would exclude them as homologous donors.[4] In fact, we showed in a preliminary study that autologous donors had surgery or received blood components more frequent than first-time donors.[5]

In that study, we saw that 67 (81%) out of 83 of autologous donors had previous surgery compared with 41 (6.6%) out of 622 first-time volunteer donors. We also saw that 56% of autologous donors had transfusion history compared with 10.6% of first-time volunteer donors. This observation is also supported by Starkey *et al.*[6] who reported a high risk ratio in units from autologous donors who were not candidates for crossover by donor history and hematocrit (range, 1.8 for elevated ALT to 8.9 to positive anti-HIV-1).

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Key words: blood transfusion, infectious disease markers, autologous blood donors, first-time blood donors.

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Blood Doping

Strengths and weaknesses of established indirect models to detect recombinant human erythropoietin abuse on blood samples collected 48-hr post administration

We studied indirect detection models of erythropoietin abuse (EPO) on blood samples collected 48-hr after administration of the drug during 6 weeks of recombinant human erythropoietin (rHuEPO) treatment. Although the efficiency of OFF-models was preserved, we found a loss of sensitivity of ON-models. This study also revealed an increased percentage of stomatocytes in athletes receiving rHuEPO.

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Recently, Gore *et al.*[1] have developed new, sensitive, mathematical models to detect current (ON-models) and recent (OFF-models) rHuEPO abuse. With ON-models, they found a better sensitivity when blood samples were collected 24hr post injection than with unstandardized protocols (25 min to 72 hr between injection and blood sampling). As the half-life time of rHuEPO is very short,[2] we hypothesized that ON-models might fail to detect subjects abusing rHuEPO when injection and blood sampling are performed 48hr apart. We also examined blood smears for abnormally shaped red blood cells (RBC), particularly stomatocytes, because EPO may affect the synthesis of some membrane proteins involved in RBC morphology.[3]

In brief, we studied scores and sensitivity of indirect detection models and stomatocyte counts in athletes receiving moderate doses of rHuEPO.

Fourteen endurance-trained athletes were randomly assigned to receive either EPO or placebo. The EPO group received subcutaneous injections of rHuEPO (Eprex® Janssen-Cilag, France) 3 times a week for 6 weeks as follows: 50 U/kg during the first 4 weeks (*acceleration phase*) and 20 U/kg the next 2 weeks (*maintenance phase*). The PLA group received subcutaneous injections of NaCl (0.9%). The time between injections and blood sampling was 48 hours. Blood samples were taken before any injection (day 0), during both the acceleration and maintenance phases, and then over the following 3 weeks (*wash-out phase*). Hematocrit (Hct), hemoglobin concentration (Hb) and percent of reticulocytes (%Rets) were determined in blood samples collected in EDTA tubes (PENTRA 120 Retic Hematology Analyzer). The coefficients of variation (CV) were 1.18%, and 14.8% for Hb and %Rets measurements, respectively. EPO and serum transferrin receptor (sTfr) levels were measured in serum (Quantitative IVD human EPO and Quantitative IVD human sTfr Elisa kits, R&D System, Inc.). Intra and inter-assay CV were 4.4% and 6.5% for EPO and 5.7% and 5.8% for sTfr. Blood samples were taken between noon and 2 PM from the athletes in a supine position. Hb, %Rets, EPO and sTfr concentrations were used to calculate the scores of the different models (he and hes On-model or hr and hre Off-model). ON-models scores were calculated during the acceleration (day 11 and day 25) and maintenance (day 32) phases and OFF-models scores during the wash-out phase (day 54 and day 61). Values from the placebo group were then used to establish the mean score, the standard deviation (SD) and the 95% confidence interval for each model. Scores greater than the mean of placebo group ± 1.96*SD indicated a probable intake of rHuEPO. Blood smears were prepared using the glass slide method and examined by light microscopy. Manual counting of stomatocytes (%Stom)[4] was performed by two investigators. ANOVA for repeated measurements was used to compare results from the two groups. The rates of detection with ON-models ranged from 13 to 63% during the acceleration and maintenance phases (Table 1). Detection was excellent (100%) for OFF-models 14 days after the end of treatment (day 54) and low at day 61 (38 to 50%). During rHuEPO treatment, Hb and sTfr concentrations, as well as %Rets (except at day 32) were significantly higher than basal and placebo values (Table 2). By contrast, EPO levels were similar to basal (except at day 11) and placebo values. During the wash-out period, Hb, %Rets and EPO values were significantly different from those at the basal measurement while sTfr was not statistically different. %Stom increased with rHuEPO injections and remained elevated until the end of the wash-out phase in 8/9 athletes.

