

Athlete Biological Passport: Practical Application in Sports

J Kumaravel¹, Dhruv Mahendru², Vidya M Mahalmani³, Phulen Sarma⁴, Ajay Prakash⁵, Bikash Medhi⁶

ABSTRACT

The role of athlete biological passport (ABP) is the longitudinal tracking of the doping biomarkers. There are three modules of this program: hematological, steroidal, and endocrinological. As of now, only the first two modules have been executed. The ABP program has become stronger since the introduction of the hematological module and is a vital instrument to combat doping malpractices. Since the ABP program focuses on the longitudinal tracking of various parameters that are affected by the use of performance-enhancing substances, it may be called an indirect tool for the detection of doping. The Athlete Passport Management Unit (APMU) linked with the laboratories performing the antidoping testing takes care of the administrative side of this program for ensuring an unbiased analysis of the outcomes after the evaluation of the passport. The results of the ABP profile of an athlete might be influenced due to some factors leading to misinterpretation of the data, which is a challenge for the authorities and needs to be looked upon. It is an important tool in the fight against doping. Recent developments in metabolomics show the ABP's tremendous potential. ABP's further progress with close analysis of biological parameters is now the most effective approach to stop athletes using undetectable banned substances.

Keywords: Antidoping, Athlete biological passport, Athlete passport management unit, Challenges, Modules.

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INTRODUCTION

Doping has been a major concern threatening the integrity of sports and requires serious attention. It has a long history going back to the ancient Olympics in Greece. The main issue is that it is very difficult to estimate the true prevalence of doping, as the doping test results do not reflect the actual prevalence and the samples from all the athletes engaged in doping for increasing their performance do not reach the laboratory.¹ Specialists are striving hard to adopt different methods that can identify and reduce the menace of doping. To overcome this issue, World Antidoping Agency (WADA) was established in the year 1999. The term "Athletes Biological Passport" was introduced in the year 2000 by the scientific community where the evaluation of an individual's hematological profile was performed by the detection of markers of blood doping. Later, the WADA along with various stakeholders and experts in the field of medicine further developed and validated the concept. As a result, "Athletes Biological Passport" was first published in the year 2009 along with the operating guidelines, which solely concerned with the hematological module. In the year 2014, the steroidal module was also incorporated for monitoring the steroidal parameters. The process set out in the guidelines laid out by WADA adds to the existing antidoping system and thereby increases the efficacy of antidoping activities.^{2,3} The endocrinological module mainly focuses on the monitoring of growth factors such as growth hormone (GH), insulin-like growth factor-1, and GH-releasing peptide. Traditional analytical techniques involve a "direct" method of detection of doping markers in urine and blood, whereas athlete biological passport (ABP) follows an "indirect" approach which involves tracking the parameters that might be affected by doping. Thus, one of the main advantages of ABP is the identification of any suspicious profile resulting due to doping thereby improving the sensitivity of doping test.⁴ More than 100 international federations and antidoping organizations (ADOs) have utilized the ABP program for the development of the antidoping strategies.⁵

¹⁻⁶Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Corresponding Author: Bikash Medhi, Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, Phone: +91 9815409652, e-mail: drbikashus@yahoo.com

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With the advancement in "-omics" approaches such as transcriptomics, proteomics, and metabolomics, ABP can be more successfully utilized to detect some regimens that cannot be detected by conventional approaches. Therefore, ABP is a powerful tool for controlling doping as well as monitoring, which has considerably improved the effectiveness of WADA's efforts in combating doping practices in elite sports.

MODULES

Hematological Module

This module intends to identify blood doping including the usage of erythropoiesis-stimulating agents or homologous blood transfusion which are used to improve O₂ carrying capacity, thereby increasing the performance of an individual.⁴ In the year 2008, the Union Cycliste Internationale, a sports organization, implemented the module for blood doping detection in elite cycling.⁶

Apart from identifying the use of erythropoietin-stimulating agents listed, it also intends to classify the use of methods which are prohibited under section M1 of the Prohibited List (Blood and Blood Products Manipulation).³ Differences in the plasma volume is the key confounding factor in the measurement of the concentration of biological markers in the blood (e.g., hemoglobin). However,

hemoglobin (Hb) levels are altered not only by doping but also by alteration in the fluid component of blood such as the volume of plasma. The fluctuations in plasma volume may be due to physical activities or other environmental conditions like temperature or altitude to which the athletes are generally exposed. The inclusion of new markers in the module is a major challenge as only a few variables can be longitudinally tracked, and a majority of them play a role in the metabolism of iron. Therefore, further research is essential before the incorporation of any of these markers.⁷

Steroidal Module

The steroidal module in the ABP program monitors the longitudinal variation in the steroid markers over a period. A steroidal profile is formed with the help of steroidal markers obtained from urine samples of athletes.⁸ Testosterone/epitestosterone ratio was used since the 1980s as an indirect marker of doping. If exogenous testosterone has been injected, this ratio increases, and a ratio of more than 6:1 is suspected of doping. Since then, the T/E ratio test is being utilized for screening purposes and a confirmatory procedure is performed with the help of gas chromatography (GC)/C/isotope ratio mass spectrometry (IRMS). This GC/C/IRMS method helps in distinguishing ¹³C/¹²C ratios of metabolites of testosterone. The synthetic testosterone used for exogenous administration has a distinct ¹³C content when compared to that of human testosterone.⁹ An analysis of the IRMS was done from 2004 to 2013 when an athlete had a T/E ratio greater than 4:1. The ABP uses the adaptive model for replacing the population-based reference approach with an individual-based reference approach allowing for a more precise assessment.⁸ The parameters within the hematological and steroidal modules of ABP have been given in Table 1.

