The influence of exercise on serum markers of altered erythropoiesis and the indirect detection models of recombinant human erythropoietin abuse in athletes

Our investigation reports changes related to endurance exercise in the hematologic variables currently used for indirect detection of erythropoietin abuse in athletes and the impact on the model scores of the detection algorithms. The model scores are altered through exercise-related plasma volume shifts, but the changes are mostly opposite to those expected to be induced by erythropoietin abuse.

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The abuse of recombinant human erythropoietin (rhEPO) and other artificial stimulants of the red blood cell system to improve athletic performance is nowadays a major concern in endurance sports and the detection of such abuse a new challenge to Hematology. Although there is a direct test that can detect artificial erythropoietin in urine samples, this test is limited: the brief period of detectability of rhEPO (3-5 days) is far outlasted by its performance enhancing effects (2-4 weeks). In addition, new products stimulating the erythropoietic system, such as modifications of rhEPO (darbepoetin, erythropoietin δ) might not be uncovered by the urine test. Artificially accelerated erythropoiesis leads to characteristic changes in peripheral blood variable, irrespectively of the stimulating agent. In year 2000, Parisotto *et al.*[1,2] defined mathematical models for indirect detection of current *(ON-model)* and recent (*OFF-model*) rhEPO abuse based on hematologic parameters and serum variables. Since then, these models have been refined and simplified to allow a broad application without the necessity of particularly sophisticated analyzing systems.[3] These models are independent of the substance being used to manipulate the athlete's performance, any type of altered erythropoiesis is unmasked.

Nevertheless, it is unclear how physical exercise affects the variables used in these models as it is known that exercise itself influences the erythropoietic system both in the short-term and in the long-term. Endurance trained athletes have a physiologically increased red cell mass and expanded vascular volume.[4] The physiologic responses to exercise might interfere with changes caused by artificially stimulated erythropoiesis. This might be of importance in evaluating the significance of results of indirect tests done in athletes during training and competitions.

The present study was, therefore, aimed at investigating the effects of endurance exercise on hematologic serum markers and the calculation models used for the indirect detection of rhEPO abuse in a group of highly trained athletes not using rhEPO.

Twenty-three male professional cyclists and 16 inactive control subjects were investigated during a five-day road cycling stage race at sea level. Serum- and EDTA samples were obtained every morning prior to the race and between one and three hours after the end of each stage. The following variables included in the detection models were automatically determined from the EDTA samples (Bayer ADVIA 120

Table 1. Hematologic variables, erythropoietic serum markers (mean ± SD) and changes in vascular volumes (%) in 23 professional cyclists during a five-day stage race.

	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Normal range*
Hemoglobin (g/dL)						
Morning	15.3±0.9	15.3±0.8	14.8±0.5	14.6±0.7	14.4±0.8*	*14-17.5*
Evening	14.8±0.7+	14.6±0.5	14.8±0.7+	14.1±0.8 *	14.6±0.8	
Hematocrit (%)						
Morning	43.3±2.1	42.9±1.9	41.7±1.3 *	41.2±1.8 *	41.4±2.3 *	*39-51*
Evening	41±1.7+	40.6±1.7+	41.4±2.1+	39.4±2 *	40.1±2.5	
Reticulocytes (%)						
Morning	1.4±0.3	1.5±0.3	1.5±0.3	1.6±0.3	1.4±2.2	*0.8-2.2*
Evening	1.5±0.3	1.5±0.4	1.6±0.3	1.5±0.3	1.4±0.2	
Iron (mg/dL)						
Morning	104.5±29.4	118.4±41.1	159.4±28.3*	127.4±31.2	153.1±31.6*	*50-180*
Evening	59±25.5+	104.9±22.3	88.1±28.2*+	75.5±29.3+	118.4±31*+	
Ferritin (mg/dL)						
Morning	116.3±68	116.1±72.2	121.6±71.9	121.9±71.9	130±80.6*	*12-200*
Evening	112.9±66.6	132.9±77.4	122.3±70.6	122.9±70.2	137.3±88.7*	
sTfr (mg/dL)						
Morning	3.4±0.6	3.4±0.5	3.3±0.5*	3.3±0.5*	3.2±0.5*	0.83-
Evening	3.5±0.5	3.4±0.8	3.4±0.5	3.3±0.5	3.2±0.5	2.76
EPO (U/mL)						
Morning	8.2±3	9.3±3	11.3±3.2*	11.8±3.6*	13.3±5.3*	*2.6-34*
Evening	8.1±2	11.2±3.5	11.9±4.1	15.5±5.4*+	14.7±6.2*	
Blood Volume (%)						
Change from day 1 (morning)		1.1	2.8*[C]	4.1 *[C]	5.1*[C]	
Change from morning (same day)		3.6+[C]	0.05	-0.7[C]	3.8+*[C]	-0,4[C]
Plasma Volume (%)						
Change from day 1 (morning)		2.0	5.6*[C]	7.9*[C]	8.4*[C]	
Change from morning (same day)		8.0+[C]	-0.9+	-1.5[C]	6.3+*[C]	0.6[C]

