Hematologic passport for athletes competing in endurance sports: a feasibility study

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Background and Objectives. Strategies based on the use of upper thresholds of hemoglobin or hematocrit to detect blood doping in endurance sports have essentially failed to deter this malpractice. With the aim of establishing a more effective strategy, we analyzed the biological variations of hematologic parameters in professional athletes and investigated the possibility of defining subject-specific reference ranges that could distinguish between physiologic and abnormal variability.

Design and Methods. Hemoglobin concentration, hematocrit, reticulocyte count, serum ferritin and soluble transferrin receptor levels were sequentially evaluated in 923 professional football players. Using the analysis of variance we tested the effect of age, ethnicity, exercise modalities and training phases on hematologic parameters and then estimated components of variation. The significance of the difference between two measures was obtained from the distribution of the within-subject variance (the so-called *reference change*). Subject-specific reference ranges were centered around the individual mean value with dispersion based on the 95th percentile of the coefficient of variation distribution.

Results. A total of 2,506 hematologic determinations were made. Exercise modalities were found to have important effects on hematologic parameters. Hemoglobin and hematocrit values were higher at the beginning of the competition season, and then declined in well-trained athletes. Aerobic exercise was clearly associated with lower values, suggesting that marginally low hemoglobin and hematocrit values should physiologically be found in endurance sports. At least five determinations were required to define subject-specific reference ranges reliably. Considering athletes showing normal indices of red cell production (i.e., reticulocyte count and soluble transferrin receptor), the 95th percentile of the coefficient of variation distribution was lower than 5% for both hemoglobin and hematocrit. Increases exceeding 10% in these latter parameters should to be considered abnormal. Score systems capable of efficiently detecting non-physiologic increases in red cell production were developed.

Interpretation and Conclusions. Using proper sequential determinations of hematologic variables subject-specific reference ranges can be defined for hemoglobin and hematocrit. Thus, the hematologic passport is feasible and might be employed to exclude athletes with non-physiologic increases in hemoglobin and hematocrit from competitions. The hematologic passport should be used within a global strategy to deter blood doping.

Key words: blood doping, anemia, erythrocytosis, erythropoietin, iron.

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Blood doping is an illicit practice aimed at increasing tissue oxygen delivery and aerobic performance, mainly but not exclusively in endurance sports. Over the decades, various approaches have been used, including homologous[1] and autologous[2] red blood cell transfusion, recombinant human erythropoietin (rHuEpo) or related erythropoietic stimulants,[3] rHuEpo-enhanced autologous transfusion,[4] and hemoglobin-based oxygen carriers (HBOCs).[3] Although blood doping is particularly exploited in endurance disciplines (e.g. cycling, track and field events, and cross-country skiing), it can confer significant advantage in recovery after intermittent efforts[3] and, therefore, other disciplines must also be considered at risk of this practice.[5]

Blood doping is not only unethical but may represent a serious risk to the athlete's health. For instance, epoetin-induced autoimmune pure red cell aplasia is a matter of great concern.[6] Athletes who use epoetin are clearly procuring it illegally, so that non-optimal conservation of the drug is more likely. Should this be a factor in inducing drug antigenicity and antibody formation, the risk of pure red cell aplasia would be higher in this group of users.

Detection of these unacceptable practices is very difficult. Fixing arbitrary limits in critical hematologic parameters in order to evaluate a person's eligibility to compete is neither a very specific nor a very sensitive strategy.[7] The wide biological variance increases the risk of false positive cases, while intentional expansion of plasma volume allows dishonest athletes to elude the upper limits.

A group of Australian researchers developed a model including indices of accelerated erythropoiesis[8] and defined algorithms capable of detecting the abuse of rHuEpo either during the administration phase (ON-model) or during the wash-out phase (OFF-model).[9,10] This approach was employed during the Sidney Olympics and has been recently refined.[11,12]

An alternative approach involves sequential evaluation of hematologic parameters to define an individual's hematologic profile, i.e., that athlete's hematologic passport. Combining the hematologic passport with the Australian approach might be an effective strategy to detect current blood doping and deter future abuse.

In this work we analyzed sequential measurements of hematologic variables in professional football players with the aim of quantifying the components of the biological variation and defining subject-specific reference ranges for the hematologic parameters of interest.

Design and Methods

Data were obtained from professional football players enrolled within the campaign *Io non rischio la salute! (I take care of my health!)* promoted by the Italian National Olympic Committee (Comitato Olimpico Nazionale Italiano, CONI) and the Italian Football Federation (Federazione Italiana Gioco Calcio, FIGC).[13] Sportsmen involved in this campaign had regular hematologic investigations including hemoglobin (Hb), hematocrit (Hct), reticulocyte percentage (Ret%) and absolute count, serum ferritin (Ferr), soluble transferrin receptor (sTfR) measurements, collected in the years 1999 and 2000. These determinations were performed by laboratories with quality control programs to reduce analytical errors. All the atheletes participated voluntarily after giving their informed consent. Data obtained from players of top football teams, monitored over a 3-year period from September 1999 to July 2002, were also included in the analysis.

