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Influence of Laboratory Index on match performance. A comparison study to evaluate physical performance in professional soccer players of an Italian Elite Team

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Abstract. – OBJECTIVE: It was hypothesized that activity exercise at professional levels could lead to an increase in metabolic levels and a decrease in performance parameters. These trends are explained by physical activity as a cellular stressor.

SUBJECTS AND METHODS: We used an algorithm, Laboratory Index which evaluates salivary cortisol, CK and d-ROMs, collected previously from elite Italian soccer players, compared to InStat Index. The last one estimates analytically the athlete's performance in soccer pitch, applying the Heath Maps.

RESULTS: A good agreement between the two Index was obtained, especially for two players, who showed an ideal combined trend.

CONCLUSIONS: We would investigate the clinical and activity profile of soccer players with the aim of providing information for the development of training strategies. Also, the performances, during training and match time, are an objective evaluation of the athlete's physical preparation. As a consequence, the combination of two Index could be used for a new approach to the sports world.

Key Words:
Athletic performance, Laboratory Index, InStat Index, Cellular stress, Oxidative stress, Reactive Oxygen Species, Physical exercise.

Introduction

Physical exercise strongly affects human metabolism and causes biochemical changes. This is reflected in the results of laboratory analyses of changes in serum parameters, which indicate degrees of fatigue. The many faces of different types of training and the variety of physiological reactions of trainees explain why sport medicine is still looking for a perfect way to monitor the effects of training[1].

Muscle metabolism parameters, like creatine kinase (CK), derivates of reactive oxygen metabolites (d-ROMs) and salivary cortisol, are usually increased after exercise. Creatine kinase (CK) is an enzyme present mainly in skeletal muscle and heart muscle. An elevated creatine kinase value may be due to heterogeneous causes, including muscle fatigue following intense sports training. CK is a dimeric globular protein consisting of two subunits with a molecular mass of 43 kDa. It buffers cellular ATP and ADP concentrations by catalysing the reversible exchange of high-energy phosphate bonds between phosphocreatine and ADP produced during contraction[2]. The serum level of skeletal muscle enzymes is a marker of the functional status of muscle tissue and varies widely in

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both pathological and physiological conditions. An increase in these enzymes may represent an index of cellular necrosis and tissue damage following acute and chronic muscle injuries. The study of CK in sports medicine allows to obtain information on the state of the muscle. High levels of serum CK in apparently healthy subjects may be correlated with physical training status. However, if these levels persist at rest, it may be a sign of subclinical muscle disease, which training loads may evidence through the onset of symptoms, such as profound fatigue[3]. D-ROMS reactive oxygen metabolites (ROS) evaluate oxidative stress by measuring the different species of oxygen reactive radicals, or free radicals. The ROM level increases following muscle exercise, compared to baseline values measured at rest: it could indicate a pathological condition, poor recovery capacity after intense effort or inadequate training. ROS production plays a key role in regulating signals required for muscle adaptation in response to exercise. Oxidative stress is a condition that occurs when ROS generation gets over the antioxidant system which may lead to oxidizing cellular components[4]. In particular, some studies[5,6] have recently focused on the role of oxidative stress in sport. In fact, it is well established that muscle contractions during exercise lead to elevated levels of ROS in skeletal muscle[5]. On the other hand, experimental evidence supports that moderate and regular physical exercise reduces ROS. Paradoxically, accumulating evidence for the past few years has shown that higher muscle contraction during exercise leads to in-creased ROS production[7]. Salivary cortisol is a hormone produced by the adrenal glands on the pulse of the brain and is the first indicator of physiological stress, but a high value is also associated with muscle fatigue due to excessive training (overtraining). High performance athletes are constantly facing different situations involving mental and physical stress. Both promote the same physiological response, which results in hypothalamic pituitary-adrenal (HPA) axis activation. The physiology of the HPA axis ensues when a stimulus perceived by the hypothalamus induces the secretion of corticotropin-releasing hormone (CRH), that in turn, causes the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which stimulates the secretion of glucocorticoids (corticosteroids in animals and cortisol in humans) in the cortex of the ad-renal gland as an end-product of the HPA axis. However, if the stress response is prolonged and chronic, the HPA axis can become with inadequate cortisol responses to a certain stimulus or at rest. Although physical exercise is considered a stressor stimulus[8].

