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APPLICATION OF CELLULAR INTEGRATIVE IMMUNE MARKERS FOR THE DIFFERENTIATION OF NON-SPECIFIC ADAPTIVE RESPONSES IN ELITE BIATHLETES

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Abstract. **Aim.** This study aimed to investigate the application of cellular integrative immune markers as a methodological framework for differentiating non-specific adaptive immune responses, with the goal of enhancing the monitoring of training efficacy and assessing physical performance in elite biathletes. **Materials and methods.** This study was conducted over a 2-year period and involved 42 elite male biathletes (mean age: 23.43 ± 3.38 years; weight: 74.97 ± 7.19 kg; height: 178.70 ± 6.71 cm; body mass index: 23.44 ± 1.28 kg/m^2). Participants underwent comprehensive cardiopulmonary exercise testing with a treadmill protocol eliciting cardiorespiratory exertion. Preceding the exercise testing, peripheral blood analysis and body composition assessments were performed. **Results.** The study revealed a significant positive correlation between the systemic immune-inflammation index and heart rate at the first ($r = 0.281$, $p < 0.001$) and second ventilatory thresholds ($r = 0.276$, $p < 0.001$). Furthermore, the aerobic capacity of athletes, as indicated by oxygen uptake at the second ventilatory threshold, maximal oxygen uptake, and running time to failure, was found to be influenced by the type of non-specific adaptive immune response. **Conclusion.** The results of the study suggest that the differentiation of non-specific adaptive immune responses with respect to cellular integrative immune markers provides a valuable tool for preventing declines in aerobic capacity among endurance athletes.

***Keywords*:** biomarkers, athletes, aerobic exercise, immune system, inflammation

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ПРИМЕНЕНИЕ КЛЕТОЧНЫХ ИНТЕГРАТИВНЫХ МАРКЕРОВ ИММУНИТЕТА ДЛЯ ДИФФЕРЕНЦИАЦИИ НЕСПЕЦИФИЧЕСКИХ АДАПТАЦИОННЫХ РЕАКЦИЙ У БИАТЛОНИСТОВ ВЫСОКОЙ КВАЛИФИКАЦИИ

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Аннотация. **Цель:** оценить применимость клеточных интегративных маркеров иммунитета в качестве базиса для дифференциации неспецифических адаптационных реакций с целью мониторинга эффективности тренировок и оценки функционального состояния биатлонистов высокой квалификации. **Материалы и методы.** В исследовании, проводившемся на протяжении 2 лет, приняли участие 42 биатлониста экстра-класса мужского пола (возраст: 23,43 ± 3,38 года, вес: 74,97 ± 7,19 кг,

рост: 178,70 ± 6,7 см, индекс массы тела: 23,44 ± 1,28 кг/м^2). Участники выполняли кардиопульмональный нагрузочный тест на беговом тредбане с регистрацией показателей сердечно-легочной нагрузки, которому предшествовали анализ периферической крови и состава тела. **Результаты.** Выявлена значимая положительная корреляция между индексом системного иммунного воспаления и частотой сердечных сокращений на уровне первого ($r = 0,281$, $p < 0,001$) и второго ($r = 0,276$, $p < 0,001$) вентиляторных порогов. Установлена зависимость между аэробной способностью спортсменов, определяемой потреблением кислорода на уровне второго вентиляторного порога, максимальным потреблением кислорода, временем бега до отказа и типом неспецифической адаптивной реакции. **Заключение.** Дифференциация типов неспецифических адаптационных реакций, построенная на основании клеточных интегративных маркеров иммунитета, может предотвратить снижение аэробных способностей спортсменов, специализирующихся в видах спорта на выносливость.

