Correspondence case report

Drug Testing and Analysis

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Case studies on ESA-doping as revealed by the Biological Passport

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Blood doping, through the increase of red cells, induces changes of hematological parameters. The aim of the Biological Passport is first to analyse individual longitudinal profiles in order to identify, through variations of the specific parameters, doping manipulations. Additionally, on the basis of abnormal values or profiles, athletes can be targeted for traditional anti-doping tests in order to detect forbidden substances or methods.

We report the experience of the International Cycling Union in applying the Biological Passport to target athletes for the presence of erythropoiesis stimulating agents. All positive results which have been reported between 2008 and 2010 concerning athletes enrolled in the Biological Passport program are presented. Four cases are discussed more in details. To conclude, we propose possible ways of using the Biological Passport in order to better understand athletes' doping modalities, so that testing programs efficiency can be improved. Copyright © 2012 John Wiley & Sons, Ltd.

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Introduction

One of the major determinants of endurance performance is the ability of the cardio-circulatory system to deliver oxygen (O_2) to the exercising skeletal muscles which depends on the available amount of circulating red cells carrying O_2.[1]

Blood doping includes all substances and methods enhancing O_2 transfer, including the artificial increase of red cells, and can be achieved through different means. The two major classes of blood doping agents are erythropoiesis stimulating agents (ESA), among which recombinant human erythropoietin (rhEPO) has been the precursor since the end of the 1980s, and blood transfusion, either homologous (HBT) or autologous.

Hematological parameters and blood doping

Since the end of the 1990s, several groups of researchers have tried to identify specific biological parameters susceptible of being modified by blood doping. Several publications have described the behaviour of hematological parameters (mainly hemoglobin (HGB), percentage reticulocytes (%RET) and the OFF-HR score which is the combination of HGB and %RET) in conjunction with blood doping administration.[2–9] For a long time, abnormal values have been used by anti-doping organizations as a screening test in order to decide which anti-doping samples to analyze for erythropoiesis-stimulating agent (ESA) or HBT.[10] Additionally, a no-start rule has been applied by some international federations for those athletes found with blood values beyond the imposed population limits. Finally, the analysis of these hematological variables combined with convicted athletes' testimonies or police inquiries has permitted the identification of different doping scenarios and the adaption of testing strategies accordingly in order to have more chances to catch cheaters.

The Athlete Biological Passport

The World Anti-Doping Agency (WADA) published in December 2009 the first version of the Athlete Biological Passport (ABP) Operating Guidelines, a new tool to fight doping whose aim is to detect the biological effects induced by forbidden substances/methods instead of their chemical/immunological presence in an athletes' bodily specimens.[11]

The principles of the ABP is to identify and then monitor on a regular basis a list of relevant and specific biological parameters known to be influenced by doping, so to create an individual and longitudinal profile with the athlete becoming his/her own reference. A statistical model is then applied to evaluate the likelihood of a given passport profile when assuming a natural physiological condition. In the presence of a statistically abnormal profile, based either on a single result or on the whole sequence of values, the anti-doping organization has two options: either target the athlete with traditional anti-doping tests (to look for recombinant EPO; CERA, homologous blood transfusion, Hematide, etc.) or, if the evidence is compelling, to pursue a possible anti-doping rule violation in accordance with Article 2.2 of the World Anti-Doping Code (WADC) which concerns the use of prohibited substances or methods.

The experience of the International Cycling Union (UCI)

In 2008, UCI launched a pilot project for the implementation of the hematological module of the ABP.[12] More than 800 riders were enrolled each year and from the beginning of 2008 to the end of 2010 more than 1000 profiles were established, one for each athlete. In total, more than 15 000 blood and 10 000 urine

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samples have been collected (in- and out-of-competition) and analyzed on this population of riders.

The updated anonymous profiles (all the statistically abnormal profiles and also some normal ones) are submitted weekly for evaluation to a group of independent experts. Different possible opinions are provided by the experts after reviewing the case: either the profile is considered as normal or pathological due to a medical condition; suspicious, which implies the necessity to target the athlete; or abnormal and, in the absence of other explanations, compatible with a doping manipulation. Additionally, the Athlete Passport Management Unit, which is an independent entity responsible for managing the administrative part of the ABP programme, can directly inform the anti-doping organization of some anomalies within a recently updated profile so that immediate actions in terms of targeting testing can be taken.

