Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (11)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

Sport und Buch Strauß, Köln, 2003

YANG SHENG, WU MOUTIAN, XIE MINHAO, DENG JING, YANG ZHIYONG, FANG ZILONG, GUO JIANJUN, ZHANG CHANGJIU:
Statistical Results of Routine Blood Test for Five Parameters Related to Sydney On-Model
In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (11). Sport und Buch Strauß, Köln, (2003) 355-363

Yang Sheng, Wu Moutian*, Xie Minhao[1], Deng Jing, Yang Zhiyong, Fang Zilong, Guo Jianjun, Zhang Changjiu

Statistical Results of Routine Blood Test for Five Parameters Related to Sydney On-Model

China Doping Control Center, National Research Institute of Sports Medicine, Beijing 100029, China

1.Beijing University of Physical Education, Beijing 100084, China

Abstract

Blood testing has been performed in China since the year 1998. In the earlier stage, only Haematocrit (Hct) has been tested with the UCI criteria as a health factor. After the On-model was published in the year 2000, most routine blood tests were based on combination of UCI criteria and the On-model. In this paper, results of blood tests for three years were statistically analyzed. Statistical distribution of the results of the On-model related parameters were reviewed and discussed. Non-parametric method was implemented to investigate the normal ranges of these measured blood parameters.

Preface

Although the International Olympic Committee (IOC) has banned the use of recombinant human erythropoietin (rhEPO) since 1989, the methods for detecting rhEPO were not formulated until in the year 2000 [1]. In the earlier stage, only Hct has been tested using UCI criteria for the athletic health. In 2000, with the co-operation of the Sydney IOC accredited laboratory, 24 trials were carried out in China, which together with studies in Australia allowed various models for blood EPO (such as the ON model) to be published. Since then, our routine blood tests were based on the combination of the UCI criteria and the ON-model. Results of blood tests for three years, 2000 with 141 cases, 2001 with 425 cases and 2002 with 228 cases, were statistically analyzed. The aim of this paper is focused on the reference ranges of the parameters for Chinese athletes.

Subjects

There were in total 794 Chinese national level athletes (369 males, 425 females) selected as the subjects for blood tests before sports competition in 3 years (including the 2000 Sydney Olympic Games, the 9th Chinese National Sports Games, the 2002 Busan Asian Sports Games). All the subjects were Chinese. They were selected by the testing section of the Chinese Olympic Committee Anti-Doping Commission (COCADC).

Blood Samples Collection and Measurement

Blood was drawn from the veins of the forearm by qualified medical staff in the morning before breakfast. Samples were drawn into one 8 mL serum seperation Vacuette tube with clot activator (Greiner Labortechnik, Frikenhaussen, Germany), and one 2 mL K_3EDTA Vacuette tube. In China, all the samples were shipped to our laboratory within 8 hours by doping control officers (DCOs). Whole blood samples were measured for Hct, Macro%, and RetHct using the Advia-120 analyzer (Bayer, USA). Soluble transferrin receptor (sTfR) was tested using an ELISA kit (R&D systems, USA). Serum EPO was measured using the Immulite Chemiluminescent instrument (DPC, USA).

Statistics

Independent sample t-test (2-tailed) was performed to evaluate the annual variance. Gaussian distribution was tested using Kolmogorov-Smirnov test as well as coefficients of skewness and kurtosis. Analyses were conducted using SPSS for Windows software with release version 10.0.1 (SPSS Inc., USA). The non-parametic method were used to develop the 95% reference ranges.[2]

Results and Statistical Tests

1. The results of female (Table 1), male (Table 2) and Total (Table 3) of Hct, Macro%,

RetHct, EPO and sTfR were given by means and standard deviations.

Table 1 Annual Female parameters expressed in Means and SDs.