Statistical analysis

In order to evaluate factors affecting hemoglobin, hematocrit, reticulocyte count, serum ferritin, and soluble transferrin receptor levels, a main effects ANOVA was performed. All the variables of interest were log-transformed to normalize their distribution. The predictive factors examined were age (<25, 25-30, ≥30 years), ethnic group (Caucasian, South-American, African) and role (goalkeeper, defender, midfielder, forward).

Analysis of variance was carried out to evaluate the long-term effect of training on hematologic and erythropoietic indices. The sporting period was categorized into low (from July to September), intermediate (from October to January) and high season (from February to May).

In order to evaluate the between-subject and within-subject components of variance, a nested analysis of variance with unequal numbers of observations per subject[14] was performed for hemoglobin, hematocrit, reticulocyte percentage and absolute count, serum ferritin and soluble transferrin receptor. An *F*-test of the ratio of the mean square between-subjects to the mean square within-subjects tested the null hypothesis that the two components of variance were equal.

Statistical model of intra-individual variation in blood constituents

A strictly homeostatic model was assumed in order to estimate the individual variation over time of the hematologic parameters of interest. For each individual *i*, all the measurements were assumed to be random fluctuations around a mean value: $m_{it} = \mu i \pm e_{it}$, where m_{it} denotes the actual value of the constituent in individual *i* at time *t*, μi represents the homeostatic individual mean value, and e_{it} the deviation of the constituent value at time t from this set-point.[15,16]

Reference change

The significance of the difference between two consecutive measurements in an individual series was assessed using a method described by Harris and Yasaka.[17] This allows population *reference changes* to be estimated based on the distribution of the within-subject variances. The main assumptions of this model are the normality of differences between two consecutive measurements (for each individual separately) and the log-normality of the within-subject variance distribution. The small number of subjects who had a sufficient number of consecutive measurements prevented the normality of the differences from being tested, so this normality was assumed *a priori*. A Kolgomorov-Smirnov test was performed to assess the assumption of normality of the log-variances. The population variance of the within-subject variances was estimated as previously reported by Harris and Browne.[18] Finally, for each critical hematologic parameter, the cumulative probability that a given change between two consecutive measures would be statistically significant at the 5% level was calculated.[17,18] The model was then tested on the first four measures of the individual series.

Subject-specific reference range

Subject-specific reference ranges were calculated considering the difference (in percentage) between a new measurement (m_i) and the mean of the previous values of an individual series. The within-subject coefficient of variation ($CV_i=SD_i/\mu_i$) was used as a measure of dispersion. The distribution of CV_i was calculated in athletes for whom four or more repeated measures were available and the 95th percentile was used to define the width of fluctuation of hemoglobin and hematocrit around the homeostatic set-point. The new measurement m_i falls within the subject-specific reference range (SR) if $|(m_i-\mu_i)/\mu_i| \leq 2\ CV95^{th}$. The individual range can be expressed as $R_i = \mu_i*(1\pm 2CV^{95th})$. The width of this reference range depends on the individual mean and, more precisely, tends to become larger as the mean increases.

Score system

We combined the evaluation of each hematologic parameter of interest to obtain a score based on the number of parameters falling out of range. Since hemoglobin, hematocrit and reticulocyte count can be quickly determined at the same time with a new generation cell counter, we defined a three-variable score by simply counting the number of out-of-range parameters. Once the results of serum ferritin and soluble transferrin receptor

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assays become available, a five-variable score can be obtained from assigning a weight of 0.5 to out-of-range values of the two additional parameters. For both scores, a threshold value of two was chosen to indicate a strong alteration of the hematologic parameters.

We tested the specificity of the proposed scores by comparing every determination of each athlete to the individual range based on the athlete's previous determinations and then calculating the three-variable and the five-variable score.

The sensitivity of the scores was defined on the data from a double-blind, randomized trial performed by the Australian Institute of Sport,[9] evaluating the effect of rHuEpo administration on a group of voluntary, recreational athletes.

Results

A total of 2,506 determinations were obtained from 923 professional football players from 39 teams of the FIGC. The mean number of determinations per subject was 2.71, the range was from 1 to 16. The median age of these sportsmen was 27 years (range 16 to 38 years); 746 athletes were Caucasian, 50 South-American, 18 African, and 1 was Asian, while in 108 subjects the ethnicity was unknown. Considering the footballers' role, 91 were goalkeepers, 264 were defenders, 271 midfielders 173 were forwards, while in 124 cases it was unknown.

Hemoglobin and hematocrit

Thirty athletes (3.25%) showed a constant microcytosis (MCV < 80 fL) without any evidence of iron deficiency, likely due to thalassemic trait since this prevalence is very close to the rate of heterozygous β-thalassemia in the general population.