Results

In the first three years of the UCI ABP project, 26 athletes who were part of the programme were found positive for the presence of ESA in their bodily specimens (Table 1): 10 in 2008, 8 in 2009, and 8 in 2010. It is a very significant increase compared to 2007, the year prior to the introduction of the ABP, where only 3 athletes were convicted for blood doping (1 for ESA and 2 for HBT). In most cases, it was the abnormal blood profile which raised suspicions leading to a targeted anti-doping urinary or blood test; only in very few exceptions was the athlete found positive in routine random tests, either because of the classification in a competition or as part of the normal testing programme.

Table 1. Positive cases for ESA between 2008 and 2010 on athlete within the ABP programme.

#	ESA	TEST	REASON FOR TESTING	TESTING STRATEGY	Year
1	CERA	OOC	INTELLIGENCE	TARGET	2008
2	EPO	IC		RANDOM	2008
3	CERA	IC	HB	TARGET	2008
4	CERA	IC		RANDOM	2008
5	EPO	OOC		RANDOM	2008
6	EPO	OOC		RANDOM	2008
7	EPO	IC	RET	TARGET	2008
8	CERA	IC	HB, RET	TARGET	2008
9	CERA	IC	RET, HB	TARGET	2008
10	CERA	IC	RET, HB	TARGET	2008
11	EPO	OOC		RANDOM	2009
12	EPO	OOC	RET, OFF	TARGET	2009
13	CERA	IC	RET, OFF	TARGET	2009
14	EPO	OOC	RET	TARGET	2009
15	CERA	IC + OOC	RET	TARGET	2009
16	EPO	OOC	RET	TARGET	2009
17	EPO	OOC	RET	TARGET	2009
18	EPO	OOC	RET, HB	TARGET	2009
19	EPO	OOC	HB	TARGET	2010
20	EPO	IC	HB	TARGET	2010
21	EPO	OOC	RET	TARGET	2010
22	EPO	IC	HB, RET	TARGET	2010
23	EPO	IC	RET, OFF	TARGET	2010
24	EPO	OOC	RET, OFF	TARGET	2010
25	EPO	OOC		RANDOM	2010
26	EPO	OOC	HB, OFF, RET	TARGET	2010

Several different cases are presented indicating the testing strategy (in-competition *vs* out-of-competition test) applied in the specific situation. Generally it emerges that %RET is a very sensitive parameter in relation to blood doping. Very often it is the only parameter showing significant variations and it has the advantage of being independent from hydration status and manipulations related to that.

Case No. 1

The athlete had shown an abnormal profile during a stage race the previous year (increased HGB values during a stage race – samples 1–3) (Table 2, Figure 1). A testing programme prior to his major competition the following year (day 0) was put in place and he was tested in the weeks preceding the competition. What drew attention was the increase of almost 100% of %RET in only 7 days (−7 to 0) prior to the start or the competition. This was followed, during the competition, by a typical off-scenario, with a decrease of the %RET and increase of OFF-HR.

Traditional and special anti-doping tests were conducted on several days: urine EPO analysis was requested on days 5, 6, 12, 20, and 24; serum CERA test was performed on days 0, 13, 21, 23, and 24. Finally, samples collected on days 13 and 21 were reported positive for CERA. It is worth mentioning that the sample collected on day 0 also reported positive for CERA, but because the sample was collected for the purpose of the ABP, a B sample was not available, and therefore it couldn't be considered as an adverse analytical finding. Since then, UCI has modified its policy by systematically collecting two whole blood tubes instead of one as recommended in the WADA ABP Operational Guidelines.

This profile is interesting because it shows that despite extremely stable and low HGB values, %RET vary a lot. Additionally, it demonstrates the importance of the evaluation of the values in conjunction with the competition/season calendar, in order to identify possible doping scenarios which could be repeated by the athlete. This information is used to conduct targeted and intelligent tests prior to similar competitions. Furthermore, the increase of OFF-HR confirms the hypothesis of blood manipulation. Finally, it is probably due to its long half-life that the athlete was found positive for CERA while being in-competition and in an off-scenario. This is substantiated by the positive result for CERA on day 0, which makes the hypothesis of a transfusion bag containing CERA having been received during the competition unlikely.

