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Blood Monitoring in Anti-Doping Setting

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Abstract

We report some considerations about the possible use of simple haematological parameters, easy to measure, in the setting of the fight against doping. These parameters measured on each athlete may be analysed on a single basis, or, in a more efficient way, put into the perspective of an individualised monitored evolution, as defined by the concept of the haematological passport¹. In case of abnormal absolute values or abnormal evolution, this should lead to targeted anti-doping tests on those athletes showing such results.

Introduction

One of the purposes to fight against doping is to ensure health protection to athletes taking part in competitions, through the establishment of a list of prohibited substances and methods, the Prohibited List, which includes substances or methods dangerous for the health and performance enhancer or contrary to the ethic of sport².

Blood doping defines all substances and methods enhancing oxygen transport (i.e. rhEPO, NESP, autologous or homologous blood transfusion, hemoglobin based oxygen carriers, and others). It is one of the most dangerous forms of doping, because in general it has a great effect on the performance and can induce major health risks^{3,4}, immediately or on long term (e.g. the appearance of transfusional accidents or of anti-erythropoietin antibodies). Furthermore, some forms of blood doping can be difficult to detect or even be undetectable (autologous blood transfusion). On the other side, it is important to note that blood doping is probably the only form of doping where easy measurable indicators of its use, are available: haematological parameters like haemoglobin, haematocrit, reticulocytes or free plasma haemoglobin.

Methods

In January 1997, the International Cycling Union launched a program to protect and prevent health risks to the riders⁵. The first part of the program was the introduction of unannounced blood tests before the competition, which started in March 1997⁶, followed then by the medical monitoring program in January 1999.

The aims of both programs were to protect the athletes' health, to promote fair competition and to improve the fight against doping by using these tests as screening tests for targeted anti-doping controls.

During unannounced bloods tests, several haematological parameters are measured, which include haematocrit, haemoglobin, reticulocytes, free plasma haemoglobin and the calculation of the stimulation index or OFF-hr score⁷ ($Hb [g/L] - 60 * \sqrt{Ret[\%]}$). Cut-off limits have been established in order to temporarily stop from competing (for 15 days) those athletes found with values beyond those limits. These latter are represented in Table 1; the parameters and limits have changed through the years, in order to adapt to new blood doping habits, because, as mentioned before, these parameters can be good indicators of the use of pharmacological blood boosters (rhEPO, NESP; CERA and other EPO-mimetic) as well as blood transfusions (homologous or autologous) or synthetic haemoglobins (HBOC).

Blood parameters are measured on site of the competition, in the morning before the departure of the race, on an unannounced way. Samples are immediately analyzed in a nearby hotel, where the instrument has been installed. Actually, we rely on a Sysmex XT-2000i (Sysmex Corporation, Japan) to analyze all the parameters, including reticulocytes determination. For the determination of free plasma haemoglobin, the HemoCue® Plasma/Low Hemoglobin (HemoCue AB, Sweden) system can easily be utilized, as well as the instrument cited above.

Results are available about one hour after blood collection, and athletes are notified of the decision about their "aptitude" or "inaptitude" to compete.

Table 1: Measured blood parameters, year of introduction and their limits for men and women

Blood parameters	Year of introduction	Limit for men	Limit for women
Haematocrit [%]	1997	50	47
Haemoglobin [g/dl]	2000	17	16
Free plasma haemoglobin [mg/dl]	2003	300	300
Stimulation index	2004	133	123
Reticulocytes [%]	2005	0.2	0.2

Results

In the Figure 1, the mean values of haematocrit, haemoglobin, reticulocytes and stimulation index are reported since 2001, the year when reticulocyte measurements were introduced.

It can be noted (unpublished data), that since 1997, when the first unannounced blood tests were conducted, there was a progressive and continuous decline of mean haematocrit and haemoglobin values, which, however, started to increase again in 2003 and 2004. Even though the measured values were still in the normal and physiological range that it should be expected within professional endurance athletes, this new fact was surprising. At the same time, the reticulocytes means decreased from 2001-2002 to 2003-2004. More than that, two distinct reticulocyte distribution curves were observed, one for 2001-2002 and a different one for 2003-2004, in a situation where the population of athletes submitted to the health controls had not changed, the modalities of the controls were the same through the four years and no changes had occurred in the technology used to perform the analyses.