Mean values of hemoglobin and hematocrit of normal subjects were 14.8 g/dL (range 12.1-17.8 g/dL) and 43.7% (range 34.6-52.6%), respectively (Table 1). Six out of 923 subjects (0.65%) had a hemoglobin above 17 g/dL on at least one determination; no other unusual hematologic results were observed in this group except for in one subject who had a borderline absolute reticulocyte count (104×10^9/L) and another who had a high serum ferritin value (289 ng/mL). A hematocrit value above 50% was observed on one or more occasions in 11/923 (1.19%) athletes, four of whom had a hemoglobin level above 17 g/dL; one subject, mentioned above, had an high absolute reticulocyte count.

Reticulocyte count

The mean percentage of reticulocytes was 1.01%, the range was from 0.2% to 3.9%, with a mean absolute count of 52.6×10^9/L ($13.4\text{-}117.0\times10^9$/L). Thirty-six subjects had a reticulocyte percentage below 0.4%, with hemoglobin values ranging between 13.1 g/dL and 15.7 g/dL, while 5 subjects had an absolute reticulocyte count below 20×10^9/L. Thirty-eight athletes showed a reticulocyte percentage above 2%, although hemoglobin and hematocrit were within the normal population range. Forty-nine athletes showed an absolute reticulocyte count above 100×10^9/L on at least one determination, with normal hemoglobin and hematocrit values in all but one subject, who had both parameters above the range for the population studies.

Table 1. Descriptive statistics of hematologic parameters of the normal population of football players.

Variable	*Mean*	*Minimun*	*Maximum*	*Std. Dev.*
Hb (g/dL)	14.82	12.1	17.8	0.87
Hct (%)	43.68	34.6	52.6	2.69
RBC ($\times10^{12}$/L)	4.90	4.00	6.75	0.32
MCV (fL)	89.25	80	102.1	3.65
MCH (pg)	89.25	80.00	102.10	3.65
MCHC (g/dL)	33.95	28.8	39	1.10
RDW (CV%)	12.92	9.60	21.4	0.93
Reticulocyte (%)	1.01	0.20	3.90	0.47
Reticulocyte ($\times10^9$/L)	52.36	13.4	117.00	18.87
WBC ($\times10^9$/L)	6.03	3.10	12.24	1.25
PLT ($\times10^9$/L)	210.75	92.00	419.00	41.50
MPV (fL)	9.35	3.70	15.50	1.49
Iron (µg/dL)	96.42	11.00	231.00	32.51
Ferritin (ng/mL)	84.19	14.90	249.00	49.75
Transferrin (mg/dL)	237.25	122.00	386.00	40.01
Soluble TRF receptor (mg/L)	1.25	0.50	3.20	0.26
Bilirubin (mg/dL)	0.86	0.24	4.37	0.45
LDH (IU/L)	346.06	89.00	751.00	92.77

Iron status parameters

Fifteen players demonstrated iron deficiency in at least one analysis, while 44 subjects (4.8%) showed high ferritin values. Considering only the 34 athletes within this latter group who had two or more determinations, 25 showed constant iron overload (2.7% of the global population) with blood count and reticulocyte values within the normal ranges. The remaining 9 subjects showed occasional iron

