guidelines for biomedical research, which ensured the reliability and reproducibility of the findings.

THE RESULTS

The analysis of immune biomarkers of soccer players of different competitive levels showed certain variations in leukocyte subpopulations, but without statistically significant differences between groups. Descriptive data (Table 1) indicate that football players from the third and fifth leagues had slightly higher average values of total leukocytes compared to players from the first league ($6,243 \pm 1,269$ and $6,233 \pm 1,243$ compared to $5,999 \pm 1,221$, respectively).

However, the analysis of variance (ANOVA) did not show a statistically significant difference between the groups ($F = 0.243$, $p = 0.785$). A similar trend was recorded in lymphocytes, where football players of the third league had the highest average values (2.476 ± 0.638), while players of the first league had the lowest (2.166 ± 0.443). Despite these differences, statistical analysis did not confirm a significant effect of the level of competition on the number of lymphocytes ($F = 1.734$, $p = 0.186$). In terms of monocytes, football players of the first league had slightly higher values (0.640 ± 0.201) compared to the third (0.535 ± 0.130) and fifth league (0.526 ± 0.162).

Although the differences were more pronounced compared to other biomarkers, the analysis of variance did not

pouzdanost i tačnost rezultata. Sve analize krvi obavljene su u akreditovanoj laboratoriji, uz strogo pridržavanje standardizovanih procedura za uzorkovanje, obradu i skladištenje. Metodologija analize usklađena je s međunarodnim hematološkim smjernicama, čime je osigurano da izmjereni imunološki parametri precizno odražavaju fiziološki status ispitanika.

Statističke analize

Sve statističke analize sprovedene su korištenjem softvera IBM SPSS Statistics 20 for Windows (IBM Corp., Armonk, NY, USA). Za sve ispitivane varijable izračunati su deskriptivni statistički pokazatelji, uključujući srednju vrijednost, standardnu devijaciju, minimalne i maksimalne vrijednosti, kako bi se sažeto prikazao imunološki status fudbalera različitih takmičarskih nivoa. Normalnost distribucije podataka testirana je pomoću Shapiro-Wilk testa. Homogenost varijansi procijenjena je korištenjem Leveneovog testa. Za utvrđivanje razlika u imunološkim parametrima između tri takmičarske lige korištena je jednosmjerna analiza varijanse (ANOVA). Prag statističke značajnosti postavljen je na $p < 0.05$. Sve analize sprovedene su u skladu sa standardnim statističkim smjernicama za biomedicinska istraživanja, čime je osigurana pouzdanost i ponovljivost nalaza.

REZULTATI

Analiza imunoloških biomarkera fudbalera različitih takmičarskih nivoa pokazala je određene varijacije u leukocitnim subpopulacijama, ali bez statistički značajnih razlika između grupa. Deskriptivni podaci (Tabela 1) ukazuju na to da su fudbaleri iz treće i pete lige imali nešto veće prosječne vrijednosti ukupnih leukocita u odnosu na igrače prve lige (6.243 ± 1.269 i 6.233 ± 1.243 u odnosu na 5.999 ± 1.221 , respektivno).

Međutim, analiza varijanse (ANOVA) nije pokazala statistički značajnu razliku među grupama ($F = 0.243$, $p = 0.785$). Sličan trend zabilježen je i kod limfocita, gdje su fudbaleri treće lige imali najviše prosječne vrijednosti (2.476 ± 0.638), dok su igrači prve lige imali najniže (2.166 ± 0.443). Uprkos ovim razlikama, statistička analiza nije potvrdila značajan efekat nivoa takmičenja na broj limfocita ($F = 1.734$, $p = 0.186$). Kod monocita, fudbaleri prve lige su imali nešto više vrijednosti (0.640 ± 0.201) u poređenju sa trećom (0.535 ± 0.130) i petom ligom (0.526 ± 0.162).