However, the current literature[9-11] is mainly focused on acute effects of one or two exercise's stressor parameters, although this does not represent the typical situation. To overcome this limitation and to provide new references for athletes during continuous long term high-intensity training, we have chosen professional soccer team. This discipline contains high muscular demand of different kinds (from locomotor activity, change of directions, jumping, and from contacts) and is characterized by frequent competitions[11], with the possibility to monitor stressor parameters in long time terms.

The aim of our study is to compare the Laboratory Index (salivary cortisol, CK and d-ROMs) with physical training in elite professional soccer players. The Laboratory Index could be useful to regulate the athletes training in order to achieve better performances on the match time. Also, we put in relationship the clinical algorithm Laboratory Index and InStat Index values to evaluate player's performance and physical training, so as could be a predictive tool for the athlete's performance.

Subjects and Methods

Participants

For this study, an Italian First Division team was considered, with 11 professional soccer players. The athletes who participated in the study were all male aged between 25 and 35 years old. They were non-smokers and had no history of metabolic syndrome or cardiovascular diseases. They took no medication or supplements, which may have impact on their body metabolism. Sadly, we carried on the continuity of monitoring biochemicals and athletics parameters with six soccer players.

Unfortunately, the timing of biochemical analysers collection was not so frequent as the time of periods of training, because of professional training of soccer players. However, each athlete was tested once a month for three consecutive months. Each test was two days after the match-day. Blood samples were collected by capillary-puncture in tubes, less invasive than venipuncture, for serum and K2DTA tubes for plasma, meanwhile

the salivary cortisol was collected by a non-invasive method. The biological specimens were analysed immediately.

The players were fully informed in advance of any risks and discomfort associated with the blood sampling procedures before giving their written consent to participate. The study has been carried out in accordance with the Helsinki Declaration and approved by the Ethics Committee of University Hospital of Tor Vergata, Rome, Italy (Registration number R.S.41.17 on 22nd September 2019).

Biochemical Analyses

In the d-ROMs Test (Diacron International s.a.s., Italy), ROMs (hydroperoxides, ROOH, primarily) of a biological sample, in presence of iron can generate radicals, according to the Fenton's reaction. Such radicals, in turn, oxidize an alkyl-substituted aromatic amine thus transforming them in a pink-coloured derivative, which is photometrically quantified. Indeed, the intensity of developed colour is directly proportional to the concentration of ROMs, according to the Lambert-Beer's law. For more details, follows the manufacturers' instructions.

Salivette sampling devices (Sarstedt AG & Co., Nümbrecht, Germany) were used for saliva collections according to manufacturers' recommendation. The time of sample collection must be considered when interpreting results due to the cortisol secretion circadian rhythm.

Salivary cortisol kit (Elecsys Cortisol II, Roche Diagnostics, Germany) with competitive immunoenzimatyc colorimetric method was used for quantitative determination of the concentration of cortisol in saliva, according to manufacturers' recommendation.

Creatine kinase (CK) was measured via capillary blood samples using a test in vitro for a quantitative determination (Roche Diagnostics, Germany). The principle of test is based on a UV determination of CK, measured by quantities of NADPH. The photo-metrically measured rate of reduction of NADP+ is directly proportional to the CK activity. For optimum performance of the assay, follow the directions given from manufacturers (Roche Diagnostics, Mannheim, Germany).

Match Analysis and Performance Analysis

Match Analysis is the attitude analyses which describes the behavioural analysis in sport performance by codifying the actions of individuals or groups in specific terms useful for the practice[12] and consists of two components: Notational Analysis and Motion analysis. Notational Analysis is identifying, booking and accurate analysis of critical event happening during the sport performance. It elaborates the team collaboration and efficiency under a competitive attitude. Motion Analysis objectively evaluates the sport/motion features, without a qualitative score/vote.

Match analysis has been extensively used[13] in soccer to evaluate player motions during the game. Match-motion data, once obtained, are extremely useful to the exercise and sport physiologist for the implementation of proper training.

Instead, performance analysis is the process of assessing performance in a sport and it consists of tactical assessment, movement analysis, video and statistical databasing and modelling and coach and player data presentations[14].

Our analysis was elaborated due to match analysis software, LongoMatch (https://longomatch.com/en/) and due to statistical online platform, InStat (https://InStatsport.com/football). LongoMatch consents to analyse live sports competition and recorded competition, in a post-match moment.

The parameter of InStat used in this study is the InStat Index, which is an automatic algorithm that considers a series of parameters as player's involvement in team's performance, as outcome of his actions, as level of the opponent and as level of the championship in which he plays.

