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NLR: An incipient marker to monitor inflammation, overtraining, and recovery in athletes

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Abstract
Neutrophil-to-Lymphocyte ratio (NLR) is a simple parameter used for easy assessment of the inflammatory status of a subject. NLR is calculated by dividing the absolute neutrophil count by the definite lymphocyte count. NLR reflects the balance between the innate and adaptive immune systems. Recently, NLR has been used as an essential biomarker of inflammation, infections, and postoperative complications and has displayed a correlation with the circulating level of C-reactive protein. More recently, NLR is also being used to assess the systemic inflammatory status in athletes. In athletes, altered neutrophil and lymphocyte counts ensued during and after the exercise, resulting in the altered neutrophil-lymphocyte ratio (NLR). Usually, NLR returns to the normal range within 6-9 hours after the cessation of training, but it takes more than 24 hours to reach the basal state if the exercise is prolonged and stressful. Thus, NLR seems to be a good measure of exercise stress and subsequent athlete recovery. Moreover, estimation of NLR requires no exertion from the athletes, and it can be assessed within a minimum time frame and is cost-effective. So, the assessment of NLR is advantageous in monitoring the post-exercise recovery and exercise-induced inflammation in athletes.

Keywords: Neutrophil to lymphocyte ratio, exercise-induced inflammation, overtraining, C-reactive protein

Introduction
White blood cells, also known as leukocytes, protect our bodies from infectious diseases and clean up cell debris. Depending on the granules present inside the cells, leukocytes are divided into granular and agranular leukocytes [1]. Granular leukocytes are further divided into neutrophils, eosinophils, and basophils. Agranular leukocytes are subdivided into monocytes and lymphocytes [2].

Neutrophils: These granular leukocytes typically comprise 50-70% of the WBC count. They contain a distinct lobed nucleus, and with the age of the cell, the number of the lobes of the nucleus increases from two to five. Thus, these are also called polymorphonuclear leukocytes (PMNs). Neutrophils protect our body from pathogens such as bacteria by phagocytosis [3].
Eosinophils account for about 2–4% of the total leukocyte count. They provide defense against parasitic infections and intracellular bacteria and modulate immediate hypersensitivity reactions. Eosinophils control the immediate hypersensitivity reactions by degrading or inactivating the various mediators released by the mast cells (histamine, leukotrienes, lysophospholipids, heparin. etc.) [4].
Basophils are the rarest type of leukocyte, making up less than one percent of the total leukocyte count. Their function is unclear, but most scientists agree that they play a significant role in host defense against parasites and release histamines and anti-clotting chemicals in response to wounds and potential infections/pathogens [5].

Lymphocytes: Though all lymphocytes originate from the lymphoid stem cells of bone marrow, their maturation occurs in distinct lymphatic tissues such as the thymus (T-lymphocytes) and bursa equivalent, i.e., gut-associated lymphatic tissue (B-lymphocytes). During regular exercise, the lymphocyte count might increase in the vascular compartment, but after prolonged exercise, it decreases [6].

Monocytes: Account for approximately 2-8% of total leukocytes in the blood. These cells are usually quite large and can be differentiated into macrophages and dendritic cells. The primary function of macrophages includes the phagocytosis of pathogens. Monocytes also secrete chemicals that will bring other types of leukocytes to the area of infection or wound [7].

Neutrophil to Lymphocyte Ratio (NLR): The neutrophil to lymphocyte ratio (NLR) is a simple parameter used to assess a subject's inflammatory status. NLR can be evaluated by dividing the absolute neutrophil count by the total lymphocyte count. The NLR is assumed to reflect the balance between the innate and adaptive immune systems [8]. Recently, NLR has been applied as a diagnostic marker of inflammation, infections, and postoperative complications as it has shown a correlation with the circulating level of C-reactive protein [9]. More recently, NLR is also being used to access the systemic inflammatory state in athletes.

Application of NLR in sports coaching and sports medicine: The NLR and monocyte to lymphocyte ratio (MLR) are inexpensive parameters and considered as relevant biomarkers in various fields of medicine. For coaches to understand the recovery status of athletes, it is essential to understand the impact of the initial exercise session on their bodies. This monitoring could help the coaches to develop a more suitable training program that effectively stimulates the athlete's body and allows adequate recovery time besides limiting the possibility of overtraining while maximizing the intensity of the workout. Currently, coaches are using some functional tests that evaluate strength, peak power output, and fatigue as primary tools to assess the recovery of athletes, and the application of biochemical markers to understand exercise recovery is very much limited. However, this approach requires time to observe noticeable alterations in an athlete's performance. Alternatively, they can use some blood biomarkers which give accurate information about exercise-induced muscle damage, inflammation, and recovery without any delay.