Iako su razlike bile izraženije u odnosu na druge biomarkere, analiza varijanse nije pokazala statistički značajan efekat ($F = 2.841$, $p = 0.067$). Vrijednosti neutrofila bile su relativno ujednačene između grupa, sa

Table 1. Descriptive statistics of immune parameters in football players of different competitive levels

Parameter / Parametar	Competitive rank / Takmičarski rang	N	M (SD)	Min	Max
Leukociti ($10^9/L$)	First league / Prva liga	20	5.999 (1.221)	4.2	8.8
	Third league / Treća liga	20	6.243 (1.269)	4.39	8.84
	Fifth league / Peta liga	19	6.233 (1.242)	4.15	9.27
Limfociti ($10^9/L$)	First league / Prva liga	20	2.166 (0.444)	1.50	3.10
	Third league / Treća liga	20	2.476 (0.638)	1.55	3.79
	Fifth league / Peta liga	19	2.405 (0.552)	1.41	3.61
Monociti ($10^9/L$)	First league / Prva liga	20	0.640 (0.201)	0.36	1.00
	Third league / Treća liga	20	0.535 (0.130)	0.32	0.84
	Fifth league / Peta liga	19	0.526 (0.163)	0.35	0.86
Neutrofili ($10^9/L$)	First league / Prva liga	20	3.036 (1.145)	1.03	6.00
	Third league / Treća liga	20	3.006 (0.790)	1.99	4.78
	Fifth league / Peta liga	19	3.091 (0.962)	1.84	5.81
Eozinofili ($10^9/L$)	First league / Prva liga	20	0.439 (0.720)	0.08	2.05
	Third league / Treća liga	20	0.206 (0.186)	0.05	0.89
	Fifth league / Peta liga	19	0.183 (0.075)	0.06	0.33
Bazofili ($10^9/L$)	First league / Prva liga	20	0.009 (0.007)	0.00	0.02
	Third league / Treća liga	20	0.018 (0.011)	0.00	0.05
	Fifth league / Peta liga	19	0.045 (0.089)	0.01	0.41

N-number of respondents, M-arithmetic mean, SD-standard deviation, Min- the lowest score, Max-the highest score

Tabela 1. Deskriptivna statistika imunoloških parametara kod fudbalera različitih takmičarskih nivoa

N-broj ispitanika, M-aritmetička sredina, SD-standardna devijacija, Min- najmanji rezultat, Max-najveći rezultat

Table 2. Results of ANOVA analysis of immune parameters in football players of different competitive levels

Parameter / Parametar	F	p
Leukociti ($10^9/L$)	0.243	0.785
Limfociti ($10^9/L$)	1.734	0.186
Monociti ($10^9/L$)	2.841	0.067
Neutrofili ($10^9/L$)	0.038	0.962
Eozinofili ($10^9/L$)	1.995	0.148
Bazofili ($10^9/L$)	1.527	0.229

F-F statistic, p-significance level

Tabela 2. Rezultati ANOVA analize imunoloških parametara kod fudbalera različitih takmičarskih nivoa

F-F statistik, p-nivo značajnosti

show a significant effect ($F = 2.841$, $p = 0.067$). Neutrophil values were relatively uniform between groups, with the lowest value in the third league (3.006 ± 0.790) and slightly higher averages in the first (3.036 ± 1.144) and fifth league (3.091 ± 0.961). However, ANOVA did not indicate significant differences ($F = 0.038$, $p = 0.962$). In the case of eosinophils, a slightly higher value was observed in the first division (0.439 ± 0.719) compared to the third (0.206 ± 0.185) and fifth division (0.182 ± 0.074). However, statistical analysis did not confirm a significant difference between the groups ($F = 1.995$, $p = 0.148$). Basophils showed certain variations between the groups, with the fifth league soccer players having the highest values (0.045 ± 0.089), while the first league players had the lowest (0.0086 ± 0.0069).