The numerical value of the InStat Index is obtained from an average of about 12 or 14 factors multiplied each other, calculated during matches in long-term periods.

Also, the final value obtained allow you to predict any possible performance drops of the athlete, such as to change the intensity of his training and his distribution during the match[15].

Heat Maps

To make inferences about the frequenting of specific zones of the soccer pitch, the playing surface was divided into 14 zones and grouped into 8 sections. With dedicated software, it was possible to calculate the distances covered by each player within each of the 14 zones. Sections were then ranked according to space covered in-side each of them and then compared for statistical significance.

Laboratory Index

Our Laboratory Index was calculated by an algorithm formula based on blood levels of CK and

d-ROMs and salivary levels of cortisol. To avoid perturbation of circadian rhythm of cortisol, the biological samples have been collected always in the morning after two day from the day match. Also, the algorithm was designed to adjust the levels of cortisol between males and females, anyhow it is not concern of the study.

Once all the biochemical data were analysed, the algorithm process a score. The score, which is from 0 to 10, investigates on athlete's physical stress levels. This score could predict the future player's performance. We associated scores with a proper training, identifying the optimal one athlete specific. We suggest from 0 to 2 low-intensity training; from 3 to 4 a low-intensity training with progressive workload recovery; from 5 to 6 a light-effort training; from 7 to 10 routine training.

Statistical Analysis

The relationship between Laboratory Index and InStat Index was evaluated by Spearman's coefficient correlation. A p-value lower than 0.05 was considered statistically significant.

Results

Our data shows a good correlation between the InStat Index, as described in materials and methods, and the Laboratory Index.

We calculated the laboratory's athletic vote for three times after two days from the day match and compared it with the InStat Index obtained. In the three different times (Time 1, Time 2, Time 3) they show a significant Spearman's coefficient correlation of 0.82, 0.79, and 0.97 respectively (Table I). The total correlation of the InStat Index vs Laboratory Index was 0.68 with a significance of p=0.002.

Figure 1 shows the data of the InStat Index and of the Laboratory Index at different times of 6 professional soccer players. As we can see from Figure 1, Laboratory Index correlates well with the performance of each player, except for player 4 and player 6 in Time 2.

Player 2 and player 5 highlight an optimal correlation between the two Index. We were not able to calculate InStat Index because player 5 in Time3 remained on the bench for whole time match due to low athlete's physical performance during the training. The Laboratory Index predict the low performance, at Time2 the Laboratory Index scored 3. In fact, Player 5 shows very low athletic lab scores.

Finally, in Figure 2 we can see the Heat Maps of player 2 and player 5. In soccer as in any other sports discipline, they are used to evaluate the frequency of the analysed events that occur in a certain area. The Heat Maps are displayed in grey tone and they allow us a visually player's activity during the match time. Player 2 shows a high frequency of events at Time 1 and Time 3, instead was observed a sharp decrease in the intensity of activity at Time 2. Instead, player 5 reveals a constantly decline of the athlete's performance.

Discussion

As it known in literature, oxidative stress is involved in many physiological (physical exercise, intracellular signalling, immune responses etc.) and it becomes pathological when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of cells to detoxify[16,17]. Even in our study was observed that physical exercise typically increases the production of reactive oxygen species and free radicals, in elite sport athletes. Also, we confirmed previous studies[18] about single bouts of strenuous physical exercise are also harmful to trained individuals, such as soccer players[6]. Intense physical exercise causes muscle injuries that lead to the on-site recall and to the activation of neutrophils and macrophages that produce additional ROS in the hours following the effort. Furthermore, the increased secretion of catecholamines induced by physical activity also plays a role in the generation of ROS[19-22]. In particular, the eccentric muscle contractions are those that cause the

Table I. Spearman's coefficient correlation between Laboratory Index and InStat Index at three different times and for total data.