Year	Hct (L/L)	Macro%*	RetHct (L/L)	EPO (mIU/ml)	sTfR (nmol/L)
2000, n=106, 105*	$0.40\pm0.028^{\#}$	$2.25\pm1.61^{\#}$	$6.68\times10^{-3}\pm4.09\times10^{-3}$	9.15 ± 4.11	18.53 ± 5.31
2001, n=209	$0.41\pm0.027^{\$}$	$1.05\pm1.03^{\&}$	$6.21\times10^{-3}\pm2.33\times10^{-3\$}$	$9.16\pm5.35^{\&}$	$19.70\pm5.51^{\$}$
2002, n=110	$0.39\pm0.024^{\psi}$	$0.75\pm1.17^{\psi}$	$8.46\times10^{-3}\pm2.70\times10^{-3\psi}$	$10.49\pm4.06^{\xi}$	$22.57\pm5.08^{\psi}$

Symbol * indicated that there was one data missing of Macro%; Symbol # represented $p<0.01$, using data of the year 2000 compared with the year 2001; Symbol $ represented $p<0.01$, Symbol & represented $p<0.05$, using data of the year 2001 compared with the year 2002; Symbol ψ represented $p<0.01$, ξ represented $p<0.05$, using data of the year 2000 compared with the year 2002.

Table 2 Annual Male parameters expressed in Means and SDs.

Year	Hct (L/L)	Macro%*	RetHct (L/L)	Epo (mIU/mL)	sTfR (nmol/L)
2000, n=35	0.45 ± 0.026	$1.46\pm1.12^{\#}$	$6.27\times10^{-3}\pm1.85\times10^{-3@}$	9.24 ± 3.51	18.16 ± 4.61
2001, n=216	$0.44\pm0.027^{\$}$	0.66 ± 0.72	$7.19\times10^{-3}\pm2.38\times10^{-3\$}$	$8.87\pm3.37^{\$}$	$19.69\pm4.35^{\$}$
2002, n=118	0.44 ± 0.023	$0.61\pm0.92^{\psi}$	$8.64\times10^{-3}\pm2.69\times10^{-3\psi}$	10.00 ± 4.26	$22.83\pm5.52^{\psi}$

Symbol * indicated that there was one data missing of Macro%; Symbol # represented $p<0.01$, Symbol @ represented $p<0.05$, using data of the year 2000 compared with the year 2001; Symbol $ represented $p<0.01$, using data of the year 2001 compared with the year 2002; Symbol ψ represented $p<0.01$, using data of the year 2000 compared with the year 2002.

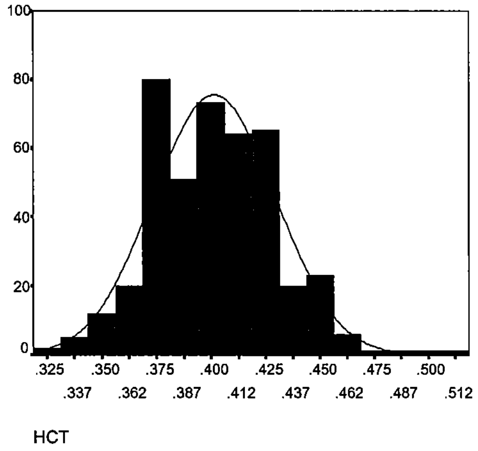
Table 3. Total Data of Female and Male parameters expressed in Means and SDs

Gender	Hct (L/L)	Macro%*	RetHct (L/L)	Epo (mIU/mL)	sTfR (nmol/L)
F N=425, 424*	0.40 ± 0.028	1.27 ± 1.36	$6.91\times10^{-3}\pm3.09\times10^{-3}$	9.5064 ± 4.78	20.15 ± 5.55
M N=369	0.44 ± 0.026	0.72 ± 0.86	$7.56\times10^{-3}\pm2.56\times10^{-3}$	9.27 ± 3.72	20.55 ± 5.03

Symbol * indicated that there was one data missing of Macro%

2. Histogram and Normal distribution of the On-model related parameters were provided.

(1). Female Hct



Skewness=0.236 Std.Error=0.118,

Kurtosis=0.383 Std.Error=0.118,

Kolmogorov-SmirnovTest Results:

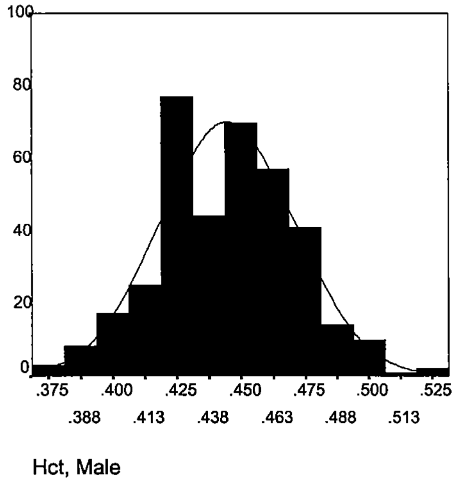
$Dmax=max(D^+,D^-)=max(0.079,-0.058)$,

$D_{0.01}=1.031/Sqr(425)=0.050$,

$Dmax> D_{0.01}$, $P<0.01$,

The hypothesis that Hct values fit a Gaussian distribution is thus rejected.

(2). Male Hct



Skewness=0.029 Std.Error=0.127,

Kurtosis=0.031 Std.Error=0.127,

Kolmogorov-SmirnovTest Results:

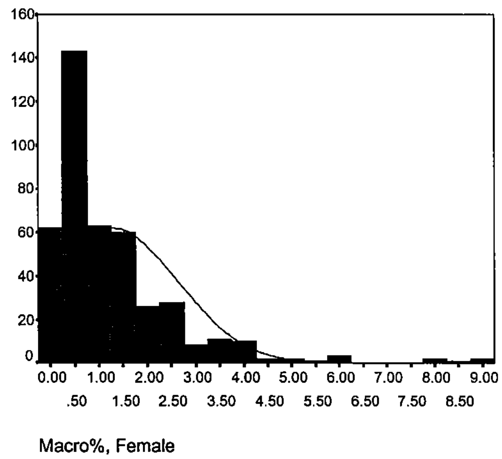
$Dmax=max(D^+,D^-)=max(0.062,-0.083)$,

$D_{0.01}=1.031/Sqr(369)=0.054$,

$Dmax> D_{0.01}$, $P<0.01$,

The hypothesis that Hct values fit a Gaussian distribution is thus rejected.

(3). Femal Macro%



Skewness=2.533 Std.Error=0.119,

Kurtosis=8.934 Std.Error=0.119,

Kolmogorov-SmirnovTest Results:

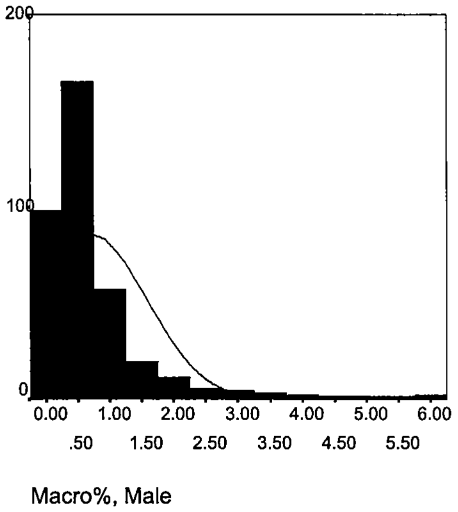
$Dmax=max(D^+,D^-)=max(0.153,-0.183)$,

$D_{0.01}=1.031/Sqr(424)=0.050$,

$Dmax> D_{0.01}$, $P<0.01$,

The hypothesis that Macro% values fit a Gaussian distribution is thus rejected.

(4). Male Macro%



Skewness=3.244, Std.Error=0.127,

Kurtosis=13.161 Std.Error=0.253,

Kolmogorov-SmirnovTest Results:

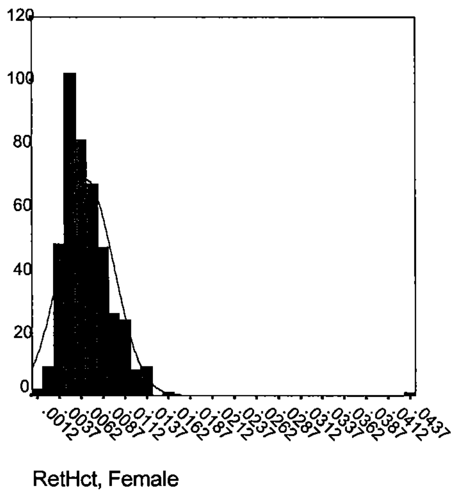
Dmax=max(D^+,D^-)=max(0.223,-0.224),

$D_{0.01}$=1.031/Sqr(369)=0.054,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that Macro% values fit a Gaussian distribution is thus rejected.