*Normal ranges are those generated by our laboratory's internal and external quality controls and suggested by the manufacturers of the assay kits. +: significant difference from morning value (ANOVA); *: significant difference from day 1 value (ANOVA); [C] significant difference from control group (ANOVA).*

Table 2. Model scores for the indirect detection of artificially stimulated erythropoiesis (mean, range) during a five day stage race in 23 professional cyclists. The variables used for the calculation are shown in brackets. Limits for positive testing for normal subjects at sea level as suggested in refs. 1-3, risk of false positives set at 1:1000).

	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Limit*
ON Score (Hct, RetHct, EPO, sTfr, %Macro)						
Morning	2.14 (1.81-2.36)	2.18(1.99-2.45)	2.16 (1.94-2.37)	2.15 (1.85-2.39)	2.16 (1.98-2.44)	2.66
Evening	2.04+ (1.86-2.26)	2.10+ (1.85-2.30)	2.15 (1.91-2.65)	2.08+ (1.92-2.30)	2.10 (1.9-2.25)	
OFF Score (Hct. RetHct. EPO)						
Morning	1.7 (1.38-1.96)	1.62* (1.34-2.01)	1.53* (1.23-1.88)	1.45* (1.25-1.80)	1.53* (1.22-1.87)	2.47
Evening	1.56+ (1.27-1.89)	1.46+* (1.11-1.81)	1.45+* (1.05-1.83)	1.37+* (1.16-1.80)	1.46* (1.24-1.83)	
ON HE Score (Hb. EPO)						
Morning	172.8 (151.8-188.2)	172.6 (163-187.4)	170.8 (160.7-182.8)	169.5 (154.6-182.9)	168.8 (158.5-184.3)	200
Evening	167.8 + (158-184.6)	168.7 + (157.4-181.4)	170.8 (159.8-185.6)	167 (156-181.2)	171.6 (158.3-178.9)	
ON HES Score (Hb. EPO. sTfr)						
Morning	173.6 (153.4-192.8)	172.8 (151.8-188.2)	169.9 (160.8-185.4)	168.3 (153.2-186)	167.7 (154.9-185.9)	200.9
Evening	168.8 + (158.6-185.4)	168.3 (155.3-185.4)	170.5 (188.5-159.3)	165.1+* (153.6-183.2)	169.7 (155.2-180.7)	
OFF HRE Score (Hb. %Retic. EPO)						
Morning	80 (63.3-97.7)	76 * (60.1-91.5)	70.4 * (53.5-84.4)	65.9 * (52.6-84)	67 * (47.7-86.7)	113.3
Evening	72.9 + (58.2-88.2)	67.3 +* (52.5-81.9)	67.2 +* (44.6-86.9)	61.2 +* (39.2-89.7)	68.8 * (53-88.6)	
OFF HR Score (Hb. %Retic)						
Morning	82.5 (62.5-99)	79.2 * (65.3-93.3)	75 * (59.3-90)	70.2 * (56.5-85.0)	72.6 * (53.5-86.3)	119.8
Evening	75.4 +(58.5-90.3)	71.5 +* (55-87)	71.3 +* (51.2-86)	67.7 +* (47.7-93.1)	75.3 (60.7-97)	

*+: significant difference to morning value (ANOVA); *: significant difference to day 1 value (ANOVA).*

Hematology Analyzer): total hemoglobin concentration (Hb), hematocrit (Hct), reticulocyte hematocrit (RetHct), percentage macrocytotic red cells (MCV > 120 fL) (%Macro). In the serum samples, iron, ferritin, transferrin, soluble transferrin receptor and serum erythropoietin concentration (EPO) were determined using commercial assay kits. Changes in blood-volume (BV), plasma-volume (PV) and red cell volume (RCV) were estimated.[5] The results were submitted to the calculation models for EPO-abuse: ON and OFF model-scores were determined. ANOVA for repeated measurements was used to calculate differences between cyclists and controls, changes between morning and evening samples and variations from stage one to stage five.

Prior to the race, all variables were within the normal range for all tested subjects. No statistically significant difference was found between cyclists and inactive controls. During the race, Hb, Hct and iron were significantly lower in samples obtained from cyclists after every stage. Hct and Hb decreased significantly from day 1 to day 5 whereas iron, EPO, ferritin, BV and PV increased over the same period. The erythrocyte matrix, reticulocyte variables and RCV remained unchanged. Model scores for EPO-abuse were significantly altered, but never exceeded the threshold values for positive testing. No significant changes were observed in the samples obtained from control subjects. The results are illustrated in Tables 1 and 2.