Ключевые слова: биохимические маркеры, спортсмены, аэробная нагрузка, иммунная система, воспаление

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Introduction. In contemporary elite sports, the training system of athletes is characterized by a marked intensification of the training process, often involving workloads that approach the physiological limits of the human body. To achieve peak competitive results while minimizing the risk of injuries and illnesses, it is of utmost importance to establish an optimal balance between training load and recovery. The immune system plays a critical role in this context, as acute physical exertion elicits a complex immune response involving both humoral and cellular components. The humoral compartment, including cytokines and acute-phase proteins, and the cellular compartment, primarily leukocytes, have been extensively studied in relation to the intensity and duration of physical stress [1]. However, the total leukocyte count fails to capture the distinct kinetic behaviors of various leukocyte subpopulations.

In recent years, integrative cellular immune markers (IIMs), which incorporate multiple immune cell populations, have been introduced in clinical science, thereby providing a comprehensive, multifactorial perspective on inflammatory processes.

The neutrophil-to-lymphocyte ratio (NLR) is an IIM that reflects the interplay between two critical aspects of the immune system: acute and chronic inflammation, represented by neutrophil counts, and adaptive immunity, represented by lymphocyte counts [9]. Neutrophils, as the first line of immune response, mediate innate immune responses through mechanisms such as chemotaxis, phagocytosis, the release of reactive oxygen species, granular proteins, and cytokine production [10]. In contrast, lymphocytes play a central role in adaptive immunity, serving as key effector cells in systemic inflammatory reactions. The NLR, as a ratio of the two largest leukocyte subpopulations, holds significant potential as a marker for inflammation induced by physical exercise and ongoing inflammatory processes.

Another significant IIM is the platelet-to-lymphocyte ratio (PLR). Unlike NLR, PLR incorporates not only leukocyte subpopulations but also platelet counts. While platelets are traditionally recognized for their role in primary hemostasis, they also exhibit diverse pro-inflammatory properties, underscoring their relevance as a marker of inflammation [7]. To date, PLR has received limited attention in the context of exercise physiology, likely due to the perceived dissimilarity between platelets and lymphocytes in immune function. Similar to neutrophilia, which is commonly observed during exercise, platelet counts also increase significantly in response to physical exertion (thrombocytosis). This rise is attributed to the rapid release of platelets from the spleen, intravascular pools in the lungs, and bone marrow.

Hu et al. [5] introduced the systemic immune inflammation index (SII), calculated as NLR multiplied by platelet count, acting as a marker of cellular immune inflammation. This index integrates three distinct blood cell populations – neutrophils, lymphocytes, and platelets – thereby

amplifying the effects of exertion-associated neutrophilia and lymphocytopenia through the additional influence of thrombocytosis.

In light of these considerations, the primary objective of this study was to investigate the influence of IIMs (NLR, PLR, and SII) on the aerobic capacity of competitive biathletes.

Materials and methods. The current study, approved by the Ethics Committee of the Federal Science Center of Physical Culture and Sport, Moscow, Russia (approval protocol No. 2, dated April 1, 2021), was conducted in accordance with the principles outlined in the Declaration of Helsinki. This two-year research (2022–2023) involved 42 elite male biathletes undergoing centralized training as part of the national team (age: 23.43 ± 3.38 years; weight: 74.97 ± 7.19 kg; height: 178.70 ± 6.71 cm; body mass index: 23.44 ± 1.28 kg/m^2, mean ± standard deviation). All volunteers provided written informed consent, agreeing to participate in the study, undergo medical interventions, perform exercise testing with peak loads, and allow the use and publication of their anonymized results for scientific purposes. Prior to functional testing, and following a 12-hour fasting, blood samples were collected from the median cubital vein using K3-EDTA vacuum tubes (VACUETTE®, Greiner Bio-One, Austria). The Bod Pod plethysmography system (Cosmed, Concord, CA, USA) was used for body composition analysis. Cardiopulmonary exercise testing (CPET) was conducted on a Venus 200/100r running machine (H/P/Cosmos Sports & Medical GmbH, Nussdorf, Traunstein, Germany).

Cardiorespiratory parameters were continuously recorded throughout the test using the Metalyzer 3B gas analysis system (Cortex Biophysik GmbH, Leipzig, Germany). Heart rate (HR) was recorded using a chest strap as part of the Polar RS800CX training system (Polar Electro Oy, Kempele, Finland), which has a manufacturer-declared accuracy of 1 bpm.