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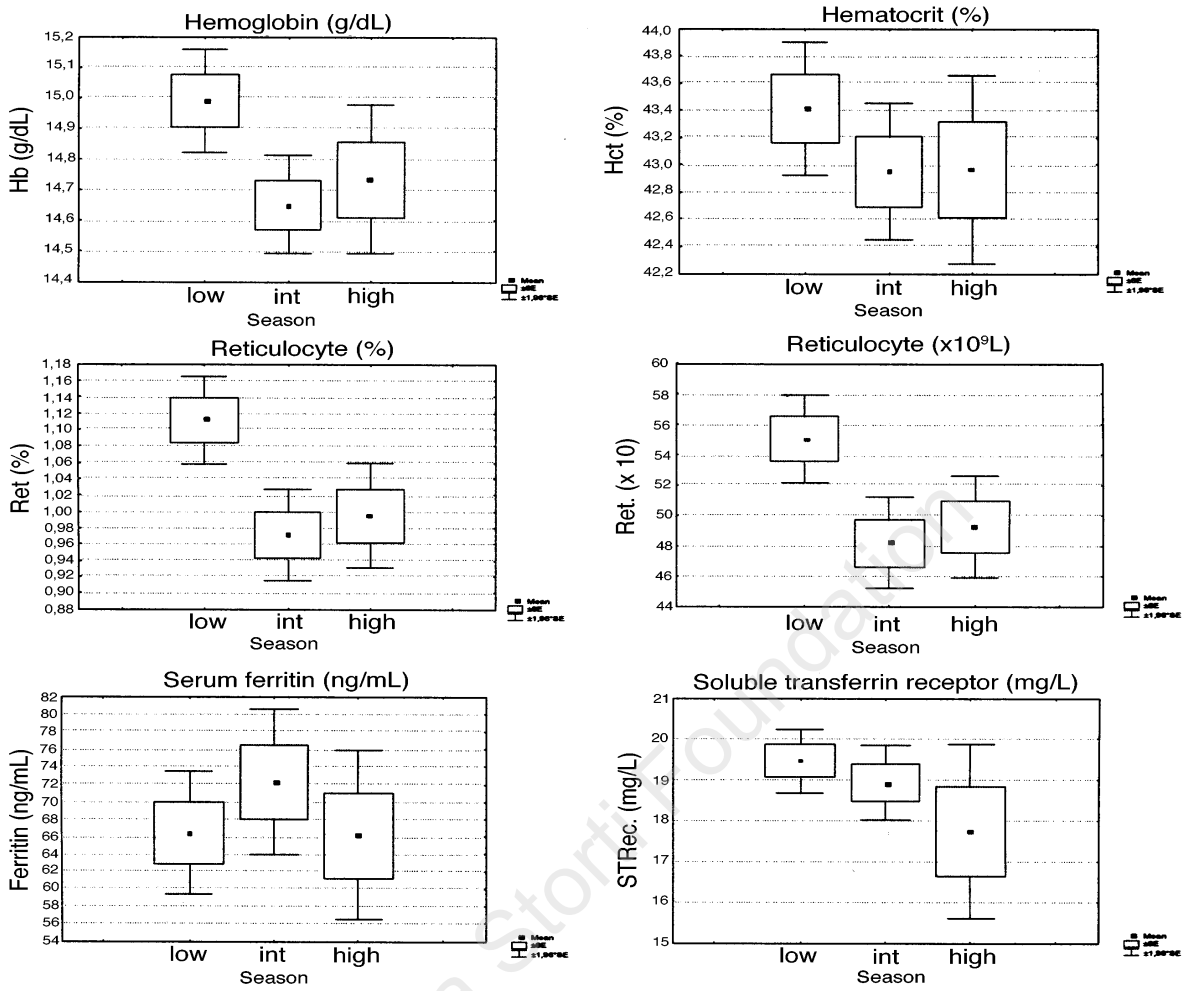

Figure 1. Variation of critical hematologic parameters in different phases of the sport season.

overload, with outlier ferritin values ranging from 256 to 414 ng/mL.

As far as soluble transferrin receptor is concerned, 6.2% of subjects had values above the upper normal limit. Two of them had iron deficiency and 3 had severe microcytosis and likely a thalassemic trait. No unusual hematologic values were observed in the remaining subjects except in one athlete who had iron overload. Only two subjects had a hemoglobin concentration exceeding16 g/dL.

Analysis of variance

A main effect analysis of variance was performed to evaluate the influence of ethnic group (Caucasian, South-American, African), age (<25, 25-30, ≥30 years) and role (goal-keeper, defender, mid fielder, forward) on hemoglobin, hematocrit, reticulocyte percentage and absolute count, serum ferritin, and soluble transferrin receptor levels. The analysis was limited to the 436 athletes who had measures, including blood count, associated with normal erythropoietic indices (total number of determinations: 793). A log transformation was used to normalize the distribution of all the variables of interest.

As far as ethnicity is concerned, the analysis showed significant differences (p<0.001) in sTfR levels between ethnic groups, the African athletes having the highest values (1.65 mg/L vs 1.25 mg/L in Caucasians and 1.24 mg/L in South-Americans). A borderline significance was also observed for hematocrit (p=0.05); in this case African subjects had the lowest values. Considering the age effect, the analysis showed that mean values of ferritin increased significantly in 25-30 and >30 year old athletes (69 ng/mL in subjects <25 years old, 84 ng/mL 25-30 years, 83 ng/ml ≥ 30 years; p<0.001).

The role of the footballers was also considered and found to have a significant influence on mean values of hemoglobin and hematocrit (p<0.05).

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Table 2. Nested analysis of variance of critical hematologic parameters.

Source of variance	*Hb (g/dL)²*	*Hct (%)²*	*Ret (%)²*	*Ret (×10⁹/L)²*	*Ferritin (ng/mL)²*	*STfR (mg/L)²*
Between-subject variance	1.912	18.794	0.210	566.191	4433.160	0.108
Within-subject variance	0.227	2.020	0.047	120.605	278.570	0.027
Variance of the true means of subject*	0.573	5.716	0.056	153.633	1414.340	0.033
F ratio (between/within)	8.389	9.300	4,394	4.694	15.913	3.876
p value	2.747^{-103}	1.172^{-112}	3.190^{-51}	1.231^{-55}	5.935^{-166}	3.876^{-25}

Table 3. Distribution of within-subject coefficient of variation in athletes having four or more repeated measures.