najmanjom vrijednošću u trećoj ligi (3.006 ± 0.790) i nešto višim prosjecima u prvoj (3.036 ± 1.144) i petoj ligi (3.091 ± 0.961). Međutim, ANOVA nije ukazala na značajne razlike ($F = 0.038$, $p = 0.962$). Kod eozinofila je primijećena nešto viša vrijednost u prvoj ligi (0.439 ± 0.719) u poređenju sa trećom (0.206 ± 0.185) i petom ligom (0.182 ± 0.074). Ipak, statistička analiza nije potvrdila značajnu razliku između grupa ($F = 1.995$, $p = 0.148$). Bazofili su pokazali određene varijacije među grupama, pri čemu su fudbaleri pete lige imali najviše vrijednosti (0.045 ± 0.089), dok su igrači prve lige imali najniže (0.0086 ± 0.0069). Uprkos ovim razlikama, ANOVA nije pokazala statistički značajan efekat ($F = 1.527$, $p = 0.229$). Ukupno gledano, analiza leukocit-

Despite these differences, ANOVA did not show a statistically significant effect ($F = 1.527$, $p = 0.229$). Overall, the analysis of leukocyte subpopulations did not reveal significant differences between first, third and fifth league football players. Although certain variations in certain biomarkers were observed, they did not reach the threshold of statistical significance. These findings suggest that the level of competition does not significantly affect immune biomarkers in stable conditions, but additional research is needed to examine the longer-term effects of different training regimens and loads on the immune system of soccer players.

DISCUSSION

The results of this study did not show statistically significant differences in immune biomarkers between football players of the first, third and fifth leagues, although certain variations were observed between the groups. To some extent, these findings contradict previous research that indicated more pronounced changes in leukocyte subpopulations in athletes of a higher competitive level due to greater training stress and load (Fernández-Lázaro et al., 2022; Díaz et al., 2022). In the context of the total number of leukocytes, football players of the third and fifth league had slightly higher values compared to the players of the first league, but these differences were not significant. Previous research suggests that training intensity can cause acute changes in leukocyte counts, especially after strenuous matches and training, but that values usually return to reference limits after adequate recovery (Díaz et al., 2022). The absence of significant differences in this study can be attributed to the fact that the blood samples were taken during the period between seasons, when football players are in a phase of reduced load, which could explain the relatively stable immune parameters between the groups. Comparing lymphocytes, football players of the third league had slightly higher average values compared to football players of the first and fifth leagues, but without a statistically significant effect. Previous research has shown that elite athletes often have reduced lymphocyte values after intense efforts, which may indicate transient immune suppression ("open window" effect) and increased susceptibility to infections (Fernández-Lázaro et al., 2022). In this study, the absence of a significant difference can be explained by the stable physiological conditions during the data collection period. The values of monocytes were slightly higher in football players of the first league compared to other groups, which could indicate a slight increase in the inflammatory response in players who are exposed to a higher intensity of training during the season. Previous studies suggest that monocytes may reflect a state of chronic inflammation in athletes exposed

nih subpopulacija nije otkrila značajne razlike između fudbalera prve, treće i pete lige. Iako su primijećene određene varijacije u pojedinim biomarkerima, one nisu dostigle prag statističke značajnosti. Ovi nalazi sugeriraju da nivo takmičenja ne utiče značajno na imunološke biomarkere u stabilnim uslovima, ali je potrebno dodatno istraživanje kako bi se ispitali dugoročniji efekti različitih trenažnih režima i opterećenja na imunološki sistem fudbalera.

