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ORIGINAL ARTICLE

The impact of blood sampling location on results in the athlete's biological passport

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ABSTRACT

BACKGROUND: The aim of this study was to assess whether the arm from which the blood sample was taken could potentially affect the results of the athlete's biological passport (ABP) data.
METHODS: The study involved 25 adult male athletes aged between 20 and 35 years old. The analysis took place in an accredited laboratory, where all participants had their blood collected in the morning after fasting from both the right and left arms. Regarding the evaluation, we focused on the parameters monitored in the ABP (hemoglobin, reticulocytes, Off-score). The sampling was carried out at 8:00 a.m. The parameters analyzed were those monitored in the athlete's biological passport: hemoglobin and reticulocytes, from which the Off-score was derived (Off-score = Hgb × 10–60 × (√reticulocytes).
RESULTS: The research results suggest that the sampling location – that is, from which arm the blood was sampled, may have a significant impact on the data interpreted in the ABP.
CONCLUSIONS: Based on our findings, we recommend further research focused on investigating potential differences caused by the location of blood sampling. Future research should involve conducting similar measurements on a larger sample size of both athletes and non-athletes.

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KEY WORDS: Arm; Athletes; Hemoglobins; Reticulocytes.

The athlete's biological passport (ABP) can be seen as one of the most groundbreaking instruments for detecting dopers, but there are many cases that raise questions about its reliability[1]. For illustration, while several global studies validate the substantial impact of hypoxic training, detraining, or diurnal variation on hematological parameters observed in the ABP[2-5] it turns out that these facts can sometimes be disregarded when interpreting the results. It is also worth noting that many studies[1, 4, 6-9, 1, 4, 10, 11] raise controversy about the reliability of the values for ABP purposes or even highlight shortcomings in their interpretation. On the other hand, it is paradoxical that many times when accusing athletes, the evaluators cited publications questioning the influence of hypoxic training, dehydration and diurnal variation on the hematological variables monitored in the ABP,[12-15] creating a need for further research in this area. Studies have given relatively little consideration to the analytical, physiological, or methodological factors that influence the interpretation of results in the ABP. Within the framework of the project VEGA1/0707/22 (Specifics of variability of hematological markers in Athlete Biological Passport) our aim was to assess whether the arm from which the blood sample was taken could potentially affect the results of the ABP data. Studies carried out by Morris *et al.*,[16] Wiehl,[17] Lima-Oliveira *et al.*,[18] Killilea *et al.*[19] indicate that the sampling location (side) may affect hemoglobin level determinations, but we have not encountered similar perspectives regarding the interpretation of the ABP results.

Materials and methods

The study involved 25 adult male athletes aged between 20 and 35 years old. The analysis took place in an accredited laboratory, where all participants had their blood collected in the morning after fasting from both the right and left arms. Regarding the evaluation, we focused on the parameters monitored in the ABP (hemoglobin, reticulocytes, Off-score). The sampling was carried out at 8:00 a.m. The parameters analyzed were those monitored in the ABP: hemoglobin and reticulocytes, from which the Off-score was derived (Off-score = Hgb × 10–60 × (√reticulocytes). The sampling adhered to the methodology used in data collection for the ABP.[20] We used highly reliable analyzers, for reticulocytes Mindray BC 6200 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China; precision: ≤15.0% red blood cell [RBC] ≥3×1012.L^{-1}; ≤1.0% reticulocytes % ≤4.0%), for hemoglobin Mindray BC 6000 (precision ≤1.0% [110-180 g.L^{-1}]). The study protocol and procedures were approved by the Ethics Committee of Matej Bel University and were conducted in accordance with the Declaration of Helsinki on Ethical Principles of Medical Research.[21]

The experiments reported in the manuscript were performed in accordance with the ethical standards of the Helsinki Declaration and that the participants signed an informed consent form.

Results

When comparing the measured values between the right and left arms, there was no statistically or substantively significant difference in hemoglobin, reticulocytes, or Off-score. In the context of the results interpretation in the ABP, data aggregated for a group or population cannot be considered as decisive, as individual characteristics have to be taken into account. The readings indicated that there was no clear trend showing significantly higher or lower values in either arm for hemoglobin (right 161 g.L^{-1} [SD±8.231], left 160.24 g.L^{-1} [SD±8.237]). As depicted in Figure 1, the individual differences measured between the right and left arms in several cases reached a level that can be considered significant from the perspective of the ABP.