Parameters	*Median*	*Coefficient of variation* *Minimum*	*Maximum*	*5th Percentile*	*95th Percentile*
Hemoglobin (g/dL)	2.892	0.687	4.775	0.980	4.444
Hematocrit (%)	3.107	1.016	4.552	1.192	4.451
Reticulocyte (%)	16.388	4.022	38.758	6.292	33.812
Reticulocyte (×10⁹/L)	17.469	4.510	37.481	6.518	34.094
Ferritin (ng/mL)	23.511	13.608	58.508	14.126	45.089
Soluble TfR (mg/L)	7.016	4.802	19.443	4.911	15.833

Midfielders, who perform the greatest endurance effort, had the lowest levels of hemoglobin (14.5 g/dL vs 14.9 g/dL in goalkeepers; 14.7 g/dL in defenders and 14.8 g/dL in forwards).

To evaluate the long-term effect of exercise, the sporting season was categorized into three periods: July-September (low or *training* phase), October-January (intermediate phase) and February-May (high phase) and an analysis of variance was performed on 27 athletes for whom repeated measures were available over three years. The test showed significantly higher levels of hemoglobin, reticulocyte percentage and absolute count, and soluble transferrin receptor level in the *training* period than in October-January ($p<0.01$). An opposite trend was demonstrated for serum ferritin, which showed its lowest concentration in the first period of the sporting season ($p<0.01$) (Figure 1). Non-significant increases in hemoglobin, hematocrit, and reticulocytes were observed in the February-May period, when a marked decrease of serum ferritin was recorded. No significant interactions between period of the season and role was detected.

In order to evaluate the source of variance in hemoglobin, hematocrit, reticulocyte percentage and absolute count, serum ferritin and sTfR values of our population, a nested analysis of variance with unequal numbers of observation per subject was performed on athletes for whom three or more determinations were available. The results are shown in Table 2.

An *F*-test of the mean square between-subjects to the mean square within-subjects variance, tested the hypothesis that the two sources of variance were comparable. The ratio was highly significant for all the hematologic parameters analyzed ($p<0.001$), demonstrating that the between-subject variance is the major source of variation.

Reference change

In order to assess the statistical significance of a difference between two consecutive measurements in an individual series in the selected population, a *reference change* method based on the distribution of within-subject variances was applied. The cumulative probabilities that a change in the critical hematologic parameters would be significant at the 5% level are plotted in Figure 2.

The method was then tested on the first two measurements of each individual series: the percentage of differences above the reference change for the six critical variables analyzed ranged from 0 to 1.3%. Comparable results were obtained considering the successive pairs of measurements.

Subject-specific reference range

A subject-specific reference range was defined using the mean value of the individual series as the homeostatic set-point and the within-subject coefficient of variation as an estimate of the individual variability. Considering that there was a significant correlation between mean values and standard deviation for all the hematologic parameters but hemoglobin, a CV-based reference range might be preferable to a standard deviation-based model, resulting in narrower ranges in sub-

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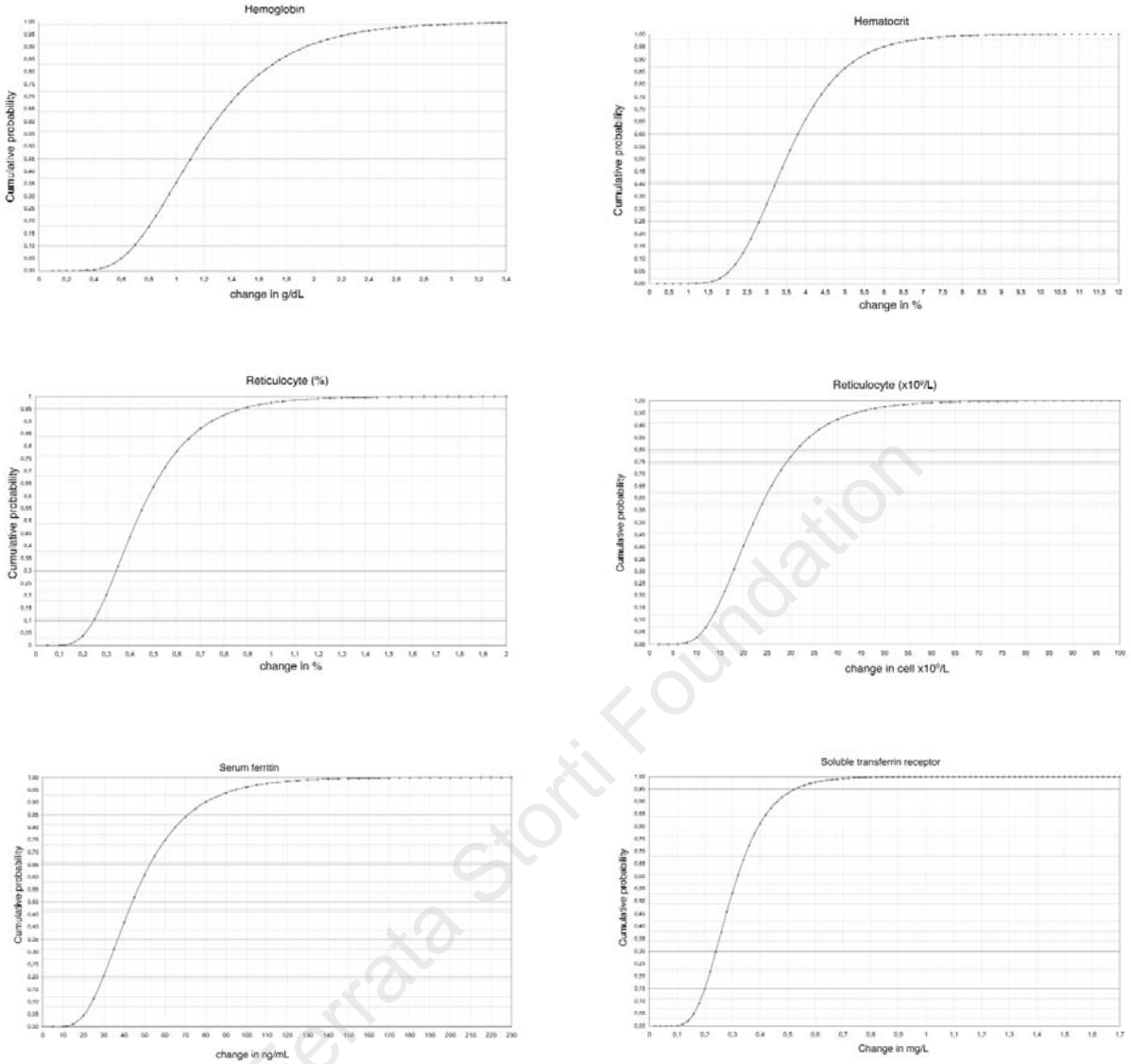

