

Indirect Markers of Blood Doping

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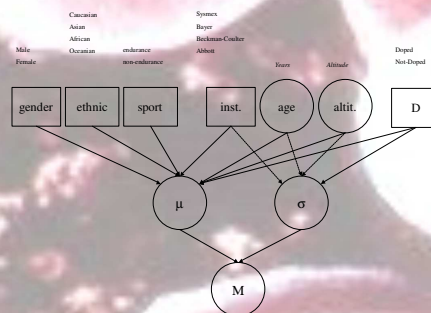
I – SUMMARY

Today, only the discovery of an exogenous substance in the body of the athlete can lead to a disciplinary sanction. However, the level of evidence provided by indirect markers of altered erythropoiesis can be high enough to differentiate between natural variations and blood doping. Forensic techniques for the evaluation of the evidence, and more particularly Bayesian networks, allow anti-doping authorities to take into account firstly the natural variations of indirect markers of blood doping – through a mathematical formalism based on probabilities -, and secondly the complexity due to the multiplicity of causes and confounding effects – through a distributed and flexible graphical representation. The information embodied in an athlete's biological passport may be sufficient to present a case to a disciplinary panel.

II – INTRODUCTION

Up to now, sports authorities exclude athletes with abnormal levels of blood parameters (e.g. haematocrit). The consideration of longitudinal blood profiles together with heterogeneous factors produces a method with enhanced sensitivity to detect blood doping. Sports disciplines with heterogeneous populations now have a general method to introduce the no-start rule or even ban athletes for a two years period.

Here we show an example that it is possible to use indirect markers (haematocrit, haemoglobin, reticulocyte count, serum EPO, sTFR...) as a sufficient prove of blood doping (blood transfusion, EPO abuse...). A) This multiparametric approach with markers altered by blood doping B) the use of heterogeneous factors (age, gender, ethnic origin, altitude...) C) the use of previous data of an athlete to define the subject-specific reference range D) the necessity of following rigorous standardised blood collection, transport and analytical protocols E) the application of a Bayesian approach to evaluate the level of evidence F) the use of information on the prevalence of doping in the studied population are all elements which enable us to get a sufficient sensitivity and specificity to launch a disciplinary procedure from these indirect blood markers



Bayesian network with the introduction of confounding heterogeneous factors either fixed or variable from one measure to another. This network enables to analyse an individual sequence according to the information contained into the biological passport of the athlete. It also enables to analyse a population of athletes and to estimate the doping prevalence.

Bayesian approach to evaluate the level of evidence:

The blood test that combines a multiparametric approach for increased specificity, a haematological passport for individual longitudinal monitoring and the formal integration of various factors for heterogeneous populations. It is based on a global Bayesian inference approach for the detection of abnormal values over time. Hb and ABPS (Abnormal Blood Profile Score: Hct, Hb, RBC, %reti, MCV, MCH, MCHC) markers are used. Effects of gender [male, female], ethnicity [Caucasian, Asian, African, Oceanian], age [<19 years, 19-24 years, >24 years], altitude [<610, 610-1730, >1730], sport [endurance, non endurance] on the mean of each parameter were taken from published data. Except for gender, the variance of blood parameters is considered independent of the factors and modeled as a log-normal distribution with parameters estimated from data collected on control subjects.

III – MATERIAL & METHODS

Various clinical trials were needed to elaborate this approach:

1) 32 healthy Caucasian males (aged 19–36 years) participated in the EPO study (EPREX three times a week); 8 subjects received subcutaneous injections of 40 IU/kg of EPO, 8 subjects 80 IU/kg of EPO, 8 subjects received placebo and 8 subjects did not receive any treatment. Each subject of either groups treated with EPO got full dosage for Hct below 45%, half dosage for Hct between 45 and 50% and substitution by isotonic saline when Hct exceeded 50%.