The highest difference (participant 25) between the right and left arms was 7 g.L^{-1}(right – 157 g.L^{-1}, left 150 g.L^{-1}), in the other two cases (participants 10, 13) the difference was 5 g.L^{-1}. Interestingly, both participants had the same value of 163 g.L^{-1} measured on the left arm, but on the right arm, one recorded a value of 153 g.L^{-1} and the other recorded 168 g.L^{-1}. Therefore, while they had the same result on the left arm, there was a difference of 10 g.L^{-1} between them on the right arm. In three additional cases, the difference between the arms was 4 g.L^{-1}, in five cases the difference was 3 g.L^{-1} and in the remaining 14 cases the difference was less significant (0-2 g.L^{-1}). Considering that the interpretation of the ABP calculates a variance of approximately 29 g.L^{-1} for hemoglobin,[22] which is a variance from the mean predicted value of less than 15 g.L^{-1}, in 11 cases, the difference between the right and left arms exceeded 3 g.L^{-1}, representing 20% or more of the potential variance from the mean. The highest observed dif-

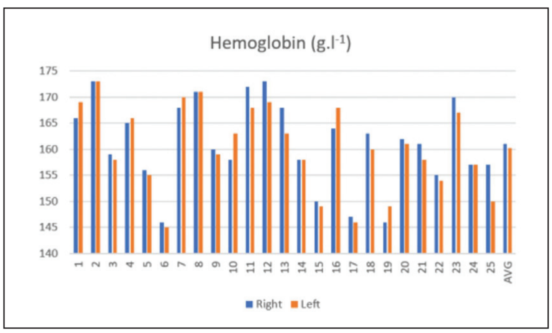

Figure 1.—The comparison of hemoglobin levels between the right and left arms.

ference of 7 $g.L^{-1}$ represented nearly half of this variance, posing a potential risk in interpreting the ABP results.

A similar trend was observed for reticulocytes, with the average value measured at 1.5352% (SD±0.2948) when collected from the right arm and 1.5536% (SD±0.3077) when collected from the left arm. However, as we can see in Figure 2, certain individual differences can be deemed significant in the context of ABP.

The most significant difference recorded (participant 24) was 0.41 percentage points, the second most significant difference (participant 22) was 0.4 percentage points, then it was 0.21 percentage points (participant 12), 0.17 percentage points (participant 10) and in the other 8 cases the difference was 0.11-0.14 percentage points. In the remaining 13 cases, the difference was between 0 and 0.1 percentage points. Given that the ABP assumes a variance of about 1 percentage point, which is 0.5 percentage points from the average of the potential variance, the two most significant differences are very close to the maximum variance from the average values. As in 12 cases a difference of more than 0.1 percentage points was observed between the arms, it is necessary to considerate this in the case of reticulocytes as well.

The third parameter assessed is the Off-score, which reflects the relationship between hemoglobin levels and the proportion of reticulocytes. As shown in Figure 3, there was no significant difference between the arms in this indicator either, with the mean Off-score for the right arm at 86.99 (SD±10.74) and for the left arm at 85.83 (SD±11.57).

The most significant difference in Off-score was 11.9 (right 94.40, left 82.50) for participant 22, followed by the second most notable difference of 10.48 (right 81.34, left 91.82) for participant 24, and the third biggest difference of 10.18 (right 90.72, left 80.54) for participant 25. Respecting the predicted variance of the Off-score of approximately 45 (*i.e.*, 22.5 from the mean),[21] a difference exceeding 5 can be regarded as a significant difference between the arms. Such a difference was recorded in 8 cases. Similar to hemoglobin and reticulocytes, our findings suggest that the arm from which the sample was taken could also influence the outcome evaluated in the ABP.