The test protocol started with a 5-minute warm-up, consisting of walking or light pedaling on a cycle ergometer. The initial running speed and treadmill incline were set at 6 km/h and 10%, respectively. The first stage lasted 2 minutes, after which the speed was incrementally increased by 0.5 km/h per minute. Ventilatory thresholds (VT1, VT2) were determined using gas exchange data, including ventilation equivalents for O_2 (VE/VO_2) and carbon dioxide (VE/VCO_2), as well as end-tidal P O_2 ($PETO_2$) and P CO_2 ($PETCO_2$) [4]. Following a 5-minute passive rest, maximal oxygen uptake (VO_{2max}) was measured. The initial running speed was set at 6 km/h and maintained for 45 seconds, while the treadmill incline was increased to 16%. The speed was raised to 9 km/h for 30 seconds, followed by a further increase to 12 km/h. Participants were instructed to run for as long as possible to accurately assess their maximal aerobic capacity [2].

Hematological parameters of EDTA-stabilized peripheral blood were analyzed using an XN-1000 automated hematology analyzer (Sysmex Corporation, Japan). IIMs were calculated using the following equations:

$$NLR[a.u.] = \frac{neutrophil\left[\times 10^9 \times L^{-1}\right]}{lymphocyte\left[\times 10^9 \times L^{-1}\right]}$$

$$PLR[a.u.] = \frac{platelet\left[\times 10^9 \times L^{-1}\right]}{lymphocyte\left[\times 10^9 \times L^{-1}\right]}$$

$$SII\left[\times 10^9 \times L^{-1}\right] =$$

$$= \frac{neutrophil\left[\times 10^9 \times L^{-1}\right] \times platelet\left[\times 10^9 \times L^{-1}\right]}{lymphocyte\left[\times 10^9 \times L^{-1}\right]}.$$

All statistical data were tested for normality using the Kolmogorov – Smirnov test. The results are presented as mean ± SD. Pearson correlation coefficients were calculated to identify potential relationships between aerobic capacity ($VO_{2\,VT2}$, VO_{2max}, and time to exhaustion) and hematological parameters, including white blood cell (WBC) count, NLR, PLR, and SII. The experimental group was divided into five subgroups based on the type of exercise-induced non-specific adaptive reaction (NAR), as classified using the method developed by Garkavi et al. [3] and further modified by Makarova [6]. Significant differences between subgroups were evaluated using one-way analysis of variance (ANOVA). The level of significance was set at $p \leq 0.05$.

Results. NLR and SII were determined for the identified NARs. The values of NLR, PLR, and SII calculated for the entire cohort and stratified by NAR type are presented in Table 1 and Fig. 1. The relative distribution of NAR types within the experimental group was as follows: chronic stress (3.6%), training (21.5%), smooth activation (24.7%), enhanced activation (35.4%), and overactivation (14.8%). The mean $VO_{2\,VT2}$ and VO_{2max} values for the entire group of biathletes

Table 1

NLR, PLR, and SII values in elite biathletes (n = 42) and frequency distribution of NAR types observed during the study

	Number of examinations	NLR, a. u.	PLR, a. u.	SII, ×103/μL
Total	**223**	**1.43 ± 0.50**	**119.6 ± 25.1**	**336.8 ± 132.5**
Type of NAR				
Chronic stress	8	2.99 ± 0.72*	149.3 ± 13.74$	750.9 ± 177.0*
Training	48	1.93 ± 0.18*	132.13 ± 22.7#	452.3 ± 75.3*
Smooth activation	55	1.50 ± 0.12*	120.0 ± 25.4$&	346.3 ± 64.0*
Enhanced activation	79	1.15 ± 0.10*	114.1 ± 22.6$#	273.6 ± 53.4*
Overactivation	33	0.85 ± 0.10*	106.3 ± 23.1$#&	203.8 ± 39.2*

*$&# – significantly differs from all other values across various types of NAR ($p < 0.000$); NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; SII – systemic immune inflammation index, NAR – non-specific adaptation reaction.

