Relationship between erythropoietin levels and reticulocyte count in sports

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ABSTRACT

The aim of the study was to determine if a direct relationship could be established between erythropoietin levels and reticulocyte counts in blood samples collected at the same time. The research was conducted as a case study on an adult endurance athlete (aged 26-27 at the time of the study), with blood samples taken over a two-year period. Erythropoietin levels and reticulocyte counts were analyzed to investigate a possible relationship between the two. Erythropoietin levels ranged from 3.24 mlU/ml to 12.5 mlU/ml, with a maximum value 3.86 times higher than the minimum. The reticulocyte count ranged from 0.94% to 1.75%. Therefore, the maximum value was 1.86 times higher than the minimum value. A negligible relation (r=0.13) was found between erythropoietin levels and the reticulocyte count. The results of this case study suggest that it is not possible to definitively identify a relationship between erythropoietin levels and the reticulocyte counts when both parameters are analyzed from a single blood draw. Based on these results, further research is needed to identify individual factors that could improve the objectivity of interpreting the results monitored in ABP.

KEY WORDS: Athlete Biological Passport, Erythropoietin, Reticulocytes

DOI: https://doi.org/10.24040/sjss.2024.9.suppl.189-193

INTRODUCTION

The fight against doping is one of the most important components of fair play and the protection of athletes' health, making it is essential to continuously improve methods used to combat doping. One option is the Athlete Biological Passport (ABP), a modern anti-doping tool that can indicate potential violations of anti-doping rules. The ABP primarily identifies variations in hemoglobin levels, reticulocyte counts, and their relationship calculated using the OFF Score (OFF Score = Hb x 10 - 60x(√reticulocyte %)). However, several cases of athletes who were accused of doping use but later proven innocent demonstrate that this model has its interpretational limitations, which can negatively affect an athlete's career in various ways. As highlighted by Mahendru et al. (1) there are many advantages with the implementation of this programme; however, there are various issues, which may lead to false interpretation of passport data that must be taken into consideration. During the process of proving their innocence, athletes are often suspended, preventing them from fully training, competing (therefore losing potential income), and often incurring significant costs for their defense (including tests and independent analyses, etc.). Furthermore, the publicity surrounding such cases often severely damages their reputation, as the public typically does not understand the difference between a ban for doping and a temporary suspension pending the outcome of a case (2). Suspension itself can be easily compared to a punishment or ban, as the athlete is prohibited from engaging in any sporting activities during this period. This is why we believe that it is necessary to continue expand the parameters evaluated in the ABP and make them more objective. This is because standard anti-doping tests typically analyze a urine sample that is either contaminated with doping substances or not, and after the analysis there are no further questions regarding the presence of doping (at most, only how it entered the body). However, the ABP remains a hypothetical predictive model that works with a specific (very high) degree of probability, although it still allows for a certain margin of error. If a result is atypical (according to the ABP prediction), the athlete is asked to explain and justify their (statistically) abnormal result. Independent evaluators who rely on their knowledge and experience, supported by scientific literature describing similar cases, will then evaluate the athlete's explanation. However, this approach sometimes minimizes the fact that elite athletes may react differently to various situations than other people 99.99% of the time. Cases such as that of Slovak Olympic champion Matej Tóth and Czech cyclist Roman Kreuziger, both of whom were cleared of doping allegations after much time and effort. These high-profile cases, which are not unique to Slovakia but are evident worldwide, highlight the need to improve the interpretation of ABP data making it more objective (2). Many experts and scientists are seeking ways to eliminate the potential risks of misinterpretation, and thus it may seem logical, in terms of understanding erythropoiesis, to look for a direct relationship between erythropoietin levels and reticulocyte counts. However, the somewhat unclear time delay is a significant limitation. Although studies (3,4,5,6,7) have described a relationship between erythropoietin levels and reticulocyte production, which is logically linked to the general knowledge about erythropoiesis, but without having precise time frames fully defined and thus complicating a potentially more effective interpretation of the relationship between erythropoietin levels and the reticulocyte count in relation to results interpreted in ABP. Within the framework of the project VEGA 1/0707/22 (Specifics of variability of haematological markers in Athlete Biological Passport), we aimed to evaluate the potential use of the dependence of erythropoietin levels and reticulocyte counts in ABP.

RESEARCH AIM

The aim of the study was to determine if a direct relationship could be established between erythropoietin levels and reticulocyte counts from a blood sample collected from an adult athlete at the same time.