	Time 1	Time 2	Time 3	Total
Spearman's coefficient correlation Laboratory Index *vs.* Stat Index	0,82	0,79	0,97	0,68
Significance level	p = 0,0458	p = 0,049	p = 0,0012	p = 0,0020
95% Confidence Interval for Spearman's coefficient	0,0243 to 0,980	-0,0482 to 0,976	0,752 to 0,997	0,310 to 0,870

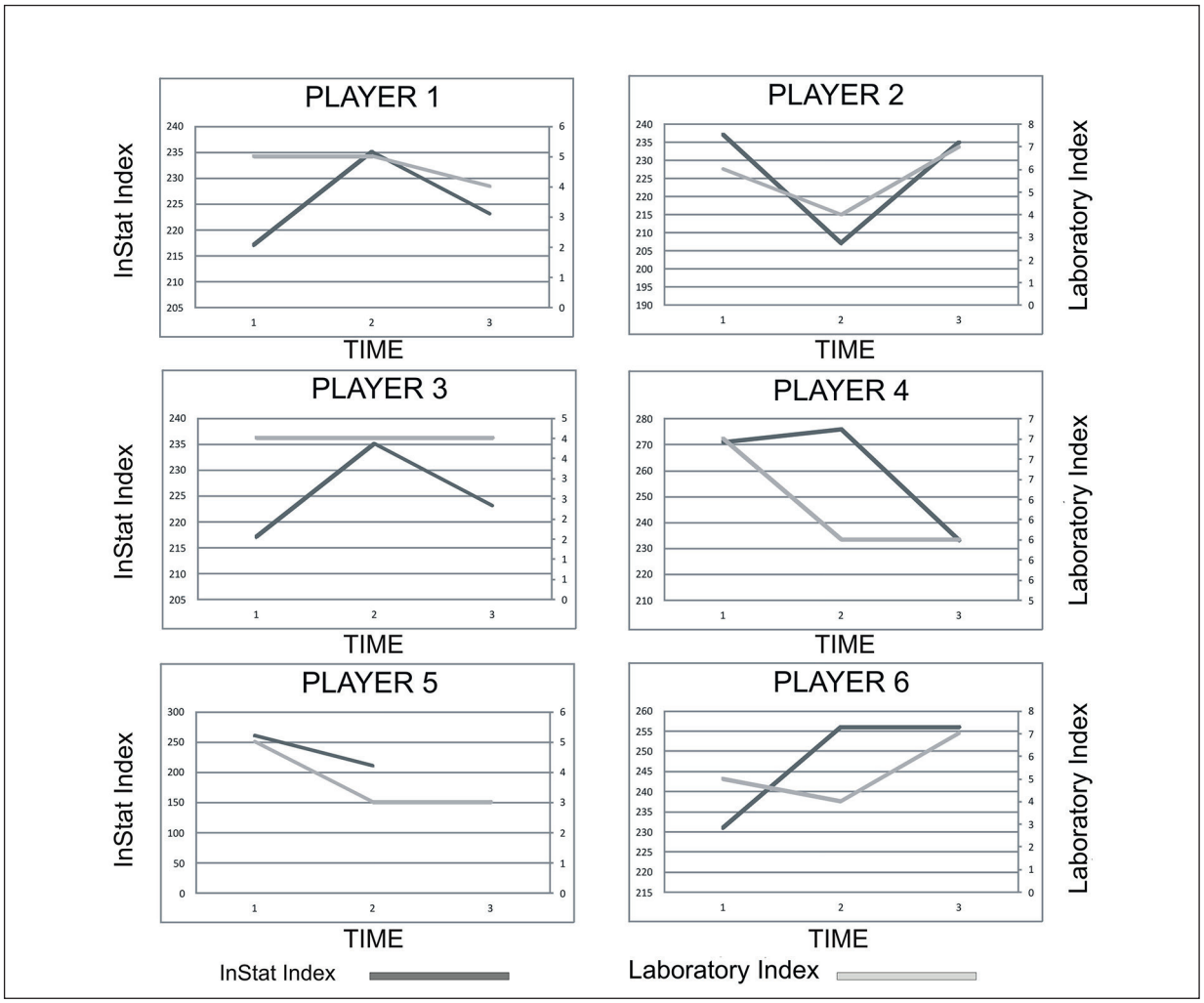

Figure 1. Relationship between InStat Index versus Laboratory Index in six elite players from Italian First Division at three different times. The light-grey line indicates Laboratory Index; the dark-grey line indicates InStat Index.

greatest oxidative stress in skeletal muscle[23,24]. If free radicals exceed the body's ability to buffer them, they negatively affect lipids, proteins and DNA[25], causing molecular damage and impaired cellular function[18,20,25]. The production of ROS and free radicals during exercise might have both positive and negative physiological effects depending on the antioxidant capacity and physical effort[26]. Many eccentric muscle contractions are performed during the soccer matches and those contractions cause bursts of oxidative stress in the skeletal muscle[23,24]. Souglis et al[27] analysed the time course of oxidative stress, inflammation and muscle damage markers for 5 days after a soccer match in both male and female players and they explored the differences between the roles of: de-fenders, midfielders and attackers. They demonstrated that male players had higher values than women for all oxidative, inflammatory and muscle damage indices and they found that the midfielders had the major laboratory abnormalities. Both female and male players experienced a significant level of muscle damage and inflammation response.