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Figure 2. Cumulative probability that a change between two successive measurements of critical hematologic parameters would be statistically significant.

jects with lower mean values and wider ranges in athletes with higher mean values. The 95th percentiles of the hemoglobin and hematocrit CV distributions, calculated in athletes with erythropoietic indices falling within the population reference ranges and more than three repeated measures, were 4.44% and 4.45%, respectively (Table 3); the value of 5% was therefore assumed as the parameter of dispersion for both variables. Figure 3 describes the estimated within-subject CVs in function of the number of determinations in 27 athletes monitored over a three-year period, showing that a relatively stable estimate of the within-subject variation can be obtained after 5 repeated measures. As shown in Figure 4, the between-subject variance for hemoglobin and hematocrit translates into markedly different subject-specific reference ranges of hemoglobin and hematocrit.

As far as erythropoietic indices (reticulocyte count, serum ferritin and soluble transferrin receptor) are concerned, the within-subject CVs were markedly greater than those for hemoglobin and hematocrit. These results might be due to a significantly higher between-laboratory analytical vari-

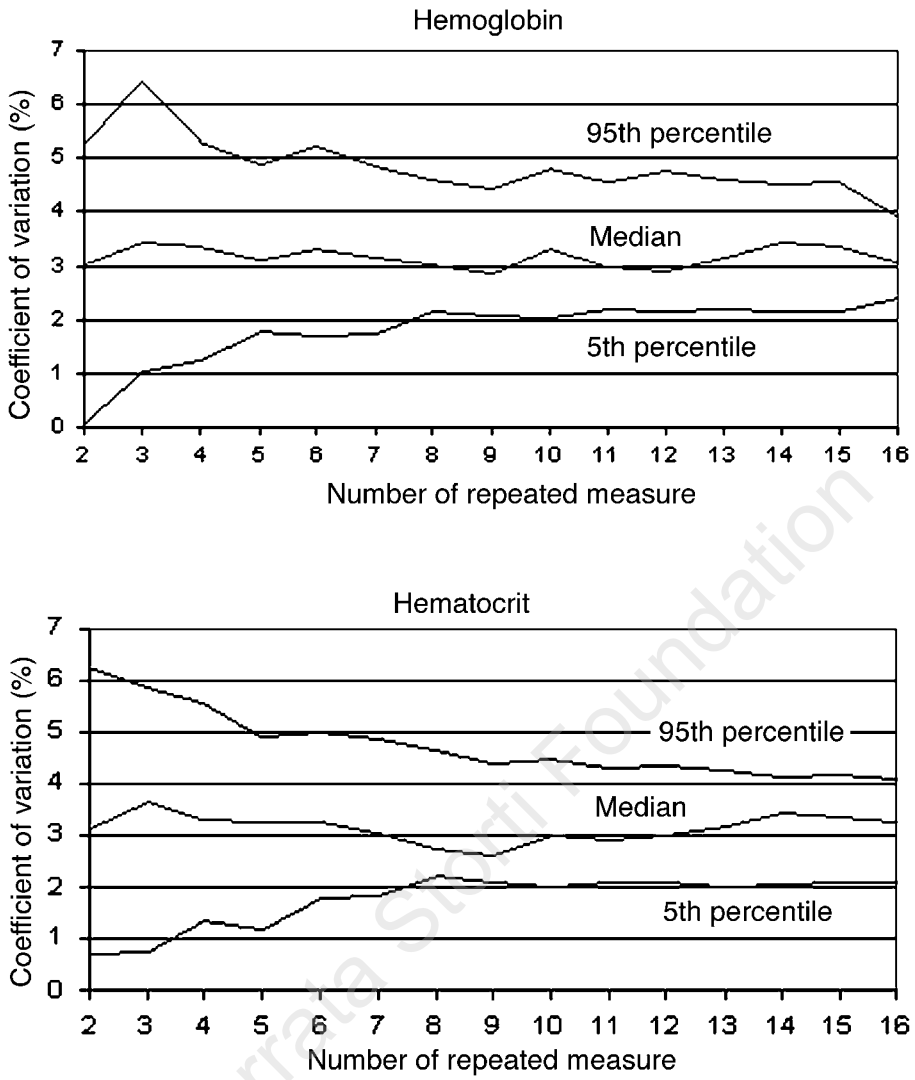

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Figure 3. Distribution of within-subject coefficient of variation of hemoglobin and hematocrit in relation to the number of repeated measures in 25 athletes studied for 3 years.

ance, which could not be estimated in this study. This result dissuaded us from defining subject-specific reference ranges for these variables.

Score system

We defined two score systems based on the number of parameters falling out of range. Individual ranges were used for hemoglobin and hematocrit, while population reference ranges were used for reticulocyte count, serum ferritin and soluble transferrin receptor.

In order to test the specificity of the proposed scores, for each parameter we compared all the successive measurements of each individual series to the subject-specific reference ranges calculated from all the previous validated determinations. A total of 284 determinations from 109 subjects were scored for each parameter

Table 4 shows all possible scenarios, with the corresponding scores and number of determinations as obtained from the test.

The sensitivity of the scores, with the cut-off derived above, was assessed on the data obtained from a double-blind, randomized trial evaluating the effect of rHuEpo administration on erythropoietic parameters, performed by the Australian Institute of