Discussion

While we consider the ABP as a valuable tool in combating doping, our research also reveals several limitations associated with this indirect method of doping detection. In the context of our research, it is important to mention that controversies about differences in hemoglobin level measurements between the right and left sides of the body were already present in the middle of the 20th century.[17] However, it must be noted that at that time, capillary blood from the finger was used, and of course, methods for determining hemoglobin levels have significantly improved since then. The differences we observed in sampling between the right and left arms are not clinically significant, but such potential influences on the results need to be considered when interpreting the results in the ABP. Highlight possible discrepancies in different blood collection locations

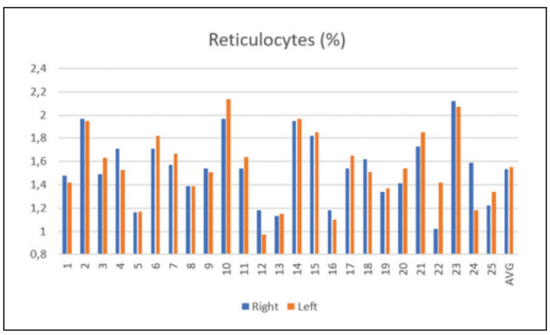

Figure 2.—Comparison of the proportion of reticulocytes between the right and left arms.

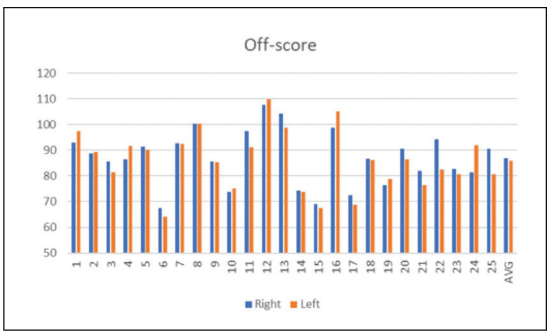

Figure 3.—Comparison of Off-scores between the right and left arms.

when determining hemoglobin levels, noting that while the differences are not large, they are statistically significant.[19] In the summary of their study, they outline interpretation boundaries that must be adhered to in order to prevent misinterpretation of their research conclusions. We consider it crucial to continue gaining new insights in the future, as we are mindful of the current research's limitations, particularly in terms of analysis. Additionally, other studies mentioned above also point out the necessity of contemplating similar issues, especially in the context of data objectivity in the ABP.[1] The limitations in interpreting the cited studies mainly stem from the use of capillary blood, as was the case in the study.[16] Their results indicate a significant impact of the capillary blood sampling location on the obtained result. Other studies most often cite the same sources as we did. In the context of our research, an interesting finding comes from Lima-Oliveira *et al*.,[18] who, in their study comparing the effect of body position (standing, lying down, sitting), show a significant impact of the body position on measured hemoglobin levels. The above information also implies that there are still several questions regarding the ABP that, while not crucial from a medical standpoint, are certainly worth further investigation in terms of interpreting results in the ABP.[22]

Limitations of the study

We view the ABP as a suitable, innovative, and modern tool for combating doping. However, we must also acknowledge its limitations and the potential risks associated with it being an indirect method of doping detection, which involves subjective opinions of evaluators. We view the most significant risk in this context the possibility of an athlete who has not violated anti-doping rules theoretically being punished, which we find unacceptable. Combating doping cannot include the risk of punishing an innocent athlete.

Conclusions

The research results suggest that the sampling location – that is, from which arm the blood was sampled, may have a significant impact on the data interpreted in the ABP. Based on our findings, we recommend further research focused on investigating potential differences caused by the location of blood sampling. Future research should involve conducting similar measurements on a larger sample size of both athletes and non-athletes. If similar findings to those we observed are confirmed, efforts should be made to determine the underlying reasons and mitigate the risks of misinterpretation, thereby reducing the possibility of false positives in non-doping athletes.

References

1. Pupiš M., Brünn D, Sýkora J, Líška D. Môže ovplyvniť výsledky v biologickom pase športovca to, z ktorej paže je odobraná vzorka? In: Kyslík, krv a šport: vedecký recenzovaný zborník vydaný za podpory GÚ [Oxygen and blood in sports training: Scientific peer-reviewed proceedings published with the support of the General Administration of Slovenia]. Banská Bystrica: Slovenská asociácia kondičných trénerov; 2023. p.51–60. [Slovak].

2. Alberty R, Pupiš M, Vachalík V, Bátovský M. Diurnal variation in red blood cell variables in athletes after single and repeated bouts of exercise. J Sports Med Phys Fitness 2021;61: 269–79.