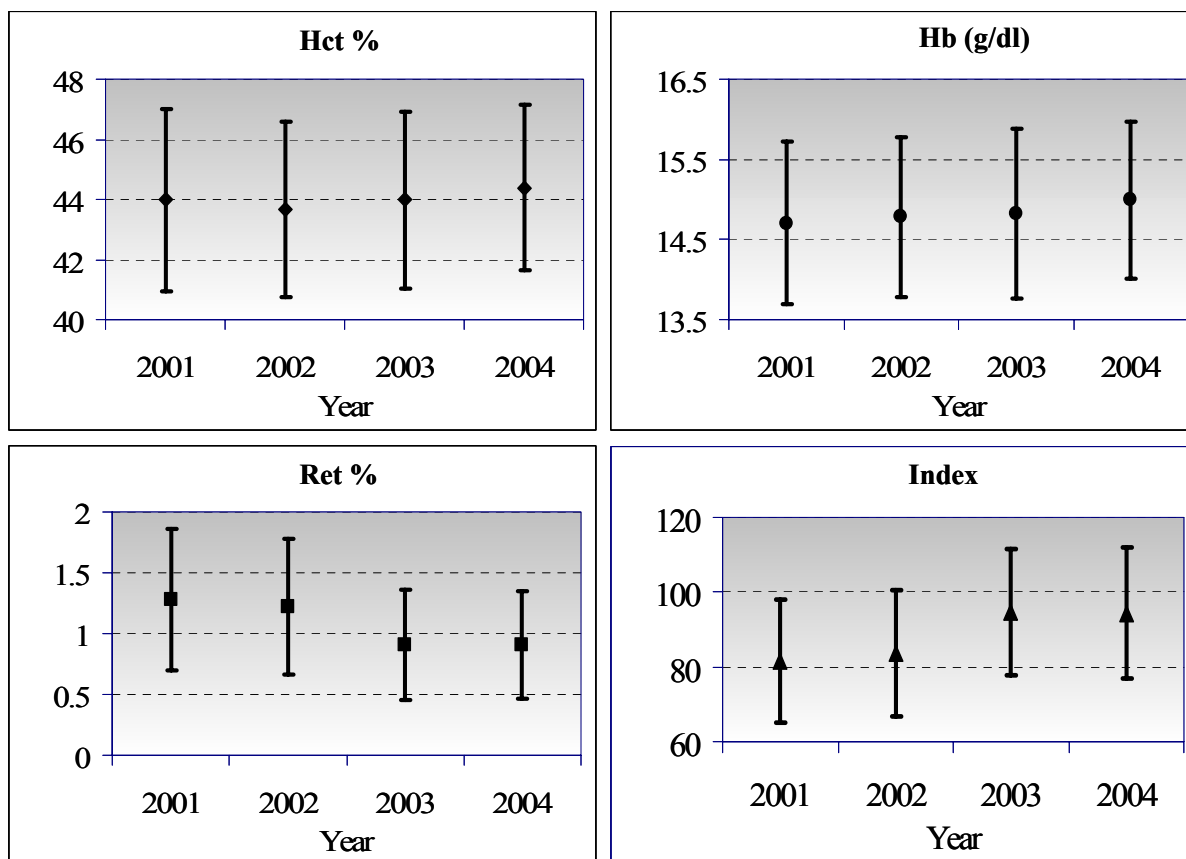


Figure 1: Haematological results from 2001 to 2004 expressed in means \pm SD. Data are obtained from male and female athletes. Number of samples analysed per year respectively for Hct-Hb and Ret%-Index: 2001 [2495/1541]; 2002 [3033/2352]; 2003 [2890/2073]; 2004 [2285/2001].

Discussion

How to explain this unexpected evolution of the blood parameters? It is important to focus on the reticulocyte results, which is a parameter very much influenced by blood doping. In fact, an increased number of circulating reticulocytes (red cells precursors) is the consequence of a stimulus of the bone marrow to increase the red blood cells production, following the administration of blood boosters (rhEPO, NESP, CERA, etc.). Of course, a reticulocytosis is not specific enough to proof the use of these forbidden drugs, because other conditions or situations can cause a similar result, but nevertheless this can raise suspicions.

On the other hand, when an increased number of circulating red cells is present, as a consequence of bone marrow stimulation, or after the administration of blood transfusion (homologous or autologous), a physiological negative feed-back on red blood cell production

takes places, which will result in a decreased number of circulating reticulocytes (reticulocytopenia). It is important to note that a very low number of circulating reticulocytes cannot be the consequence of other non forbidden ways of increasing the total number of red cells, like living in altitude or using simulated altitude devices⁸. By the way, only severe medical conditions can be responsible for an important reticulocytopenia, but these pathologies are incompatible with the practice of sport at very high levels, and therefore cannot explain such results in a competing athlete. More than that, as it is stated by Gore et al.^{7,9} “it should be noted that the haematological milieu of increased haemoglobin together with abnormally low reticulocytes levels has not been ascribed to any known pathological abnormality in the literature”. This combination of results is not known in the medical community, and is in all likelihood due to prior use of forbidden substances that induce bone marrow stimulation, or to blood transfusion.