Table 2. Haematological values of athlete in Case No. 1.

#	Day	Test	HCT	HGB	MCH	MCHC	MCV	OFF-HR	RBC	# RET	%RET
1	−364	OUT	43.3	14.3	28.8	33	87.3	83	4.96	0.0496	1
2	−351	IN	43.2	14.5	29.3	33.6	87.3	79.3	5	0.0594	1.2
3	−340	IN	40.1	12.9	28.8	32.2	89.5	51.5	4.48	0.0748	1.67
4	−332	OUT	44.9	14.4	28.3	32.1	88.4	86.8	5.08	0.0462	0.91
5	- 57	OUT	42.2	14.4	30.3	34.1	88.7	81	4.76	0.0524	1.1
6	0	OUT	44.9	14.2	27.8	31.6	88	66.1	5.1	0.0816	1.6
7	10	IN	43	13.9	27.9	32.3	86.3	93.3	4.98	0.0289	0.58
8	24	IN	44.3	14.3	28	32.3	86.7	105.5	5.11	0.0199	0.39
9	70	OUT	42.8	14.1	28.1	32.9	85.4	70	5.01	0.0701	1.4

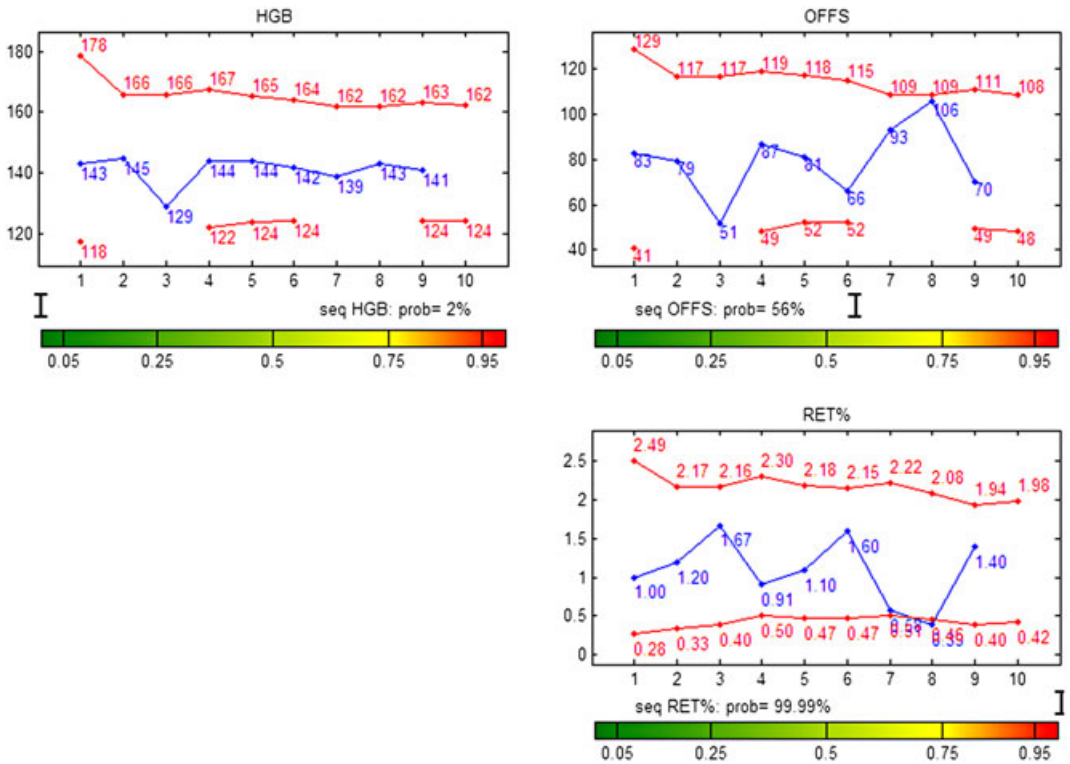

Figure 1. ABP profile of case 1 athlete.