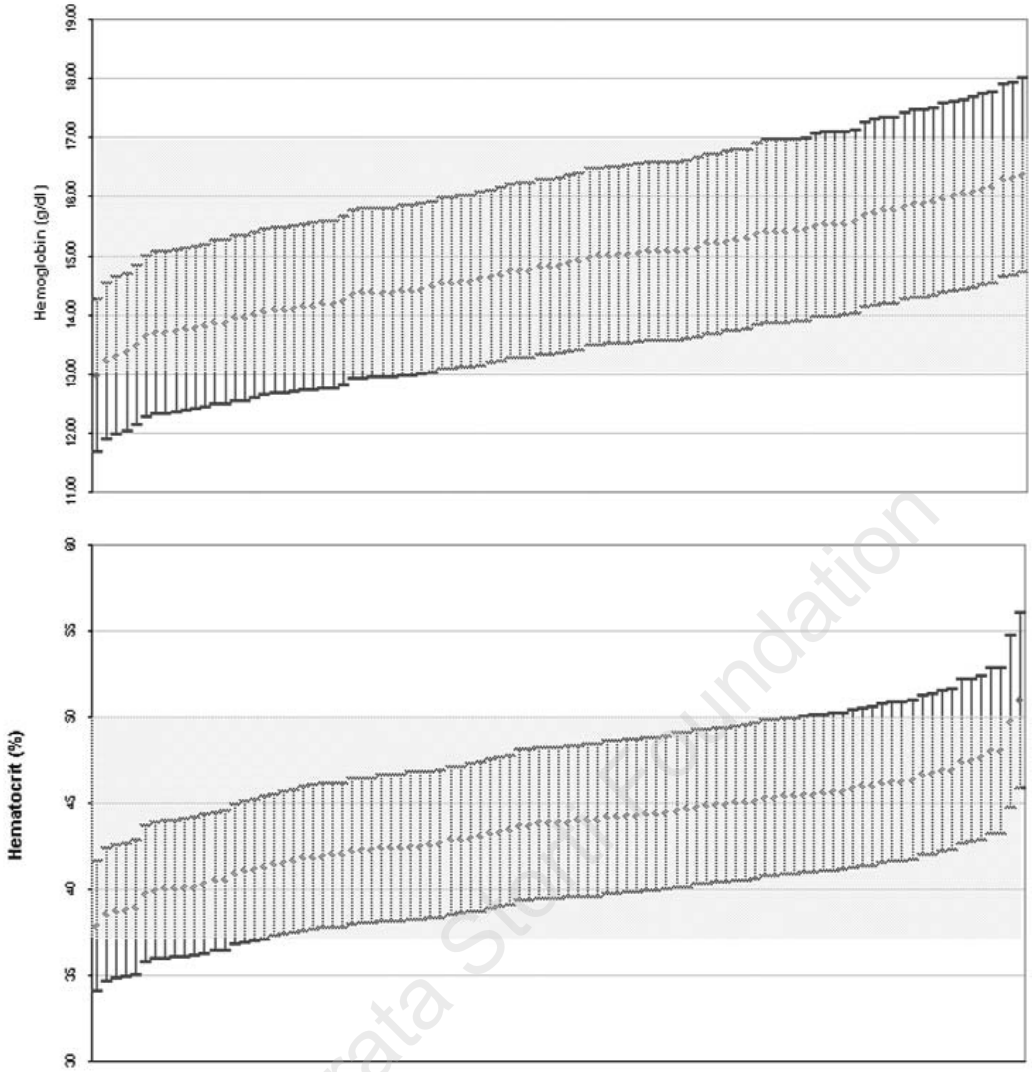

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Figure 4. Subject-specific reference ranges of hemoglobin and hematocrit in 95 football players who had more than 3 sequential blood counts. The shaded areas represent the normal reference ranges. This graph illustrates the between-subject variance for hemoglobin and hematocrit, and the need for using subject-specific reference ranges for distinguishing between normal and abnormal patterns in individual athletes.

Sport.[9] In this study, 30 voluntary, recreational athletes were randomized to receive rHuEpo (50 IU/kg subcutaneously 3 times a week) for 25 days, associated with oral or intramuscular iron administration, or placebo. Hemoglobin, hematocrit, reticulocyte count, serum ferritin and serum soluble transferrin receptor levels were monitored in the administration phase and over the 4 weeks following rHuEpo withdrawal (wash-out phase). The 3-variable score was repeatedly able to detect 12 out of 18 athletes treated with rHuEpo during the administration and the wash-out phases (sensitivity 66%), without false positive subjects in the control group. The five-variables score repeatedly detected 14 of the 18 treated athletes (sensitivity 77%), with a 100% specificity.

Table 4. Score systems and distribution of the tested determination.

n. Major positives	*n. Minor positives*	*3-variable score*	*5-variable score*	*N. determinations*
3	2		4.0	0
3	1	3	3.5	0
3	0		3.0	0
2	2		3.0	0
2	1	2	2.5	0
2	0		2.0	0
1	2		2.0	0
1	1	1	1.5	0
1	0		1.0	4
0	2		1.0	1
0	1	0	0.5	14
0	0		0	265

Discussion

In this study, serial determinations of blood count and erythropoietic indices were obtained from a large group of professional football players. Most of these data were collected within the campaign *I take care of my health!* promoted by the *Italian Olympic Committee* and the *Italian Football Federation*. Participation in this campaign was voluntary and this, although open t0o question, may itself represent a quality control of the data. These data were integrated with a long series of repeated measures obtained from players of a top football team during a three-year period.

Our analysis indicates that, despite the homogeneity of biological characteristics (age, sex) and sport discipline in this population of athletes, there is a considerable variation in hematologic parameters from subject to subject. This confirms the need for the use of subject-specific reference ranges.