3. Eastwood A, Boudron PC, Snowden KR, Gore CJ. Detraining decreases Hb (mass) of triathletes. Int J Sports Med 2012;33:253–7.

4. Pupiš M. Biologický pas športovca – benefity a rizikáinterpretácievýsledkov [Athlete biological passport – benefits and risks of the interpretation of the results]. Českákinantropologie 2021;25:97–105. [Slovak].

5. Wilber RL, Stray-Gundersen J, Levine BD. Effect of hypoxic "dose" on physiological responses and sea-level performance. Med Sci Sports Exerc 2007;39:1590–9.

6. Banfi G. Limits and pitfalls of athlete's biological passport. Clin Chem Lab Med 2011;49:1417–1.

7. Banfi G, Lombardi G, Colombini A, Lippi G. Analytical variability in sport hematology: its importance in an antidoping setting. Clin Chem Lab Med 2011;49:779–2.

8. Lippi G, Banfi G, Maffulli N. Preanalytical variability: the dark side of the moon in blood doping screening. Eur J Appl Physiol 2010;109:1003–5.

9. Lippi G, Plebani M. Athlete's biological passport: to test or not to test? Clin Chem Lab Med 2011;49:1393–5.

10. Pupiš M, Pupišová Z, Franek V, Švantner R, Barthová M, Líška D, *et al*. Biologický pas športovca a jeho potenciálne limity [The athlete's biological passport and its potential limits]. In: Kyslík, krv a šport: vedecký recenzovaný zborník vydaný za podpory GÚ [Oxygen, blood and sport: peer-reviewed scientific proceedings published with the support of the General Institute]. Banská Bystrica: Slovenská asociácia kondičných trénerov; 2023. p.5–13. [Slovak].

11. Sanchis-Gomar F, Martinez-Bello VE, Gomez-Cabrera MC, Viña J. Current limitations of the athlete's biological passport use in sports. Clin Chem Lab Med 2011;49:1413–5.

12. Schumacher YO, Wenning M, Robinson N, Sottas P-E, Ruecker G, Pottgiesser T. Diurnal and exercise-related variability of haemoglobin and reticulocytes in athletes. Int J Sports Med 2010;31:225–30.

13. Schumacher YO, Klodt F, Nonis D, Pottgiesser T, Alsayrafi M, Bourdon PC, *et al*. The impact of long-haul air travel on variables of the athlete's biological passport. Int J Lab Hematol 2012,34:641–7.

14. Schumacher YO, D'onofrio G Scientific expertise and the athlete biological passport: 3 years of experience. Clin Chem 2012;58:979–5.

15. Schumacher YO, Garvican LA, Christian R, Lobigs LM, Qi J, Fan R, *et al*. High altitude, prolonged exercise, and the athlete biological passport. Drug test Anal 2015;7:48–55.

16. Morris SS, Ruel MT, Cohen RJ, Dewey KG, De La Brière B, Hassan MN. Precision, accuracy, and reliability of hemoglobin assessment with use of capillary blood. Am J Clin Nutr 1999;69:1243–8.

17. Wiehl DG. Accuracy of hemoglobin determinations on finger-tip blood. Milbank Memor Fund Q 1946;24:5–28.

18. Lima-Oliveira G, Guidi GC, Salvagno GL, Danese E, Montagnana M, Lippi G. Patient posture for blood collection by venipuncture: recall for standardization after 28 years. Rev Bras Hematol Hemoter 2017;39:127–2.

19. Killilea DW, Kuypers FA, Larkin SK, Schultz K. Blood draw site and analytic device influence hemoglobin measurements. PLoS One 2022;17:e0278350.

20. World Anti-doping Agency Expert Committe. Athlete biological passport operating guidelines, version 6.1; 2018 [Internet]. Available from: https://www.wada-ama.org/sites/default/files/resources/files/guidelines_abp_v61_2018_jul_en.pdf [cited 2025, Mar 27].

21. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human participants. JAMA 2013;310:2191–4.

22. Mørkeberg J. Athlete biological passport. Best anti-doping program implementation models; 2016 [Internet]. Available from: https://nada.by/en/education/mezhdunarodnyy_seminar_effektivnye_modeli_realizatsii_antidopingovykh_programm/ [cited 2025, Mar 27].

Conflicts of interest
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Authors' contributions
All authors read and approved the final version of the manuscript.

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