Endocrine Module

The steroidal module can detect several direct or indirect types of steroidal doping, but it cannot detect certain growth factors, which is the target of this endocrine module. Insulin-like growth factor 1 and procollagen III are the biomarkers assessed for many confounding factors such as sex, age, ethnicity, and exercise. Endocrine module can be used for both target testing including stimulators of GH secretion by chromatography mass spectrometry and GH by isoform immunoassay.⁷

How to differentiate the physiological level of hormone from exogenous administration?

There are two methods developed:

- Marker approach: Focused on measured serum concentrations of GH-dependent parameters.
- Isoform approach: Based on the estimates of GH, isoform spectrum changes following the administration of recombinant GH injection.

The marker approach explains better with a combination of insulin-like growth factor-1 and type III procollagen amino-terminal extension peptide; changes in concentration of these markers after GH administration surpass those seen under physiological conditions.¹⁰

Athlete Passport Management Unit

Athlete Passport Management Unit (APMU) has a significant role in the passport process. This department linked to the laboratories performing the analysis is responsible for the administrative part of this program to ensure a fair and unbiased review of the outcomes

Table 1: Different modules with biomarkers in ABP

<i>Hematological module</i>	<i>Steroidal module</i>	<i>Ratios described within the steroidal module</i>
• Hemoglobin	• Androsterone	• Androsterone to testosterone
• Hematocrit	• Epitestosterone	• Androsterone to etiocholanolone
• Mean corpuscular hemoglobin	• Etiocholanolone	• Testosterone to epitestosterone
• Mean corpuscular volume	• Testosterone	• 5 β -Androstane-3 α ,17 β -diol
• Immature reticulocyte fraction	• 5 β -Androstane-3 α ,17 β -diol	• 5 α -Androstane-3 α ,17 β -diol to epitestosterone
• Abnormal blood profile score	• 5 α -Androstane-3 α ,17 β -diol	• 5 α -Androstane-3 α ,17 β -diol to 5 β -androstane-3 α ,17 β -diol
• OFF-hr score		
• Mean corpuscular hemoglobin concentration		
• Platelets		
• Red cell distribution width		
• RBC count		
• Reticulocytes%		
• Reticulocyte count		
• WBCs		

of the passports and an appropriate follow-up. It provides a tool for tailored screening and accurate test schedule, manages laboratory records, assembles analytical reports, performs initial assessments, and cooperates for in-depth assessment of passport information between independent experts and ADOs. Athlete Passport Management Unit must report any adverse passport reports to the antidoping agency. Athlete Passport Management Unit staff must have sufficient knowledge to handle and conduct the antidoping operations, including the legal dimensions. Athlete Passport Management Unit reviews any previous report of athletes associated with passport, determines the validity of each sample, and updates the APMU report accordingly. It evaluates the requirement for urgent target testing and communicates the recommendation through the APMU report to the ADO. It also assesses the need for further research by using different analytical methods of existing samples. Athlete Passport Management Unit report is an integral part of the ABP administrative procedure entered and managed in Antidoping Administration and Management System (ADAMS) by APMU. It gives a current overview of the passport status along with suggestions that are appropriate for the passport authority to monitor effectively.¹¹

Roles and Responsibilities of Various Stakeholders

The ADOs of different countries have certain responsibilities for the successful implementation of ABP.³

- Adoption and execution of ABP program as laid out in the guidelines.
- Contracting Athlete Passport Management Unit for managing the ABP program.
- Establishment and execution of a test distribution plan in coordination with Athlete Passport Management Unit.
- Notifying the athlete if the result of the passport reveals a possible pathology as per the experts decision.
- Exchanging necessary data or information with various ADO and the personnel involved with the internal investigation.
- If the custodian of passports is the ADO, follow-up on Adverse Passport Findings according to specifications laid out in the Code and the ISTI (International Standard for Testing and Investigations) has to be done.

The responsibilities of WADA-accredited laboratories performing ABP analysis are as follows:³

- Analysis of blood sample in accordance with the guidelines.
- Analysis of urine sample in accordance with the guidelines.
- Further information for analyzing the findings.
- Providing a certificate of analysis.