(5). Female RetHct



Skewness=4.431,Std.Error=0.118,

Kurtosis=0.236, Std.Error=0.118,

Kolmogorov-SmirnovTest esults:

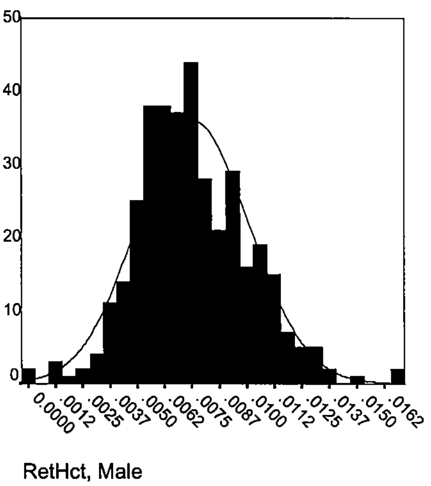
Dmax=max(D^+,D^-)=max(0.102,-0.087),

$D_{0.01}$=1.031/Sqr(425)=0.050,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that RetHct values fit a Gaussian distribution is thus rejected.

(6). Male RetHct



Skewness=0.319,Std.Error=0.127,

Kurtosis=0.658, Std.Error=0.253,

Kolmogorov-SmirnovTest esults:

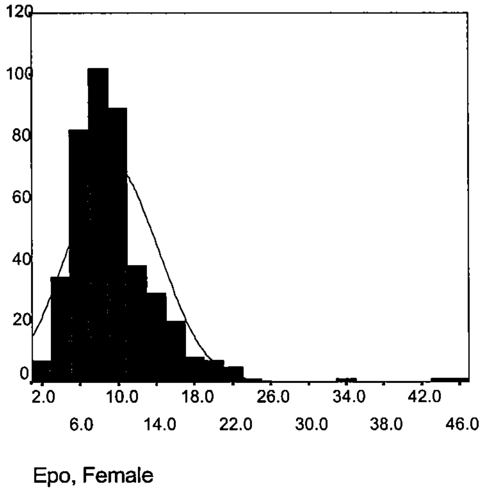
Dmax=max(D^+,D^-)=max(0.058,-0.032),

$D_{0.01}$=1.031/Sqr(369)=0.054,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that RetHct values fit a Gaussian distribution is thus rejected.

(7). Female Epo



Skewness=2.673,Std.Error=0.118,

Kurtosis=0.236, Std.Error=0.118,

Kolmogorov-SmirnovTest esults:

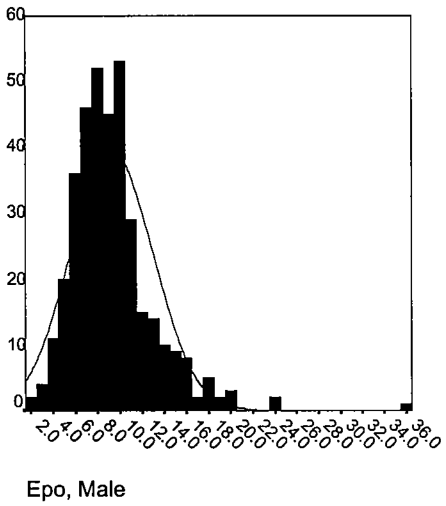
Dmax=max(D^+,D^-)=max(0.125,-0.089),

$D_{0.01}$=1.031/Sqr(425)=0.050,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that Epo values fit a Gaussian distribution is thus rejected.