METHODOLOGY

The research was conducted as a case study on an adult endurance athlete (aged 26-27 at the time of the study), with blood samples taken over a two-year period. All tests were conducted under the same conditions and according to WADA regulations. Blood samples were collected at 8:00 AM ± 30 minutes after an overnight fast. We analyzed erythropoietin levels and reticulocyte counts to determine if there was a relationship between them. We used highly reliable Mindray BC 6200 and Mindray BC 6000 analyzers.

FINDINGS

Analysis of the results showed that erythropoietin levels ranged from 3.24 mlU/ml to 12.5 mlU/ml, with a maximum value 3.86 times higher than the minimum. As shown in Figure 1, for the first ten measurements (during the first year of the study) the erythropoietin values were in a larger range (3.92-12.5 mlU/ml), whereas in the second year the range of measured values was 3.24-7.64 mlU/ml.

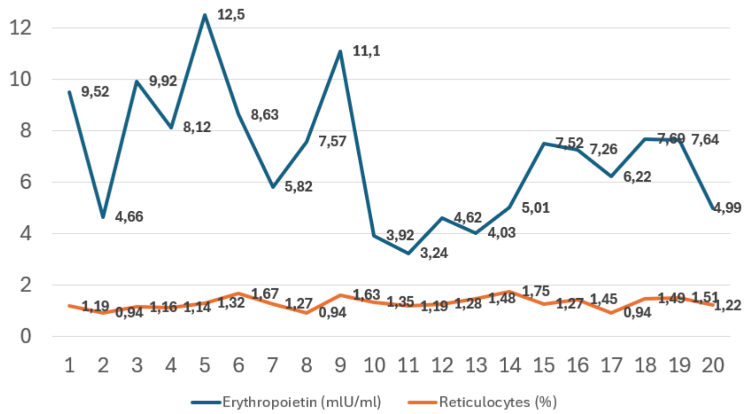

Fig. 1 Relationship between erythropoietin levels and reticulocyte counts

Figure 1 clearly shows that erythropoietin levels are relatively variable, indicating that this marker is not as stable as interpreted expectations in ABP towards reticulocytes or hemoglobin.

The second parameter monitored was the reticulocyte percentage, which ranged from 0.94% to 1.75%. Therefore, the maximum value was 1.86 times higher than the minimum value. Simply put, erythropoietin levels show greater variability compared to reticulocytes. However, from the point of view of data interpretation, it is more important to look for the relationship between them, where we found a very low correlation (r=0.13).

According to Krzyzanski & Perez-Ruixo (5) (whos administered single doses of recombinant human erythropoietin) the parameter estimates obtained by simultaneous fitting of the model to the reticulocyte and red blood cell counts data revealed that recombinant human erythropoietin transiently increased the reticulocyte lifespan from the baseline value of 1,7 days to 3,4 days and the effect lasted for 8,3 days. The dose dependent increase in the reticulocyte production had the maximal value of 77,5 10(9) cells/l/day and was followed by a rebound that was less than 9% of the baseline value. Both reticulocyte and red blood cell counts responses were preceded by a dose-independent lag time of 1,7 days. In light of this, it would be interesting to delve deeper into the potential differences between the response to natural erythropoiesis compared to the administration of recombinant human erythropoietin. However, it is also necessary to consider the views of Saugy et al. (8), who suggest that the mean effects of recombinant human erythropoietin on blood parameters are greater than those induced by hypoxic exposure (1.7 times higher for HGB and RET% and 4 times higher for hemoglobin mass). But they suggest, that recombinant human erythropoietin micro-doses have shown effects that are hardly distinguishable from those identified after hypoxic exposure.

CONCLUSION

The results of the case study suggest that it is not possible to definitively identify a relationship between erythropoietin levels and the reticulocyte count when both parameters are analyzed from a single blood draw. Although a clear relationship between these two variables illustrating the course of erythropoiesis could not be identified, it is necessary to continue the search for additional variables that could make the data monitored by ABP more objective. Our study shows that we cannot find a direct relationship between erythropoietin and reticulocytes when measured in the same sample. However, future research could explore the possible time-dependent relationship between erythropoietin production and the emergence of reticulocytes. Based on our findings, we recommend searching for individual characteristics that could contribute to a more objective interpretation of the results monitored in ABP.

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