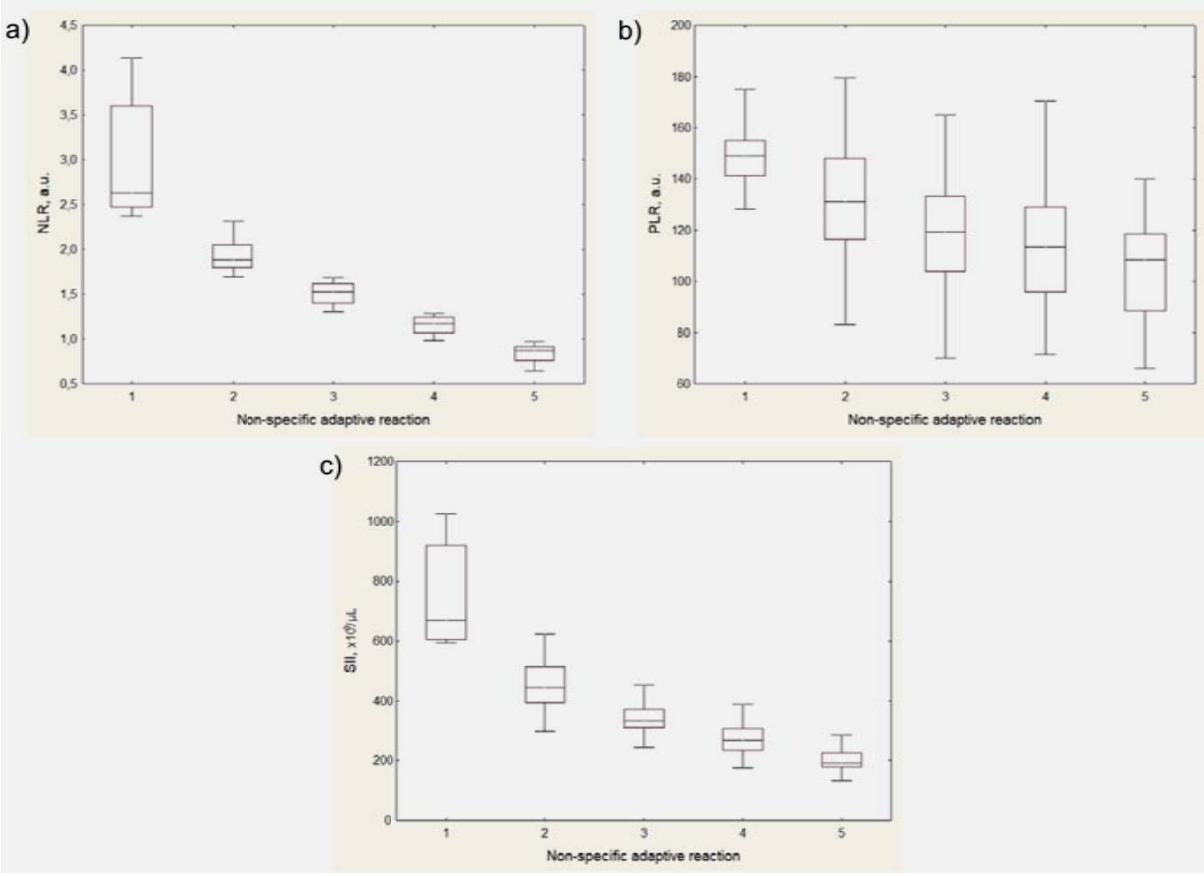

Fig. 1. IIMs in elite biathletes stratified by NAR types, median values. Whiskers indicate minimum and maximum values: a) NLR – neutrophil-to-lymphocyte ratio; b) PLR – platelet-to-lymphocyte ratio; c) SII – systemic immune-inflammation index. 1 – chronic stress; 2 – training; 3 – smooth activation; 4 – enhanced activation; 5 – overactivation

were 58.69 ± 4.47 and 67.94 ± 6.39 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively. The average running time to failure was 3:35±0:35 minutes.