Muscle damage biomarkers such as lactate dehydrogenase (LDH), aspartate aminotransferase, carbonic anhydrase isoenzyme II, and creatine kinase (CK) are widely used. Among these, creatine kinase is more reliable and is cost-effective in diagnosing exercise-induced muscle damage [10]. According to Bird *et al.*, after severe bouts of exercise such as marathons and ultra-marathons, the level of creatine-kinase increases beyond 2400 IU/L [11].

Muscle damage is often associated with an inflammatory response, which initiates a rapid invasion of inflammatory cell populations into the muscle fibers and may persist for days or weeks. After completing the high-intensity exercise, when recovery occurs, the inflammatory cells and their signaling molecules, such as reactive oxygen species (ROS) and cytokines, are thought to mediate the repair process. Thus, the post-exercise inflammatory response induced by muscle damage is functionally beneficial. Inflammation is assumed as a fundamental part of muscle repair while monitoring the biomarkers of recovery status. Therefore, Bessa *et al.* hypothesized that the altered appearance of leukocyte subpopulations and cytokines in the bloodstream could serve as biomarkers of an individual's recovery [12]. Our previous study also reported an increased neutrophil count and decreased lymphocyte counts after the intense weightlifting training session, increasing NLR significantly [13].

Sports training and Overtraining: The principal objective of training in sports is to provide the body with adequate physical stress that triggers the adaptive changes that can improve performance. Recovery between training sessions is crucial for improvement and recuperation. Sometimes, if the training load is more significant than what the athlete's body can bear or sufficient recovery is not provided, that might lead to overtraining. Intense training, characterized by long-lasting fatigue and worsening competitive performance with further attempts to improve the physical condition, is known as overtraining. Sometimes the stress response becomes too high with inadequate recovery time between the training sessions, which results in overtraining. Other stressors, such as psychological stress, malnutrition, infection, and lifestyle, may also contribute to underperformance in some cases [14].

Overtraining syndrome is characterized by chronic fatigue and underperformance, with increased susceptibility to infection leading to recurrent infections. Several factors, including psychological, endocrinological, physiological, and immunological factors, could affect an athlete's post-exercise recovery in overtraining conditions. Careful monitoring of training response will help to prevent overtraining in athletes, with a well-organized exercise regimen and recovery strategies. Generally, overtraining symptoms resolve in 6–12 weeks but may continue much longer or recur if athletes return to hard training too soon [15].

Overtraining markers: A strenuous exercise bout results in both short-term and long-term changes to the plasma volume and analyte concentrations. The concentration of circulating biomarkers such as catecholamines (adrenaline and noradrenaline) is proportionally related to exercise intensity. Since catecholamines have a short half-life, circulating concentrations usually return close to resting concentrations within 5–10 min after the cessation of exercise. They, therefore, are not likely to be substantially elevated in the samples taken after this time [16]. Cortisol levels might increase during or after high-intensity, short-duration exercise, and during low–intensity exercise is done for 2-3 hours. β-endorphins also show a similar response to cortisol. Testosterone increases with high-intensity activity and decreases if the training proceeds for a prolonged period. Testosterone and cortisol play an essential role in protein and carbohydrate metabolism [17]. The free testosterone: cortisol ratio (FTCR) is widely used in sports to screen for overtraining [18].

Several studies have reported increased levels of biomarkers, *viz*. creatine kinase, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and conjugated bilirubin, after the completion of marathon and ultra-distance events [19]. NLR is another significant parameter in understanding the balance between training load and recovery in athletes.

NLR response during sports training: An acute bout of exercise alters the counts of leukocyte subpopulations. The neutrophil count increases during exercise and continues to rise in post-exercise conditions. But there is a different picture in the case of lymphocytes. During the exercise initially, the lymphocyte count increases, but it decreases after the intense long-duration exercise. The initial elevation of lymphocytes might be due to the release of lymphocyte subpopulation into the blood. The decrease of lymphocytes in the later phase might be due to the destruction of natural killer (NK) cells and B cells. Immediately after high-intensity exercise, increased apoptosis of lymphocytes also has been observed. Periodic monitoring of differential leucocyte counts seems helpful when exercise-protocol is too stressful. After a repeated bout of strenuous exercise, many neutrophils releases from the bone marrow, but after repeated bouts of exercise for weeks or months, the neutrophil reserve of the bone marrow might decrease. Due to this, low neutrophil counts can be noticed after testing in some overtrained athletes [20].