The currently used variables for indirect detection of rhEPO abuse are partly influenced by physical exercise. These changes (Hb, Hct) might be attributed mainly to shifts in vascular volumes (increased PV) with subsequent hemodilution[4] and mild exercise-induced inflammatory reactions (iron, ferritin).[6] However, an increase in serum EPO levels was observed, possibly indicating an exercise-induced erythropoietic stimulus leading to a subsequent increase of red cell mass. The OFF-model scores were significantly affected by these changes and showed a decrease after prolonged endurance exercise, most likely because of their dependancy on volume-related variables.

Our data demonstrate that several variables used in the indirect detection models of erythropoietic stimulant abuse are altered by an endurance sport event lasting several days. The ON- and OFF-model scores are therefore altered, but the changes are mostly the opposite of those expected to be induced by rhEPO abuse. The relative stability of the model scores in in contrast with the marked variability inherent in some of the single parameter approaches (based only on Hct and Hb) currently used by several sporting federations for blood testing.[7] The sporting community should be encouraged to implement these new algorithms in their anti-doping strategies.

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Isochromosome 17q in patients with myelodysplastic syndromes: six new cases

We report six cases of myelodysplastic syndromes (MDS) with isochromosome i(17q) as the isolated chromosome anomaly. The patients shared several features, such as severe anemia, prominent pseudo-Pelger-Hüet neutrophils, increased micromegakaryocytes and poor clinical prognosis. Our data support the concept that MDS patients with i(17q) should be considered as a new, specific subset.

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The formation of an i(17q) results in loss of the p arm and duplication of the q arm, such that the affected cell has a single copy of 17p, and three copies of 17q. So far, i(17q) as the sole structural abnormality has been reported in about 80 patients with various types of hematologic diseases.[1-8] i(17q) is a common feature of myeloid neoplasms, such as myeloproliferative and myelodysplastic syndromes (MPS and MDS), blast crisis of chronic myeloid leukemia (CML-BC), acute myeloid leukemia (AML), but is also occasionally found in acute lymphoblastic leukemia (ALL). Previous studies on isolated i(17q) have suggested that this aberration is associated with chronic myeloid abnormalities that have a high rate of progression to AML. A new clinical-pathologic entity in which i(17q) is the sole abnormality has also been reported in a mixed myeloproliferative disorder/myelodysplastic syndrome with an

Table 1. Cytogenetic and clinical features of the patients.

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Karyotype {No. of karyotype}	46,XY_i_17_q10_[8]	47, XY , i_17_q10_,+13[9]/44-46, X_i_17_q10_(6),-3, -4_,+13, -15_-16_-18_-19_2_-20_-21_2 _cp6_/45Xi_q10_(5), -Y_5_-8_-10_+13_5_-22_cp5_	44-45, _i_17_q10_(6), +X_-2_-7,+12_3_, -15_3_-16_-21_-22_ cp6_45, XY_-14[1]	46, XX_i_17 _q10_[8]	47, XX_i_17_ q10_[14]/46, XX[1]	46, XY_i_17_ q10_[6]/46, XY[1]
Sex/Age (y)	M/47	M/54	M/38	F/67	F/31	M/48
Peripheral blood						
Hb (g/L)	22	54	62	36	23	70
WBC ($\times 10^9$/L)	2.1	9.8	2.5	3.6	0.7	3.8
Platelets ($\times 10^9$/L)	10	14	179	36	29	154
Blasts (%)	0	4.0	1.0	0	0	0
Pelger-Hüet neutrophils (%)	7	9	14	5	6	11
Nucleated red blood cells	0	4	1	2	0	2
Bone marrow						
Cellularity	normal	increased	increased	increased	normal	increased
Myelopoiesis (%)	70.0	67.5	58.5	64.0	16.0	69.5
Blasts (%)	6.0	10.0	7.5	2.5	1.0	3.0
Erythropoiesis (%)	14.0	25.5	26.5	8.0	66.0	22.5
Dyserythropoiesis	No	yes	yes	yes	yes	yes
Megakaryocytes	increased	increased	increased	normal	increased	increased
Dysmegakaryocytopoiesis*						
Micromegakaryocytes	yes	yes	yes	yes	yes	yes
Large mononuclear	yes	yes	yes	no	yes	yes
Multiple small nuclei	yes	yes	yes	yes	no	yes
FAB type	RAEB	RAEB	RAEB	RA	RA	RA
Survival (months)	*	15	18	*	6+	3°

**Demonstrated by immunocytochemistry; *:lost from follow-up; °:died from myocardial infarction. RA: refractory anemia; RAEB: refractory anemia with excess blasts.*