The responsibilities of the personnel, which should not be a part of the respective ADOs and are involved in evaluating the passport data, are:³

- Analysis of the results and possible confounding factors as well as pathological conditions which might impact the ABP profile of the athlete.
- It is advised to follow-up testing if required for confirmation or for verifying the role of any pathology.
- Review of the arguments provided by the athlete and make the judgement accordingly regarding a possible doping scenario.
- Operate with the APMU and giving support as and when required in the process of hearing or managing the results.

Challenges

The introduction of ABP has had a great effect on doping control that is indicated by the fact that reticulocyte percentage (RET%) has decreased significantly after the ABP program was implemented.¹² Still the ABP program is not fool-proof and certain challenges need to be overcome to make this program more accurate. There are certain conditions or factors that influence the ABP profile of the athlete which are discussed below.

Impact of Dehydration on ABP

The hematological module of the ABP contains various aspects of blood manipulation that can be monitored over time using blood-doping markers such as Hb and RET%. The ABP is the best tool as it involves longitudinal testing where the present records of an athlete are compared with his previous records, thereby reinforcing the assessment of blood biomarkers in athletes.⁹ To increase the oxygen-carrying capacity and improve the performance, athletes use performance-enhancing substances to increase the circulating red blood cells (RBC).

Exercise-induced sweating can cause intracellular dehydration where the body fluids turn out to be hyperosmotic when compared

to blood plasma. As the water moves out of the cell, this can partially explain for a decline in extracellular volume. On the other hand, illnesses like secretory diarrhea can bring about extracellular dehydration where the body fluids persist to be iso-osmotic to blood plasma, thereby causing a greater reduction in extracellular fluid volume.¹³ The impact of dehydration on Hb is not known as Hb that normally varies over time.¹⁴

Impact of Postural Changes

Physical exhaustion, psychological stress, exposure to heat along with postural changes can lead to substantial alteration in the plasma volume and lead to alterations in the ABP parameters.^{15,16} The guidelines by WADA for the collection of blood state that a waiting time of 2 hours is required after any sort of physical activity. In addition, the athletes also need to remain seated for 10 minutes before collecting the sample for the equilibration of vascular volumes.¹⁷ In a study conducted by Astolfi et al., it has been shown that Hb and hematocrit (Hct) levels were decreased if the sample was collected in a supine position which may affect the ABP profile of an athlete. The findings of this study complement the guidelines laid out by WADA.¹⁸

Impact of Hypoxic Changes

Hematological variations occur as a result of hypoxic exposure (naturally or simulated) and lead to variations in the ABP profile of an athlete. Exposure to hypoxia leads to stimulation of erythropoietin, and this practice is being misused by athletes for enhancing their performance. It leads to a variation in the ABP parameters due to hemoconcentration.¹⁹ A meta-analysis conducted by Lobigs et al. for analyzing the effect of hypoxia on the hematological parameters included 17 studies and concluded that significant variation in the predictor variables was present. However, it was not determined whether these changes led to the violation of ABP or not.²⁰ There has been a case in the Court of Arbitration for Sport (CAS) where it was stated in the defence that the abnormal finding in the hematological variables was due to high-altitude training.²¹ There are very few studies that have analyzed the effect of this method on the biological passport profile of the athlete. In one of the studies conducted by Voss et al., where 10 athletes were recruited in LHHLH (live high, train low and high) training for 2 weeks and after the interpretation of the data, there were variations in the parameters but none of them led to doping.¹⁹ Hematological findings should nevertheless still be cautiously interpreted, considering altitude/hypoxic training as a potential confounding factor.

Role of Metabolomics in Antidoping

The role of metabolomics in screening the hormones consumed by athletes for increasing their performance is based on the following: (1) hormones that affect human metabolism greatly, altering many parameters in several organs or tissues; (2) being able to depict the metabolism state of many species, tissues, and various human diseases accurately; (3) animals being treated with hormones can be differentiated from controls without prior knowledge of the metabolism of the particular substance.²²

Since the implementation of high-profile instrumentation in life sciences, "omics" has gained popularity in the scientific community. Transcriptomics, genomics, proteomics, and metabolomics have proved to be appropriate for identifying the transcripts, genes, proteins, or metabolites.^{23,24} Transcriptomics and genomics have already been used for gene doping as screening tools, while proteomics for hormonal doping has been introduced.²⁵⁻²⁹

Metabolomics can be used as a screening tool in antidoping programs to detect altered metabolisms in athletes, pointing out that athletes may have abnormal values for such metabolites due to potential doping administration.

CONCLUSION

Recent developments in metabolomics offer immense opportunities to enhance the ABP and show that ABP has tremendous potential in antidoping. Further development of ABP with close analysis of biological parameters remains the most reliable method for preventing athletes from using undetectable banned substances. However, an expert's knowledge and skill are required for evaluating the biological passport of the athlete, whereas in conventional antidoping techniques, the positivity of the test depends solely on the existence of the banned substance or the metabolite.

The consequences of introducing new highly sophisticated and complex analytical methods by a network of accredited antidoping laboratories help to combat the doping malpractices in sports. Future technological advances will play a major role in ABP to control the usage of undetectable banned substances by athletes. New research is needed to improve the specificity and sensitivity of antidoping testing.

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