Viana-Gomesa et al[28] investigated the effects of playing two soccer games during one week with a temporal distance of 72 hours between the games. They have monitored the markers of oxidative stress, muscle and liver cell damage and the results showed that all the values did not return to the basal value before the second match and some of them went further after the second match[29].

However, in our study we evaluated in addition to oxidative stress, detected as dROMs, other factors, CK and salivary cortisol, that induce physio-

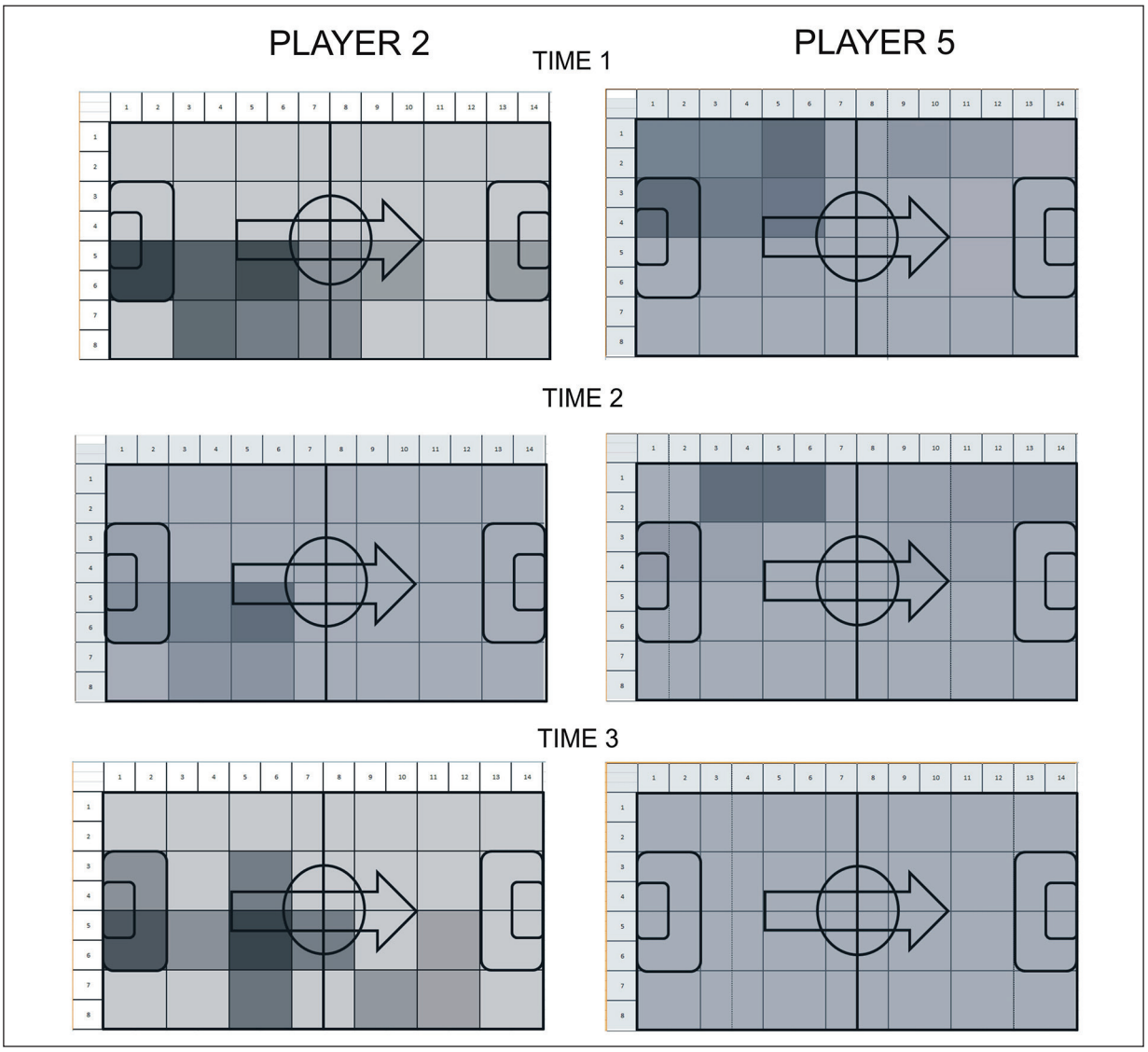

Figure 2. Heat Map of Player 2 and Player 5 at three different time. The intensity of grey tone is a measure of player's activity in the different of areas of the soccer pitch from light (*low activity*) to darkest (*high activity*).

logical stress, altering the athlete's performance. Moreover, our analysis was scattered in three times, during the soccer match season, each time after two days from day match.