The analysis of the results obtained from 2001 to 2004, shows that the number of samples with very high values of % reticulocytes (> 2.5%) decreased in a significant way from 2001-2002 to 2003-2004, and at the same time there was an increase in the reported number of sample with low to very low % reticulocytes values (< 0.4% and 0.2% respectively), as can be observed in Table 2.

Table 2: Number of blood samples analyzed, and absolute and relative values of high and low reticulocytes count from 2001 to 2004

	2001	2002	2003	2004
Samples	1541	2351	2073	2029
Ret > 2.5%	52 (3.4%)	59 (2.5%)	14 (0.7%)	9 (0.4%)
Ret. < 0.4%	33 (2.1%)	58 (2.5%)	192 (9.3%)	193 (9.5%)
Ret. < 0.2%	1 (0.06%)	1 (0.04%)	41 (2.0%)	38 (1.9%)

Two separate periods can therefore be defined: the first one, which associates 2001 and 2002, the second one, which takes into account 2003 and 2004. The differences during these two periods, concerning reticulocytes values (decreased number of samples showing reticulocytosis and increased number of samples showing reticulocytopenia), associated with increased haemoglobin, lead us to the explanation that a change had occurred in the way a limited number of athletes were using blood doping. The changes in the

reticulocytes/haemoglobin results, as well as rumors from the field, made us come to the conclusions (and other International Federations confirmed our suspicions) that:

- NESP was less administered, because of the difficulties in adapting dosage and the frequency of administration, as well as the easier and prolonged detectability. Being more potent than EPO, it has led to some abnormally high reticulocytosis (up to 3 or 4%) during the 2001-2002 period, which have disappeared in the years 2003-2004;
- EPO was used differently from previous years and blood transfusion was making its come-back. In fact, in order to avoid urinary EPO detection at the time of the competition, the new strategy consisted in decreasing the dosis of intra-venously injected EPO (so that the detection window of EPO was shorter) and performing the treatment well before the competition. Because blood transfusion was not detectable until August 2004, some athletes have decided to choose this way of doping. The consequence of these manipulations was that at the time of our tests during the competition, some athletes showed the combination of high haemoglobin and reticulocytopenia, which is a characteristic of a prior blood manipulation.

Therefore, athletes who show such abnormal results, or abnormal evolution of blood parameters, can be suspected of blood doping, and should be subject to targeted anti-doping tests, in and out-of-competition⁶.

An example is shown in Figure 2, which illustrates the blood profile of an athlete who has been convicted of using rhEPO. In the period 04.07.2002 – 22.07.2002 (grey zone), which coincides with a stage race, the results obtained at the beginning show a normal to high values of haematocrit and % reticulocytes (2.3%), which has led to a targeted urinary test for rhEPO (negative). Contrary to what is physiologically expected during such a prolonged effort, where usually a drop of the hematocrit of almost 3% is observed (e.g. from 45% to 42% at the end of the race), the successive blood profile shows an increased haematocrit associated to a very low value of % reticulocytes (0.28%). The evolution of these parameters is compatible with a bone marrow stimulation, probably by an intake of rhEPO or NESP, which has had two major consequences: increase the haematocrit and decrease the reticulocytes production due to the negative feed-back. Even though several urine tests were negative for rhEPO, we finally could obtain a positive sample almost one year later!