DISKUSIJA

Rezultati ovog istraživanja nisu pokazali statistički značajne razlike u imunološkim biomarkerima između fudbalera prve, treće i pete lige, iako su primijećene određene varijacije među grupama. Ovi nalazi su u određenoj mjeri u suprotnosti s prethodnim istraživanjima koja su ukazivala na izraženije promjene u leukocitnim subpopulacijama kod sportista višeg takmičarskog nivoa usljed većeg trenažnog stresa i opterećenja (Fernández-Lázaro i sar., 2022; Díaz i sar., 2022). U kontekstu ukupnog broja leukocita, fudbaleri treće i pete lige imali su nešto više vrijednosti u odnosu na igrače prve lige, ali ove razlike nisu bile značajne. Prethodna istraživanja sugeriraju da intenzitet treninga može izazvati akutne promjene u broju leukocita, posebno nakon napornih utakmica i treninga, ali da se vrijednosti obično vraćaju u referentne granice nakon adekvatnog oporavka (Díaz i sar., 2022). Odsustvo značajnih razlika u ovom istraživanju može se pripisati činjenici da su uzorci krvi uzeti tokom perioda između sezona, kada su fudbaleri u fazi smanjenog opterećenja, što bi moglo objasniti relativno stabilne imunološke parametre među grupama. Poredeći limfocite, fudbaleri treće lige imali su nešto više prosječne vrijednosti u odnosu na fudbalere prve i pete lige, ali bez statistički značajnog efekta. Prethodna istraživanja su pokazala da elitni sportisti često imaju smanjene limfocitne vrijednosti nakon intenzivnih napora, što može ukazivati na prolaznu imunološku supresiju ("open window" efekat) i povećanu podložnost infekcijama (Fernández-Lázaro i sar., 2022). U ovom istraživanju, odsustvo značajne razlike može se objasniti stabilnim fiziološkim uslovima tokom perioda prikupljanja podataka. Vrijednosti monocita bile su nešto više kod fudbalera prve lige u poređenju sa ostalim grupama, što bi moglo ukazivati na blag porast inflamatornog odgovora kod igrača koji su izloženi većem intenzitetu treninga tokom sezone. Prethodne studije sugeriraju da monociti mogu odražavati stanje hronične upale kod sportista izloženih dugotrajnim naporima i neadekvatnom oporavku (Fernández-Lázaro i sar., 2022). Ipak, razlike nisu bile statistički značajne, što ukazuje

to prolonged exertion and inadequate recovery (Fernández-Lázaro et al., 2022). Nevertheless, the differences were not statistically significant, which indicates the need for further research of this biomarker in different phases of the training cycle. Neutrophils, which are often associated with an acute inflammatory response to physical stress, did not show significant differences between football players of different leagues. This contrasts with some previous studies that have shown an increase in neutrophils after intense training in elite athletes (Díaz et al., 2022). Again, the period of data collection in this study, which took place between seasons, may have played a role in the relatively stable values of this biomarker. Eosinophils showed some differences between groups, with the highest values in the first league, but these differences were not statistically significant either. Eosinophils are associated with allergic reactions and can be influenced by environmental factors and stress conditions, which may be more pronounced in athletes exposed to long-term training and different living conditions (Díaz et al., 2022). Basophils had the highest values in fifth-league footballers, while the lowest values were recorded in first-league footballers. Although their function in the sporting context is less well studied, basophils are involved in hypersensitivity reactions and may potentially be indicators of immunological changes induced by physical stress. Overall, the results of this research do not confirm significant differences in the immune status of football players of different levels of competition, which may indicate that the level of competition in itself is not a key factor that determines immune responses in stable physiological conditions. One of the possible reasons for the absence of statistically significant differences is the fact that the sampling was done during the competition break, in a period when there was no training process or matches. In such a period of rest, the athlete's immune system is in a more stable homeostatic state, without pronounced acute physiological stresses, which can lead to the normalization of hematological biomarkers. Given that immune parameters show high variability depending on training load and cycle phase, future research should be conducted in periods of active training and competition, in order to more precisely see the dynamics of the immune response of athletes of different competitive levels. These findings further emphasize the importance of contextual factors such as training period, load and recovery strategies in understanding immune responses in athletes. Although the results do not indicate significant differences between the groups, it is necessary to conduct further research in different phases of the season, in order to better understand the impact of training and competition on immune biomarkers in football players of different competitive levels.