The CK provides to supply ADP to the muscle cells during the contraction, so it is a valuable biomarker to monitor the muscle cellular fatigue. Also, salivary cortisol is another biomarker to evaluate cell stress[30-32]. It is released after HPA axis activation in response to a stress situation. This biomarker could show HPA reactivity and demonstrate several stress disorders, such as overtraining syndrome[33].

In professional athletes' salivary cortisol is always higher than normal due to stressor stimulus of physical exercise and to daytime. For this, our algorithm au-to-evaluate the right score appropriately to gender and collection time. In this way diurnal variation, which may occur for cortisol level and eventually in women oral contraception, can be avoided. Anyhow, in our study we did not need these adaptations because all the samples have been collected in early morning, after two days from match and from male players.

In our work we showed that biochemicals anomalies are associated with negative physiological adaptations, that influence the player's performance during the training sessions or the match.

The clinical utility of biochemical screening using multiple parameters is a valid tool to ob-

tain objective and real results. We created an algorithm that evaluates all these biochemical parameters simultaneously, providing a score from 1 to 10. Each score is associated with a proper training session, detecting the optimal one, relying on the physiological status of the player. We called our algorithm Laboratory Index and we compared it to InStat Index, which evaluate the athlete's performance in soccer pitch. In this way, the player's performance was an objective evaluation and coach independent.

The results obtained show, in the major of the analysed cases, a good agreement between the two Index. Especially, in player 2 and player 5 an optimal relationship is observed among physiological and physical activity conditions. Moreover, player 5 achieved low scores for both Index in Time 2, pointing a low performance, also high-lighted in the Heath Map, which all these factors were led to a decrease in the physio-logical status. As it shown, player 5 remained on the bench for whole time match in Time 3. This scenario was confirmed by Laboratory Index, calculated after two days from match (Time 3) with a score of 3.

Heath Maps of Player 2 and Player 5 performed during the games at Time 2 revealed that these players tended to be static in their playing motion; they remained in small portions of the football pitch during the progress of a match. This is a pleasant confirmation of what has been seen in Laboratory Index.

Our algorithm seems to highlight the player's performance and to forecast physical preparation level relying on clinical biomarkers. Indeed, it could also suggest which athlete let pitch in, it could avoid injuries to stressed players, it could be a helpful tool to choose the optimal training session and it could be used by coaches and athletes to a professional or not professional activity. For researchers and practitioners, being able to access this information would provide a unique format to assess team readiness or recovery status between contests.

In a season long competition format, the ability to use Laboratory Index may be necessary to identify players with the best performing athletic status and biochemical profile in the match. This would allow not only to improve the performances of the players and the results of the team, but above all to safeguard the health of the players. In fact, several studies have shown that an excess of oxidative stress not only reduces the performance of athletes but is correlated with a higher incidence of muscle injuries[18]. However, further investigation, with a large number of samples, is required to demonstrate if the Laboratory Index is a real predictive tool to indicate exercise proto-cols and to expect athletic performance.

Conclusions

Overall, we have shown that our Laboratory Index is an excellent tool for monitoring athletes' performance, and it could be helpful to modify their training based on physiological parameters.

Furthermore, our algorithm, Laboratory Index, is also a useful tool combined to InStat Index. The less invasive or non-invasive collecting is practical and useful, a feature that makes Laboratory Index the best option for athletes. The combination of two Index is a valid alternative approach for a new perception of sports medicine. In this way, we could interpretate biochemicals anomalies with negative physiological adaptations, that influences the player's performance during the training sessions or the match.

The relationship between them can achieve an objective picture of the athletes' performance based on monitored biochemical parameters.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Informed Consent Statement

Patient consent was waived due to the nature of the study as the data presented here were collected during routine clinical practice; there was no requirement for informed consent.

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