For the entire cohort, correlation analysis revealed a significant positive correlation between SII and HR_{VT1} ($r = 0.281$, $p < 0.001$) and HR_{VT2} ($r = 0.276$, $p < 0.001$). There were no significant correlations between the parameters of aerobic capacity of the participants and the values of NLR, PLR, and SII.

When comparing different types of NAR, significant differences were observed between $VO_{2\ VT2}$ ($p < 0.03$) and VO_{2max} ($p < 0.008$) relative to the NAR type. Additionally, running time

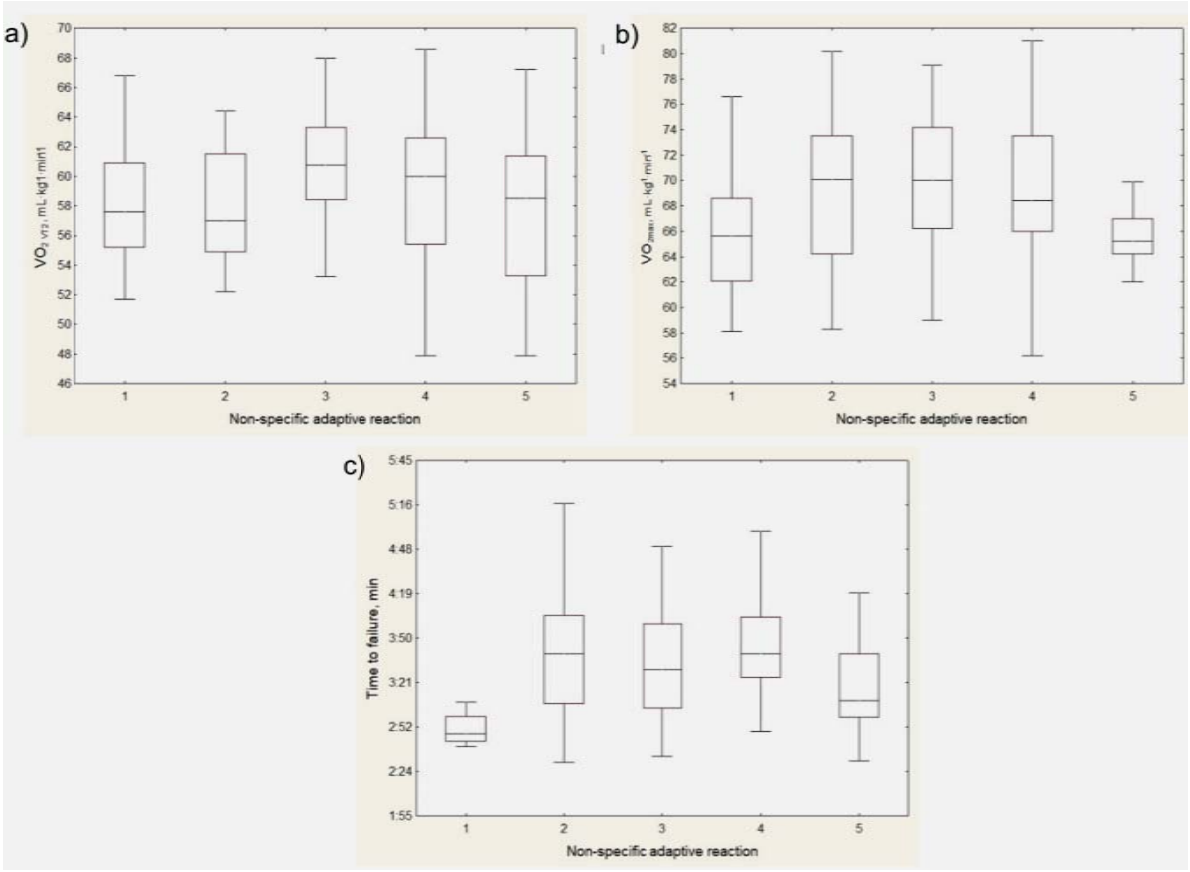

Fig. 2. Comparison of $VO_{2\,VT2}$ (a), VO_{2max} (b) and time (c) to exhaustion across various types of NAR, median values. Whiskers indicate minimum and maximum values. $VO_{2\,VT2}$ – oxygen uptake at second ventilatory threshold; VO_{2max} – maximal oxygen uptake. 1 – chronic stress; 2 – training; 3 – smooth activation; 4 – enhanced activation; 5 – overactivation

to exhaustion showed significant variation relative to the NAR type ($p < 0.007$) (Fig. 2). No significant differences were found for HR_{VT1} and VT1 and HR_{VT2} across the various NAR types.

This study focuses on the role of three clinically relevant IIMs: NLR, PLR, and SII, and their influence on physical capacity parameters in elite biathlon athletes. Although these IIMs are well-established in a number of medical fields, their predictive utility as a tool for assessing training adequacy and monitoring physical performance in elite athletes remains underexplored.

A prior pilot study [11] found no significant relationship between IIMs and key athlete characteristics, such as sex, age, BMI, and training volume. Furthermore, no significant differences in IIM levels were observed across different athletic events, including cyclic sports (n = 24), combat disciplines (n = 41), technical disciplines (n = 46), athletics (n = 42), and ball sports (n = 42). The authors attributed this observation to the small sample size of endurance athletes or to significant intra-group differences in physical fitness levels.

In contrast, the present study involved a homogeneous group of elite male biathletes undergoing centralized training as part of the national team. This selection strategy ensured that the study population accurately represented skilled athletes while maintaining sample homogeneity. Participants were selected through a competitive process, and their athletic performance can be considered representative of the best standards relative to athletes of different ages, qualifications, and athletic experience. The mean NLR, PLR, and