The results suggest that ON-models may fail to detect EPO abuse when blood sampling is performed 48 hr after injec-

Table 1. Mean scores for each model in the placebo group and reference limit (95% confidence interval). The rate of detection (sensitivity) on different days is the percentage of rHuEPO treated subjects with scores higher than those defined in placebo group.

	ON-he model	*ON-hes model*	*OFF-hr model*	*OFF-hre model*
Mean of the placebo group	162	167	76	74
Reference limit (mean of the placebo group + 1.96 SD)	181	191	102	100
Rate of detection on day 11	13%	38%	–	–
Rate of detection on day 25	33%	50%	–	–
Rate of detection on day 32	50%	63%	–	–
Rate of detection on day 54	–	–	100%	100%
Rate of detection on day 61	–	–	38%	50%

Days 54 and 61 = days 14 and 21, respectively of the wash-out phase.

Table 2. Hematologic variables in the groups treated with rhuEPO (n = 9) and placebo (n = 7).

	Day 0	*Day 11*	*Day 25*	*Day 32*	*Day 54*	*Day 61*
Hb (g/dL)						
Placebo	14.5±0.9	14.5±0.9	14.5±0.8	14.3±0.5	14.8±1.1	14.6±1.2
rhu EPO	14.6±0.8	15.4±0.9*§	16.0±1.0*§	16.3±0.9*§	16.1±0.7*§	15.4±0.9
%Rets (%)						
Placebo	1.3±0.3	1.3±0.3	1.2±0.3	1.4±0.3	1.3±0.2	1.3±0.5
rhu EPO	1.2±0.3	2.5±0.7*§	2.0±0.3*§	1.6±0.5	0.6±0.2*§	0.8±0.2
sTfr (nM)						
Placebo	20.1±4.8	19.8±6.4	20.9±6.8	20.2±8.5	23.3±7.4	22.1±4.4
rhu EPO	20.9±4.2	29.0±5.7*§	32.2±7.0*§	32.9±7.7*§	19.8±4.5	16.1±3.5
EPO (mU/mL)						
Placebo	6.4±1.4	6.9±1.9	6.3±1.2	6.4±1.8	7.7±1.9	7.5±1.9
rhu EPO	6.8±2.5	8.8±2.9*	7.5±3.2	4.9±2.0	3.6±1.2*§	5.0±1.6*§
%Stom (%)						
Placebo	2.4±1.5	2.7±1.4	2.5±1.9	1.2±1.3	3.7±2.8	3.0±2.5
rhu EPO	1.7±1.3	6.7±4.1*§	6.7±3.3*§	6.8±2.4*§	8.1±4.8*§	5.6±4.7*

*Hb: hemoglobin; %Rets: percentage of reticulocytes; sTfr: soluble transferrin receptors; EPO: erythropoietin; %Stom: percentage of stomatocytes. Values are means±SD. Day 0: baseline day; Days 11 and 25: acceleration phase; Day 32: maintenance phase; Days 54 and 61: days 14 and 21, respectively, of the wash-out phase. *Significant difference from day 0 value; §significant difference from PLA group.*

tion of moderate or low doses of rHuEPO. This is particularly true at the beginning of treatment when Hb concentration is not yet very elevated. Later on, during the treatment period, the weakness in the model may be attributed to EPO levels that were never found to be statistically higher than in the rHuEPO-treated group. By contrast, our study shows an excellent rate of detection for OFF-models after 14 days of wash-out. This shows the robustness of these models at detecting previous r-HuEPO abuse despite analytical methods that are different from those described in the original study.[1] This is important since, at competition time, an athlete is more likely to be in a wash-out phase than in treatment phase. Our study also shows a persistent elevation in the level of stomatocytes in the group treated with rHuEPO. This observation warrants further investigation, since other causes besides rHuEPO may increase stomatocyte number. Nevertheless, we believe that stomatocyte counts could add information on suspicious samples and may have a place in a passport scenario.

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