na potrebu za daljim istraživanjem ovog biomarkera u različitim fazama trenažnog ciklusa. Neutrofili, koji su često povezani s akutnim inflamatornim odgovorom na fizičko opterećenje, nisu pokazali značajne razlike između fudbalera različitih liga. Ovo je u kontrastu s nekim ranijim istraživanjima koja su pokazala povećanje broja neutrofila nakon intenzivnog treninga kod elitnih sportista (Díaz i sar., 2022). Ponovo, period prikupljanja podataka u ovoj studiji, koji se odvijao između sezona, mogao je igrati ulogu u relativno stabilnim vrijednostima ovog biomarkera. Eozinofili su pokazali određene razlike među grupama, s najvišim vrijednostima u prvoj ligi, ali ni ove razlike nisu bile statistički značajne. Eozinofili su povezani s alergijskim reakcijama i mogu biti pod uticajem ekoloških faktora i stanja stresa, što može biti izraženije kod sportista koji su izloženi dugotrajnim treninzima i različitim životnim uslovima (Díaz i sar., 2022). Bazofili su imali najviše vrijednosti kod fudbalera pete lige, dok su najniže vrijednosti zabilježene kod fudbalera prve lige. Iako je njihova funkcija u sportskom kontekstu manje istražena, bazofili su uključeni u reakcije preosjetljivosti i potencijalno mogu biti indikatori imunoloških promjena izazvanih fizičkim stresom. Sveukupno, rezultati ovog istraživanja ne potvrđuju značajne razlike u imunološkom statusu fudbalera različitih nivoa takmičenja, što može ukazivati na to da nivo takmičenja sam po sebi ne predstavlja ključni faktor koji određuje imunološke odgovore u stabilnim fiziološkim uslovima. Jedan od mogućih razloga za odsustvo statistički značajnih razlika jeste činjenica da su uzorkovanja obavljena tokom takmičarske pauze, u fazi kada nije bilo trenažnog procesa niti utakmica. U takvom periodu mirovanja, imunološki sistem sportista se nalazi u stabilnijem homeostatskom stanju, bez izraženih akutnih fizioloških stresova, što može dovesti do normalizacije hematoloških biomarkera. S obzirom na to da imunološki parametri pokazuju visoku varijabilnost u zavisnosti od trenažnog opterećenja i faze ciklusa, buduća istraživanja bi trebalo sprovesti u periodima aktivnog treninga i takmičenja, kako bi se preciznije sagledala dinamika imunološkog odgovora sportista različitih takmičarskih nivoa. Ovi nalazi dodatno naglašavaju važnost kontekstualnih faktora kao što su trenažni period, opterećenje i strategije oporavka u razumijevanju imunoloških odgovora kod sportista. Iako rezultati ne ukazuju na značajne razlike između grupa, neophodno je sprovesti dalja istraživanja u različitim fazama sezone, kako bi se bolje razumio uticaj treninga i takmičenja na imunološke biomarkere kod fudbalera različitih takmičarskih nivoa.

CONCLUSION

This research examined the immune status of football players of different competitive levels in Bosnia and Herzegovina through the analysis of key hematological biomarkers. Although certain variations were observed in the values of leukocytes, lymphocytes, monocytes, neutrophils, eosinophils and basophils between first, third and fifth league football players, statistically significant differences were not identified. These findings suggest that the level of competition is not a decisive factor in determining the immune response of athletes in stable physiological conditions. The results are to a certain extent contrary to previous research that indicated significant differences in immune biomarkers between elite and amateur athletes, which indicates the need for further research in different phases of the training cycle. It is especially important to consider the impact of acute and chronic training load, periodization of training and recovery strategies on the immune status of soccer players. The practical implications of this research emphasize the importance of regular hematological monitoring of football players of all competition levels, in order to timely identify potential changes in immune function and reduce the risk of infections and overtraining. Future studies should include a longitudinal design that would allow a more precise analysis of immune responses during different phases of the season and under different training conditions.