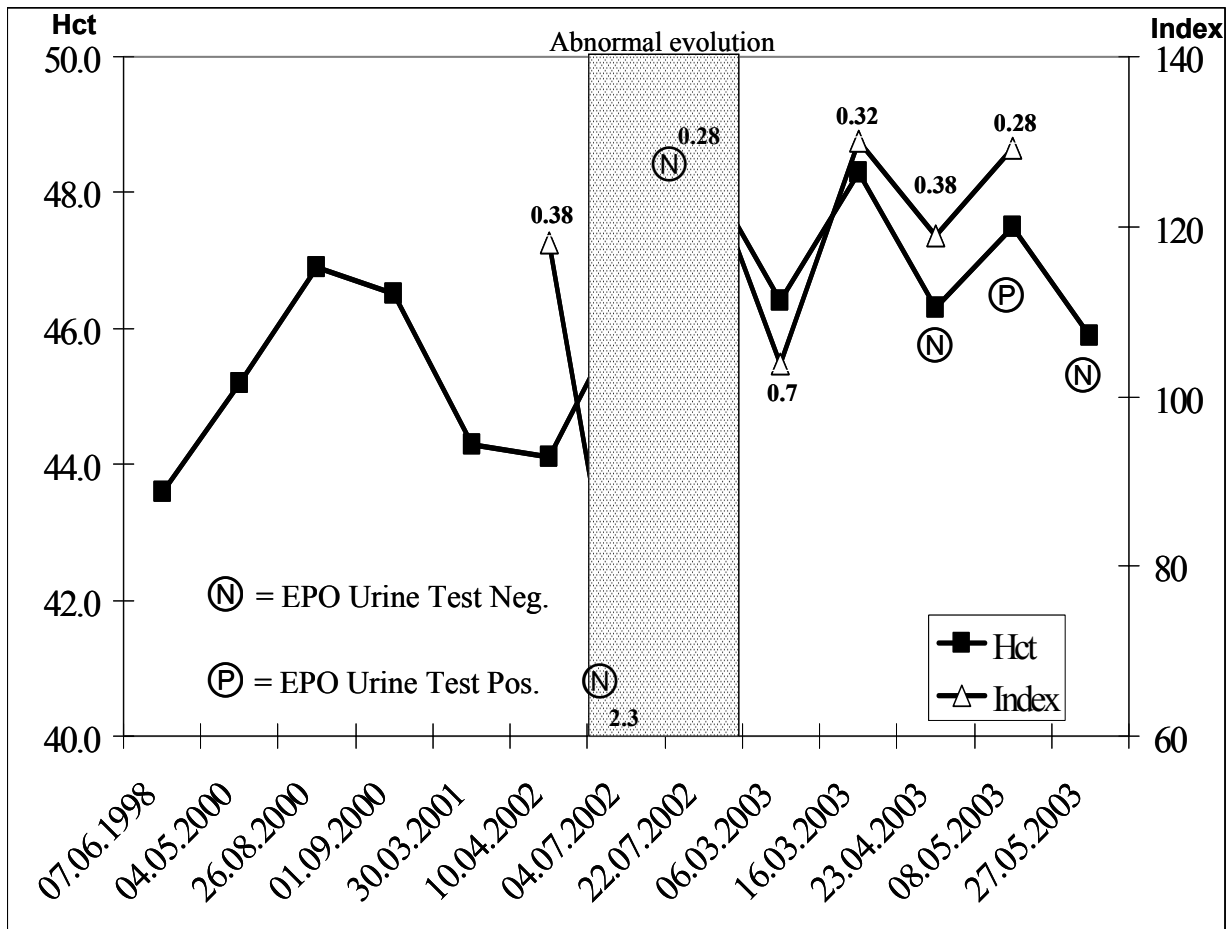


Figure 2: Example of a blood profile of an athlete (haematocrit [%] on the left; stimulation index on the right; reticulocytes in numeral [%]) and the targeted urine rhEPO tests conducted on him

Other application of longitudinal monitoring of blood parameters is shown on Figure 3, which represents the stimulation index profiles of three different athletes. Athlete A, who has been convicted of blood doping, shows highly suspicious variations (controls 5 to 10 and 10 to 16), which has lead to targeted anti-doping tests. Athlete B is considered having a normal profile, with physiological variations which are not consistent with blood doping. Athlete C has never tested positive, but has been declared inapt to compete on one occasion (control 14): since then, his values have dropped to normal range!