2) A group of 22 top-level elite endurance athletes, 11 males and 11 females, all Caucasian and aged 21–35 years participated in a study mandated by the Swiss Federal Office for Sport to assess the feasibility and utility of introducing a hematological passport in Switzerland and to promote drug free sports. Regular anti-doping tests were conducted on each athlete over a period of 2 years (6 blood and 11 urine samples on the average), returning only negative test results.

3) 10 blood samples withdrawn from 10 athletes were simultaneously tested positive to EPO in urine. 11 blood samples withdrawn from various athletes were simultaneously tested positive for the homologous blood transfusion test.

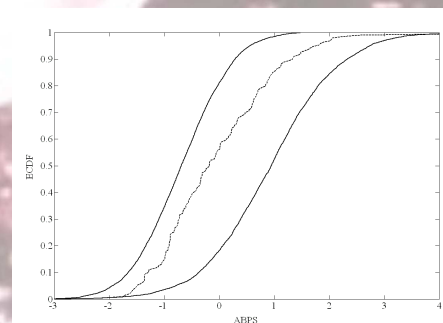
4) Two different longitudinal studies, each lasting 2 months, were conducted on, respectively, 22 and 25 male amateur athletes (Caucasian, aged 20–30 years). A total of 347 blood profiles from the first study and 225 samples from the second study were obtained.

Sample analyses

- Serum → EPO and sTFR: DPC and Nichols
- Whole blood → Full blood count + reticulocyte count (absolute, percent, IRF...); Abbott Cell-Dyn 4000, Bayer Advia 120, Sysmex XT-2000i, Beckman Coulter GenS
- All data analyses were performed on Matlab version 6.1.0. with Statistics Toolbox

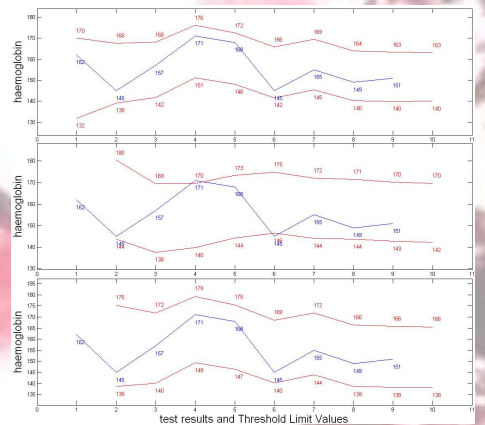
IV – RESULTS & DISCUSSION

ECDF (Empirical Cumulative Distribution Function) of ABPS enables to determine the prevalence of doping. This is possible as long as athletes dope the same way the model was elaborated (micro EPO injections, homologous blood transfusion).



Cumulative distribution function versus ABPS for a population of healthy athletes (not doped, curve on the left) and for a population of doped athletes (micro EPO injections, homologous blood transfusion) (right curve). The difference between both curves is characteristic of the discriminant power of ABPS for this type of doping of this population. The dotted line (ECDF) was obtained during a major competition in 2004. The shift on the left suggests an important number of doped athletes during this event. The prevalence is estimated to be close to 40% (95–98%).

The Bayesian approach was compared to various models actually developed and used by some federations. Here we show that the thirs generation is an exception of our model and can lead in some cases to false positive findings



First graph shows the results and cut off limits obtained by the model developed by Sottas et al. (Bayesian network). Second graph shows the results obtained with the third generation model with a universal variance as described by Sharp et al. Finally, the third graph is the same third generation model, but taking into account this time the exposure to altitude.

According to all data presented here and published into detail in various publications, it is better to use a model integrating most of the information at disposal (heterogeneous factors). The risk of false positive results will be significantly decreased.

IV – CONCLUSIONS

This new approach has many advantages. It limits the costs of the fight against doping; it enables to detect most of the bone marrow stimulators (EPO, NESP, CERA...) and according to the level of proof at disposal, the federation can decide whether or not, it should decide to launch a disciplinary procedure. Eventually, the federation could decide following the level of evidence at disposal to sanction more or less severely.

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