Bayesian detection of abnormal hematological values to introduce a *no-start* rule for heterogeneous populations of athletes

Sports authorities exclude athletes with abnormal levels of blood parameters. Here, the consideration of longitudinal blood profiles together with heterogeneous factors such as ethnicity and age produces a model with enhanced sensitivity to detect blood doping. Sports disciplines with heterogeneous populations now have a general method to introduce the *no-start* rule.

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In sports, rHuEPO, the recombinant form of erythropoietin, is illicitly used to improve physical performance. Some sports federations with a fairly homogeneous population of athletes have discouraged rHuEPO doping by introducing hematocrit (Hct) and hemoglobin (Hb) limits. Athletes tested above these limits are declared unfit for competition: the so-called *no-start* rule. Unfortunately, since Hct and Hb present elevated between-subject variations[1] and can easily be manipulated,[2] the efficiency of both variables remains limited. Models using multiple hematological variables, such as Abnormal Blood Profile Score (ABPS),[3] were proposed to improve sensitivity/specificity. Along with this, the idea of a hematological passport was also suggested.[4] Sportsmen with significant differences between new test results and an individual historical baseline could be excluded from competition.[1]

Hematological variables depend on various factors such as gender, ethnicity and age. Even though the effects of these factors on hematological parameters have been well described,[5] they have not been taken into consideration in the formulation of a blood model. This leads to an unpredictable number of false-positive findings for heterogeneous populations. Unsurprisingly, sports federations with highly heterogeneous populations refrained from introducing a *no-start* rule.

We, therefore, propose a blood test that combines a multiparametric approach for increased specificity, a hematological passport for individual longitudinal monitoring, and the formal integration of various factors for heterogeneous populations. The blood test is based on a global Bayesian inference approach for the detection of abnormal values over time.[6] Hb and ABPS3 (with Hct, Hb, RBC count, reticulocytes percentage, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration variables) 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.[5] 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. Firstly, 135 blood profiles were collected from 22 top-level elite endurance athletes, 11 males and 11 females, participating in a study commissioned by the Swiss Federal Office for Sport 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 in average) returning only negative test results. Secondly, 572 blood profiles were collected from 47 male amateur athletes over a period of two months (347 with an Advia 120, Bayer Diagnostics, Zurich, Switzerland, 225 with a XT-2000i analyzer, Sysmex, Norderstedt, Germany). Sensitivity was estimated from data collected in a two month rHuEPO clinical trial described elsewhere.[3,7] To summarized, 32 healthy volunteer males participated in this study. The aim was to reproduce rHuEPO doping habits practised in some sports. Eight subjects received subcutaneous injections of 40 IU/kg of EPREX three times a week (group R40), 8 subjects 80 IU/kg doses (group R80), 8 subjects received placebo (group P), and the last 8 subjects (group NT) had no treatment. rHuEPO administration in R40 and R80 groups was nevertheless individualized. A full dosage was administered for Hct below 45%, half doses for Hct between 45 and 50%, and substitution by isotonic saline when Hct exceeded 50%. Five hundred and ten blood samples (178 from doped

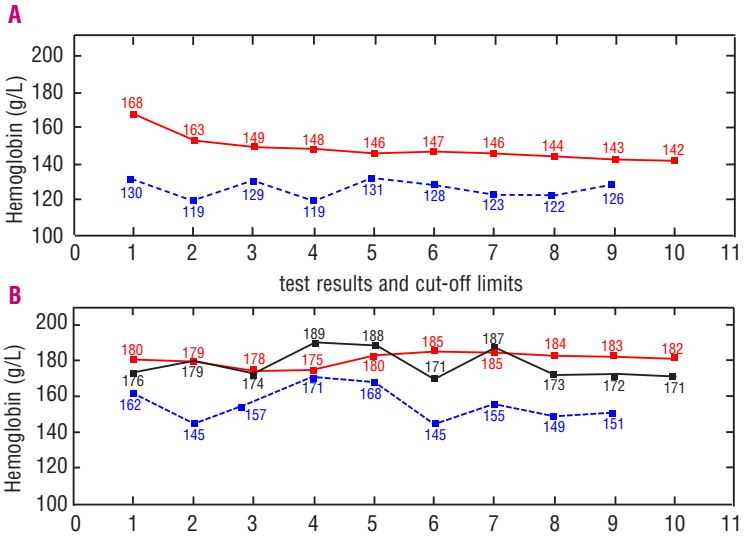

Figure 1. A. Red line represents the limits found for a specificity of 99.9% for the female subject. The first value at 168 g/L represents a population threshold: 1 in 1,000 28 year old female Caucasian endurance athletes living at low altitude should in average present a value higher than 168. The cut-off limit changes as soon as individual Hb values (blue data) are taken into account. In case of a very high number of individual test results, the last value, here 142, represents an individual limit independent of any population factor. **B.** Red line represents the limits for the male subject. Information about his location was not available. Black line represents limits, however, including the information that all tests were conducted at low altitude, except for the 4th, 5th and 7th tests which were conducted above 2,000 meters. Interestingly, the final cut-off limit was lower when an altitude model was explicitly considered (171 *vs* 182) i.e. a higher sensitivity to blood doping. Likewise, thresholds were higher when the athlete is tested in high altitude (e.g. 189 *vs* 175 for the 4th test) i.e. a lower probability of a false-positive.

Table 1. Sensitivity and specificity of the model based on 1,217 samples for selected false-positive rates (FPR) of 1/100 and 1/1,000. Second line is the 95% interval of the expected number of false-positive findings.

	FPR 1/100	*FPR 1/1,000*
95% expected	5-17	0-3
Specificity (1,039 samples)		
Hb		
Pop	17 (1.6%)	1 (0.10%)
BN	11 (1.06%)	2 (0.19%)
ABPS		
Pop	8 (0.77%)	1 (0.10%)
BN	10 (0.96%)	2 (0.19%)
rHuEPO sensitivity (178 samples)		
Hb		
Pop	18 (10%)	3 (1.7%)
BN	59 (33%)	33 (19%)
ABPS		
Pop	78 (44%)	55 (31%)
BN	119 (67%)	102 (57%)

Number of samples and the corresponding percentage are either given for a population limit (pop)[5] or the Bayesian model (BN), for hemoglobin (Hb) and the multiparametric Abnormal Blood Profile Score (ABPS)[3] model.

subjects) were analyzed on a Cell-Dyn 4000 instrument (Abbott Diagnostics Division, Baar, Switzerland). In total, 1,039 samples collected in the three studies were used to estimate the specificity. For didactic purposes, we applied the Bayesian model to two subjects (Figure 1). The first subject is a top-level female Caucasian endurance athlete, 28 years old, living at low altitude. The second subject is a top-level male African endurance athlete, 36 years old, living and training occasionally at high altitude. Consideration of a new individual Hb value induces a new distribution of possible values of Hb, and, therefore, new individual reference range. The significant decrease between the first (a population-based threshold) and the last cut-off limit (an individual threshold) can be associated to a larger between- than within-subject variance of the hematological variable.[1,6,8]

All three aspects of the model, multiparametric approach, longitudinal analysis and consideration of heterogeneous factors, lead to a decrease of the overall variance of hematological data. As shown in Table 1, a heightened sensitivity to discontinuous rHuEPO treatment was observed. A population-based strategy on Hb gives only 3 true positives for a specificity of 0.999, whereas the Bayesian model returned as much as 11 times more true positives. With the ABPS marker, sensitivity was even further improved.

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