Meanwhile, low blood leukocyte counts have been seen in overtrained athletes. So, an increase in neutrophil count and a decrease in lymphocyte count during and after the exercise will alter the neutrophil to lymphocyte ratio (NLR) in athletes. Typically, NLR returns to the normal range 6-9 hours after the cessation of exercise, but it might take more than 24 hours to reach the basal level if the exercise is prolonged and stressful. Thus, NLR seems to be a good measure of exercise stress and subsequent athlete recovery.

The NLR response to exercise-induced inflammation

Apart from well-established clinical inflammation markers such as C-reactive protein (CRP) or interleukin-6 (IL-6), white blood cell count (WBC) and erythrocyte sedimentation rate (ESR), cellular immune inflammation markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII = NLR × platelets) are also being used in sports medicine. Due to exercise stress, the stress system produces some hormones. These stress hormones affect the immune system resulting in illness. Neutrophils and lymphocytes play a significant role in both the natural and adaptive immune systems [21]. So, NLR represents the response to stress and the balance between innate and adaptive immune systems [22]. Since the NLR can be used as an indicator of systematic inflammation, it can be used to monitor the inflammatory response after exercise training [23].

Some studies have reported significant correlations between NLR and other well-established inflammation markers such as white blood cell count [24], CRP [25], IL-6 [26], and erythrocyte sedimentation rate [27]. Chen *et al*. conducted a study to examine the effect of ten-week taekwondo (TKD) specific training on aerobic capacity, body composition, hormone responses, and hematological parameters in elite taekwondo athletes with various initial inflammatory statuses. They divided twenty-two elite college TKD athletes into two equal groups based on their initial neutrophils-to-lymphocytes ratio (NLR) values (Low NLR group with NLR: 1.3 ± 0.2; and High NLR group with NLR:2.5±1.3), they detected that the high NLR group athletes exhibited relatively less adaptive response in aerobic capacity after ten weeks of TKD-specific training. They also observed a decreased dehydroepiandrosterone -sulfate (DHEA-S) to cortisol ratio (D/C ratio) in the high NLR group after the training. Chen *et al*. concluded that the fewer exercise adaptive benefits observed in the high NLR group might be due to a higher systemic inflammation state and poor anabolic capacity [28]. Sultan *et al*. investigated the role of inflammation in cardiopulmonary capacity in preoperative patients and reported that the individuals with higher NLR will have a lower anaerobic threshold (AT) value [29]. Liao *et al*. observed an increase in NLR eight weeks after the cessation of taekwondo training and reported a strong association between NLR with HOMA-IR (Homeostatic Model Assessment for Insulin Resistance), body fat mass, waist-to-hip ratio (WHR), and the decline in VO_2max [30]. Wang *et al*. investigated the effect of diet and exercise on NLR in overweight adolescents, and they reported a significant reduction in the NLR from 2.1 ± 0.7 to 1.6 ± 0.7 after a 4-week diet and physical exercise intervention, and the decrement in NLR is also significantly correlated with a decrement in Interleukin-6 (IL-6) and White blood cells (WBC) [31]. A significant increase in NLR (1.46 ± 0.64 to 2.15 ± 0.69), immediately after the high-intensity weightlifting training session had also been reported in our previous study [13]. Azam *et al*. conducted a study to investigate the individual relationships between NLR and exercise intensity and cardiac-troponin I (cTnI) after long-distance cycling and concluded that the NLR and exercise intensity are significantly associated with post-exercise cTnI levels, suggesting that inflammatory factors may influence the magnitude of exercise-induced cTnI release [32].

Conclusion

Apart from well-established clinical inflammation markers such as C-reactive protein (CRP) or interleukin-6 (IL-6), white blood cell count (WBC), and erythrocyte sedimentation rate (ESR), cellular immune inflammation markers such as neutrophil-to-lymphocyte ratio (NLR) could be used in the field of sports medicine to monitor exercise-induced inflammation, training stress in athletes. Estimation of NLR requires no exertion from the athletes, and it can be done within a minimum time frame and is cost-effective. The determination of NLR would also be advantageous in monitoring the post-exercise recovery of athletes.

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