SII values observed in biathletes were comparable to those reported in athletes specializing in non-cyclic sports. A comparative analysis of these findings with those of [11] revealed no significant relationship between IIMs and athletic events, suggesting the potential utility of IIMs as prognostic markers for identifying significant alterations in the immune status of athletes.

The pilot study [11] reported a negative correlation between aerobic fitness, measured by

peak power output, and baseline SII and NLR values. In this study, no significant correlations were found between aerobic capacity parameters ($VO_{2\ VT2}$, VO_{2max}, and time to failure) and IIMs values across the entire experimental group. However, when participants were stratified into subgroups according to the NAR type (chronic stress, training, smooth activation, enhanced activation, and overactivation), significant differences were observed in aerobic capacity parameters in all subgroups. As illustrated in Fig. 1 and Table 1, NLR and SII effectively differentiated the NAR types, whereas PLR did not provide unambiguous differentiation. Athletes classified under "chronic stress" and "overactivation" demonstrated significantly lower aerobic capacity (Fig. 2). Higher aerobic capacity observed in athletes classified under "training", "smooth activation", and "enhanced activation" is probably associated with higher functional activity of cellular immunity, while the "chronic stress" and "overactivation" responses are characterized by elevated metabolic stress compared to other NAR types [8].

Conclusion. In this work, we evaluated the adequacy and practical significance of an approach consisting of differentiating NARs based on the NLR. Our findings revealed that the aerobic capacity of athletes, as measured by $VO_{2\ VT2}$, VO_{2max}, and time to fatigue, varied significantly across NAR subgroups. No statistically significant correlations were observed between these parameters of aerobic capacity and IIMs within the general sample of athletes. Therefore, the differentiation of NAR types using IIMs (NLR, SII) could be a promising approach for assessing training loads and monitoring physical performance in athletes. A limitation of this study is the participation of exclusively male athletes. Future research could be focused on exploring the behavior of NAR types in response to exercise tests and localizing underlying inflammatory processes through the use of additional body system-specific biochemical markers.

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