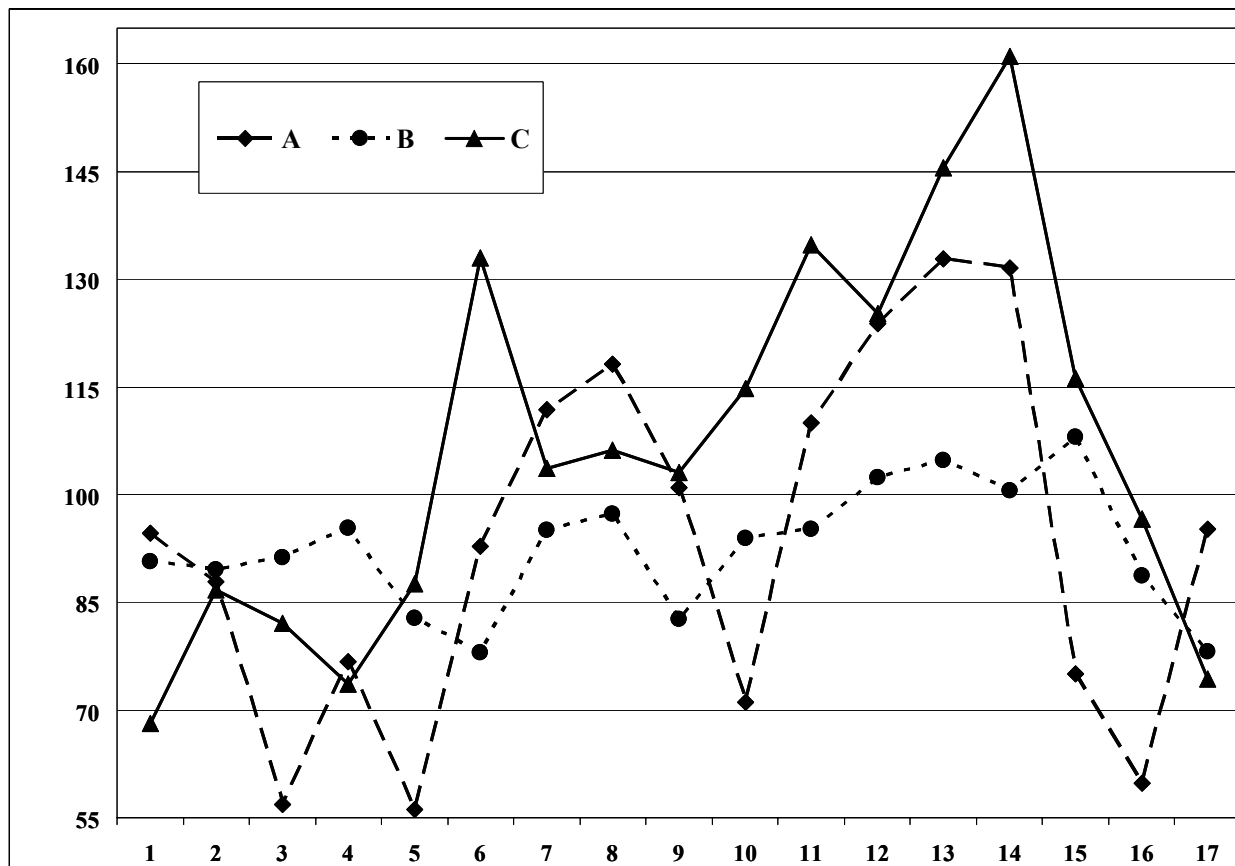


Figure 3: Stimulation index profiles of three different athletes

Schematically, it can be said that abnormal values of blood parameters should lead to targeted anti-doping tests in accordance with the following “rules” (Table 3)

Table 3: Abnormal blood parameters results and anti-doping tests

Abnormal blood parameter	rhEPO / NESP	Homologous blood transfusion	Synthetic haemoglobin
High % reticulocytes	X		
Low % reticulocytes	X ^a	X	
High / increased Hct or Hb	X	X	
High / increased stimulation index	X ^a	X	
High free plasma Hb ^b			X

^a Suspicious athlete: conduct a targeted rhEPO test in the future (in- or out-of competition). In fact, during the “OFF-phase”, it is unlikely to have a positive urinary result

^b Or pink / red plasma coloration

Conclusions

Simple blood tests, which can be performed on the field at quite low costs, provide indications of possible blood doping. Therefore, they should be utilized to target anti-doping tests on athletes showing abnormal results. This will increase anti-doping testing efficacy. Excluding from the competition those athletes who have results beyond the limits will as well increase the protection of athletes' health and the fairness of the game. It is also important to consider another effect of this rule, which is to dissuade the use also of non detectable blood doping (e.g. autologous blood transfusion), in order not to fall beyond the hematological limits. Of course, it has to be taken into consideration that a minority of the athlete population might have high natural values of haematocrit or haemoglobin, and therefore provide adapted procedures to allow them to compete, i.e establishing certificates for natural high haematocrit / haemoglobin level.

At the same time, blood tests must evolve in order to face new strategies adopted by those athletes who modify their doping practices.

To conclude, we believe that abnormal blood profile cannot be considered as a proof of doping alone, because the standards for analysis are not well defined as for anti-doping purposes and because variability is very important, due to intra- and inter-individual variations and intra- and inter-technology differences, which can be very high in case of reticulocytes measurement. Nevertheless, these results can be considered as a suspicion of doping, and be used as complementary evidence during disciplinary proceedings.

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