Case No. 2

This was the first test of an athlete from whom no previous information was available. In front of such an extreme erythrocytosis (Table 3), it is important to identify the possible origin. It is not the task of an anti-doping organization to go through the complete diagnostic algorithm of polycythemia;[13] therefore, we need to focus on the most likely causes. In this context, and because of the high %RET, blood manipulation through ESA is the most probable explanation. A hypoxia-driven stimulus (either artificial or natural) seems less realistic due to the very high %RET value measured and the origins of the athlete. Finally, a possible rare medical condition responsible for congenital or acquired polycythemia (e.g. a genetic mutation), would need to be excluded in case such pathological values persist. To confirm the first hypothesis, both a urinary EPO and a blood CERA test were conducted. The result was positive for the presence of rhEPO. Otherwise the athlete would have been subject to targeted in- and out-of-competition controls, to confirm or rule out the persistence of elevated values. If their origin was due to doping, such extreme values would have led, sooner or later, to a highly abnormal ABP profile and a possible opening of a disciplinary procedure based on the profile.

This case shows that one single sample is sufficient to assist an anti-doping organization. First, the values are used as screening results for a classical anti-doping test for the detection of ESA, similarly to the recommendations which were in place at the time of the introduction of the urinary EPO test; secondly to define a targeted intelligent testing programme on the concerned athlete.

Table 3. Haematological values of athlete in Case No. 2.

Day	Test	HCT	HGB	MCH	MCHC	MCV	OFF-HR	RBC	# RET	%RET
0	IN	59.6	19.6	29.6	32.91	89.9	106.1	6.63	0.1485	2.24

Case No. 3

This case (Table 4) refers to an athlete who has been under observation since the first test collected during the off-season. In fact, the elevated %RET raises doubt between manipulation (and we know that in the off-season a scenario of blood manipulation is to use ESA after having extracted blood to be re-infused prior to or during competitions) or a benign hematological condition influencing reticulocytes (e.g. a mild form of spherocytosis).

In this case, the global profile is consistent with ESA administration: progressive HGB increase associated with the decrease of %RET, which is the consequence of negative feedback on the production of red blood cells. Targeted EPO anti-doping out-of-competition controls were conducted on days 46, 81, and 89. The athlete was finally found positive for rhEPO on day 89.

Table 4. Haematological values of athlete in Case No. 3.

#	Day	Test	HCT	HGB	MCH	MCHC	MCV	OFF-HR	RBC	#RET	%RET
1	0	OUT	44.9	15.2	29.6	33.9	87.5	62.2	5.13	0.1149	2.24
2	46	OUT	48.8	15.8	29.8	32.4	92.1	98	5.3	0.053	1
3	81	OUT	48.8	16.2	30	33.1	90.6	98.79	5.4	0.0598	1.11
4	89	OUT	51.8	16.8	29.5	32.4	90.9	107.7	5.7	0.0576	1.01