ZAKLJUČAK

Ovo istraživanje ispitalo je imunološki status fudbalera različitih takmičarskih nivoa u Bosni i Hercegovini kroz analizu ključnih hematoloških biomarkera. Iako su uočene određene varijacije u vrijednostima leukocita, limfocita, monocita, neutrofila, eozinofila i bazofila između fudbalera prve, treće i pete lige, statistički značajne razlike nisu identifikovane. Ovi nalazi sugerisu da nivo takmičenja sam po sebi ne predstavlja odlučujući faktor u određivanju imunološkog odgovora sportista u stabilnim fiziološkim uslovima. Rezultati su u određenoj mjeri u suprotnosti s prethodnim istraživanjima koja su ukazivala na značajne razlike u imunološkim biomarkerima između elitnih i amaterskih sportista, što ukazuje na potrebu za daljim istraživanjem u različitim fazama trenažnog ciklusa. Posebno je važno razmotriti uticaj akutnog i hroničnog trenažnog opterećenja, periodizacije treninga i strategija oporavka na imunološki status fudbalera. Praktične implikacije ovog istraživanja naglašavaju značaj redovnog hematološkog praćenja fudbalera svih nivoa takmičenja, kako bi se pravovremeno identifikovale potencijalne promjene u imunološkoj funkciji i smanjio rizik od infekcija i pretreniranosti. Buduće studije trebale bi uključiti longitudinalni dizajn koji bi omogućio precizniju analizu imunoloških odgovora tokom različitih faza sezone i u različitim trenažnim uslovima.

REFERENCES

- Andersson, H., Bøhn, S. K., Raastad, T., Paulsen, G., Blomhoff, R., & Kadi, F. (2010). Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scandinavian Journal of Medicine & Science in Sports*, 20(5), 740-747. <https://doi.org/10.1111/j.1600-0838.2009.00989.x>
- Fernández-Lázaro, D., Mielgo-Ayuso, J., Córdova Martínez, A., & Seco-Calvo, J. (2023). Omega-3 fatty acid supplementation on post-exercise inflammation, muscle damage, oxidative response, and sports performance in physically healthy adults—A systematic review of randomized controlled trials. *Frontiers in Physiology*, 13, 833580. <https://doi.org/10.3389/fphys.2022.833580>
- Ghazzawi, H., Alshuwaier, G. O., Alaqil, A. I., Bursais, A. K., Al-Nuaim, A., Alhaji, J. H., ... & Shehadeh, J. H. (2023). Correlation of consumption of vegetables, fruit, and nuts with body mass index and fat deposition in Saudi elite male soccer players. *International Journal of Human Movement and Sports Sciences*, 11(2), 350-359. <https://doi.org/10.13189/saj.2023.110212>
- Mortatti, A. L., Moreira, A., Aoki, M. S., Crewther, B. T., Castagna, C., Arruda, A. F. S. d., ... & Dias, M. (2012). Effect of competition on salivary cortisol, immunoglobulin A, and upper respiratory tract infections in elite young soccer players. *Journal of Strength and Conditioning Research*, 26(5), 1396-1401. <https://doi.org/10.1519/jsc.0b013e31822e7b63>
- Nieman, D. C., & Wentz, L. M. (2019). The compelling link between physical activity and the body's defense system. *Journal of Sport and Health Science*, 8(3), 201-217. <https://doi.org/10.1016/j.jshs.2018.09.009>
- Simpson, R. J., Kunz, H., Agha, N., & Graff, R. (2015). Exercise and the regulation of immune functions. *Progress in Molecular Biology and Translational Science*, 135, 355-380. <https://doi.org/10.1016/bs.pmbts.2015.08.001>
- Tsubakihara, T., Umeda, T., Takahashi, I., Matsuzaka, M., Iwane, K., Tanaka, M., ... & Nakaji, S. (2012). Effects of soccer matches on neutrophil and lymphocyte functions in female university soccer players. *Luminescence*, 28(2), 129-135. <https://doi.org/10.1002/bio.2351>
- Walsh, N. P., Gleeson, M., Shephard, R. J., Woods, J. A., Bishop, N. C., Fleshner, M., ... & Simon, P. (2011). Position statement part one: Immune function and exercise. *Exercise Immunology Review*, 17, 6-63. <https://doi.org/10.1016/j.jshs.2018.09.009>
- Zbinden-Foncea, H., Francaux, M., Deldicque, L., & Hawley, J. A. (2013). Does high-intensity interval training elicit an acute immune response: A systematic review and meta-analysis. *Sports Medicine*, 43(9), 987-1002. <https://doi.org/10.1007/s40279-013-0063-4>

Primljen: 20. mart 2025. / Received: March 20, 2025
Prihvaćen: 19. jun 2025. / Accepted: June 19, 2025



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.