(8). Male Epo



Skewness=1.770,Std.Error=0.127,

Kurtosis=7.500, Std.Error=0.253,

Kolmogorov-SmirnovTest esults:

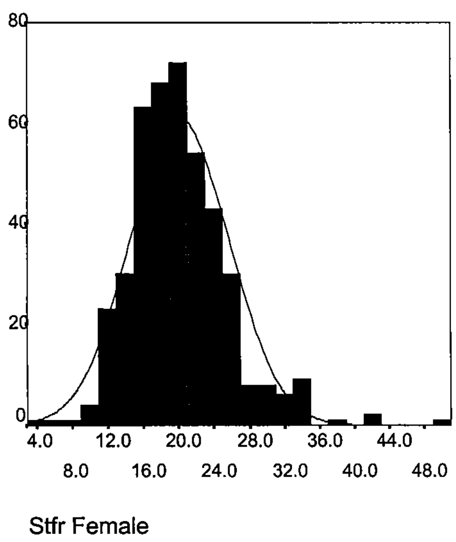
Dmax=max(D^+,D^-)=max(0.113,-0.059),

$D_{0.01}$=1.031/Sqr(369)=0.054,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that Epo values fit a Gaussian distribution is thus rejected.

(9). Female sTfR



Skewness=1.011,Std.Error=0.118,

Kurtosis=0.236, Std.Error=0.118,

Kolmogorov-SmirnovTest esults:

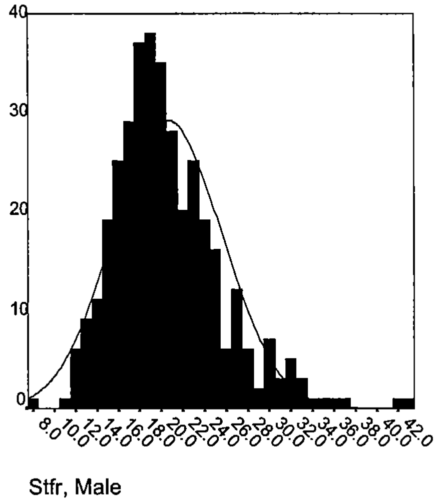
Dmax=max(D^+,D^-)=max(0.065,-0.041),

$D_{0.01}$=1.031/Sqr(425)=0.050,

Dmax> $D_{0.01}$, P<0.01,

The hypothesis that Stfr values fit a Gaussian distribution is thus rejected.

(10). Male sTfR



Skewness=1.021,Std.Error=0.127,

Kurtosis=1.992, Std.Error=0.253,

Kolmogorov-SmirnovTest esults:

$Dmax=max(D^+,D^-)=max(0.087,-0.046)$,

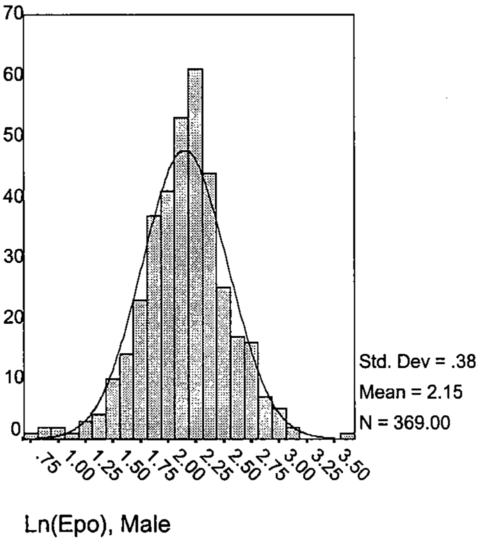
$D_{0.01}=1.031/Sqr(369)=0.054$,

$Dmax> D_{0.01}$, $P<0.01$,

The hypothesis that Stfr values fit a Gaussian distribution is thus rejected.

Since all the parameters' distribution did not fit a Gaussian distribution, we used natural logarithms to transform the parameters.[3] However, even after transformed, the variable's distribution did not fit Gaussian distribution. The following was an example.

Example: Ln(EPO)



After transformed:

(I) Kolmogorov-SmirnovTest esults:

$Dmax=max(D^+,D^-)=max(0.049,-0.044)$,

$D_{0.01}=1.031/Sqr(369)=0.054$,

$D_{0.05}=0.886/Sqr(369)=0.046$

$D_{0.05}<Dmax< D_{0.01}$

$0.01<P<0.05$,

The hypothesis that Stfr values fit a Gaussian distribution is thus rejected at level 0.05.

(II) Skewness=-0.230,Std.Error=0.127,

Kurtosis=1.206, Std.Error=0.253,

Sk>2.6,also rejected the Gaussian distribution.

Therefore, we used non-parametric methods to develop the 95% reference ranges, results see Table 4 and 5.