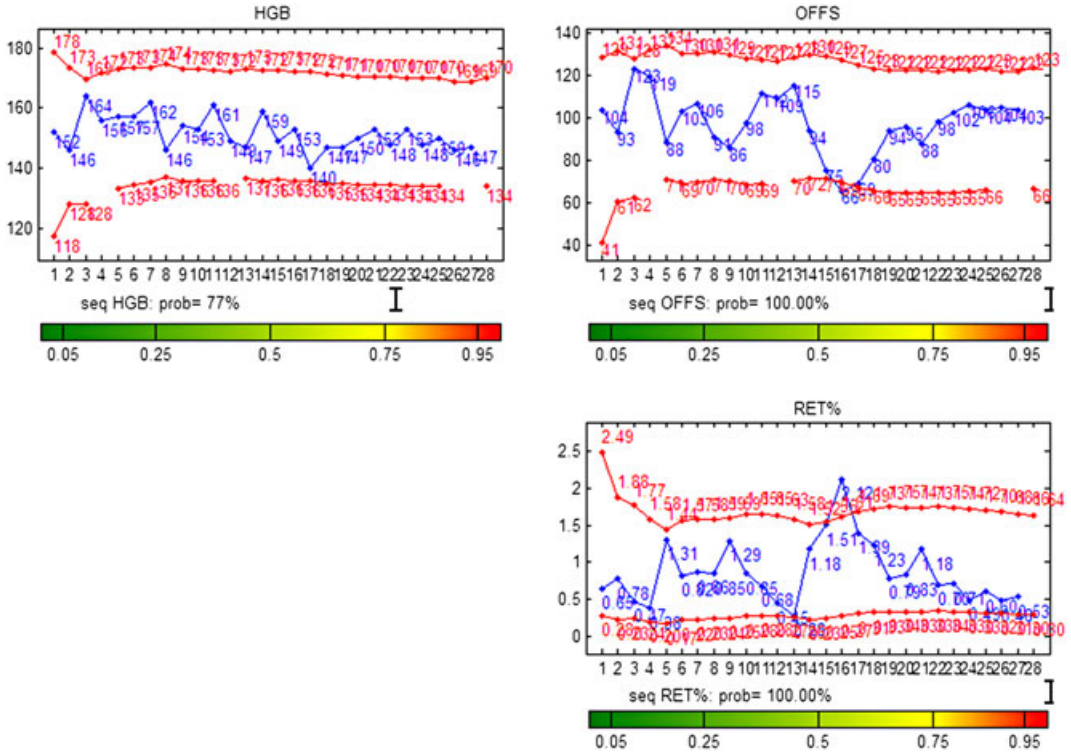

Figure 2. ABP profile of case 4 athlete.

Case No. 4

This last case (Figure 2) is presented only to show that even if an anti-doping organization has suspicions of an athlete based on an abnormal profile, it can take a long time before convicting him/her of doping. In this particular case, the rider was targeted because of his variation in %RET and OFF-HR for many months. It was only after several attempts that a urinary sample was reported positive for rhEPO during an in-competition test. Additionally, the profile was considered suggestive for doping by the independent experts and a disciplinary procedure would have probably been launched against the athlete if the adverse-analytical finding had not been reported. In this specific case, the athlete was sanctioned for two years and has not appealed the decision of the first instance disciplinary body. It is true to say that with such a scenario it would have been possible to combine both the adverse analytical finding and the abnormal profile to suggest that doping had taken place over a prolonged period of time and therefore request an imposition of a period of ineligibility greater than the standard sanction (up to four years) due to aggravating circumstances (art. 10.6 WADC).

Outlook

In addition to the direct sanctioning of athletes and the precise targeting illustrated above, the passport bears a large potential to 'fine tune' doping controls. Yet, this potential has not been fully explored. In the following, several possible applications for the use of passport data are outlined.

The multitude of data collected in connection with the passport from a limited number of athletes during a certain period might be used to estimate the point prevalence of blood manipulations in the tested athlete population.[14] Through this measure, the efficiency of anti-doping measures might be controlled.

Even in the long-term control of an individual athlete, the analysis of the data offers many possibilities. A key factor in today's doping controls is to identify athletes that very likely dope and to focus testing resources on these athletes. The passport might help in this context. In fact, many athletes will try to beat the passport by using low-dose doping or other sophisticated doping regimes that will enhance their performance, but not necessarily impact their blood values enough to make them go beyond their individual limits calculated by the passport (and thereby escape the attention of the testing authority).[15] However, mild changes in their biomarkers might be expected even in these cases. The approach to such cases must be to identify atypical patterns in the blood values of the athletes in question, such as seasonal abnormalities (atypical differences in blood values between summer and winter), differences between in- and out-of-competition tests or changes of blood values over the competitive season in relation to the competition schedule of the athlete. By these means, targeting with conventional tests can be more efficient.

Furthermore, from comparing abnormal features of different profiles, suspicious groups of athletes or teams might be identified and testing can be directed accordingly.

In a more global approach, the evaluation of overall passport data might be useful to identify new doping techniques or trends, when certain biomarkers start to shift in a large part of the investigated collective or other unusual patterns are seen in the profiles.

To fully use this large amount of information, new data processing systems and algorithms are necessary in addition to the Bayesian approach that is currently used to calculate individual limits. These algorithms should identify patterns such as described above and flag suspicious profiles for expert evaluation

or further testing. Similar systems are used by insurance and finance companies for risk assessment.

In addition to the individual limits that are nowadays used as a main indicator for further investigation of a profile, such systematic analysis of abnormal features could strengthen the sensitivity of the passport and redefine the role of the passport as central tool for targeting, testing and sanctioning in anti-doping.

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