Table 4. Reference ranges and confidence intervals.

Female data of the year 2000, 2001and 2002.

Female Parameter	2.5% Percentile	90% confid. Lower	Upper	97.5% Percentile	90% Confid. Lower	Upper	N
Hct(L/L)	0.35	0.34	0.35	0.45	0.45	0.46	425
Macro%	0.10	0.00	0.10	5.10	4.10	6.20	424
RetHct (L/L)	3.13×10^{-3}	2.45×10^{-3}	3.24×10^{-3}	1.32×10^{-2}	1.21×10^{-2}	1.34×10^{-2}	425
EPO (mIU/mL)	3.40	2.80	4.05	20.70	18.65	22.65	425
sTfR (nmol/L)	11.22	10.54	12.16	33.38	32.16	34.73	425

Table 5. Reference ranges and confidence intervals.

Male data of the year 2000, 2001and 2002.

Male Parameter	2.5% Percentile	90% confid. Lower	Upper	97.5% Percentile	90% Confid. Lower	Upper	N
Hct(L/L)	0.39	0.39	0.40	0.50	0.49	0.50	369
Macro%	0.10	0.00	0.10	3.60	2.70	4.40	369
RetHct (L/L)	2.86×10^{-3}	1.27×10^{-3}	3.74×10^{-3}	1.29×10^{-2}	1.23×10^{-2}	1.39×10^{-2}	369
EPO (mIU/mL)	3.70	2.75	4.40	18.40	17.20	19.80	369
sTfR (nmol/L)	12.57	12.16	13.24	32.70	31.22	35.00	369

Discussion

(1) The possible reasons of non-Gaussian distribution of haematological parameters

The exact reason for such a non-Gaussian distribution is not clear, yet it is also noted that the T/E ratio of the athletes is non-Gaussian distribution. From the histograms, we noticed that the skewness was mainly caused by the highest outliers. These outliers may be influenced by many factors including physiological or pathological conditions such as exercise[4], environmental factors[5] and anemia. The reasons also may be caused by non-randomly sampling.

(2) The Chinese athletes' reference ranges compared with that of foreign elite athletes

The hematological parameters' reference ranges of Chinese athletes were consistent with those reported by Australian researchers, with the ranges of female Hct (%) 34.3-45.0, RetHct (%) 0.30-1.08, Macro% 0.0-1.9 (after log-transformed), EPO (mU/mL) 4.4-23.5, sTfr (mg/L) 0.76-2.02 (after square root of the value) respectively; and with the ranges of male Hct (%) 38.8-49.6, RetHct (%) 0.31-1.12, Macro% 0.0-1.1 (after log-transformed), EPO (mU/mL) 4.6-18.6, sTfR (mg/L) 0.81-1.87 (after square root of the value) respectively.[6]

(3) Other comments

When calculating the ON-model, the unit of sTfR must be mg/L, since the results were expressed by nmol/L using the R&D ELISA kit, therefore we transformed the unit to match the Dade Behring sTfR[3]. The data presented in this paper were selected using the cut-offs (ON-model <2.5 male, ON-model <2.4 female). In our routine blood tests, once the result exceeded the cut-offs, urine sample should be analyzed to confirm the rhEPO.

---

1. Lasne F, de Ceaurriz J. Recombinant erythropoietin in urine. Nature 2000;405:635.
2. H.E. Solberg, Approved Recommendation (1987) on the Theory of Reference Values, J. Chem. Clin. Biochem. 1987;Vol.25:645-656.
3. Robin Parisotto, Moutian Wu, Michael J. Ashenden, et al. Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis, Haematologica, 2001;86;128-137.
4. Schmidt W, Biermann B, Winchenbach P, Lison S, Boning D, How valid is the determination of hematocrit values to detect blood manipulation? Int J Sports Med 2000;21:133-138.
5. Reynafarje R. Lozano R, Valdivieso J. The polycythemia of high altitudes: iron metabolism and related aspects. Blood 1959;14:433-455.
6. Ken Sharpe, Will Hopkins, Kerrv R. Emslie, Chris Howe, Graham J.Trout, et al. Development of reference ranges in elite athletes for markers of altered erythropoiesis, haematologica, 2002;87:1248-1257.