Hemoglobin and hematocrit values were higher at the beginning of the competitive season, and then declined in well-trained athletes. This trend was observed in all the athletes, independently of their role, and is probably explained by a plasma volume expansion during the sporting season.[19] In the light of these data, the detection of increased values of hemoglobin and hematocrit in endurance athletes during the competitive season should be considered abnormal. An opposite trend was observed in serum ferritin concentration, which showed its lowest values in the July-September period. Although several non-estimable variables, such as iron loss or oral intake, could have played a role in determining this trend, the low serum ferritin values observed in the *training* phase could be suggestive of an increased erythropoietic requirement because of red blood cell mass expansion, followed by a reversal of negative iron balance during the *maintenance* phase.[20] In the *high* period of the competitive season non-significant increases in hemoglobin, hematocrit and reticulocyte count were associated with a marked decrease in serum ferritin concentration that could suggest a further erythropoietic demand.

Analysis of variance showed that the between-subject variance is the major source of biologic variation and that it is significantly higher than the within-subject variance. This means that individual subjects really could be considered to provide separate sub-distributions of values. This evidence strongly supports strategies against blood doping based on the definition of subject-specific reference values.

In order to define a subject-specific profile a critical point is to validate the first measures of a series before the subject-specific pattern is clearly detectable. A direct approach could be to refer to population ranges, stratified for biological and professional variables conditioning hematologic parameters. The use of mathematical models integrating hematologic and erythropoietic indices[9,10] may provide a more reliable evaluation of a measurement, demonstrating a high sensitivity and specificity to identify rHuEpo users after 1-2 weeks of administration (ON-model) and during a 3-week period after the cessation of injection (OFF-model). We tested an alternative approach based on the within-subject variance distribution, the reference change model,[17,18] which results in the cumulative probability that the difference between successive measures would be statistically significant. It may be extended to the differences between the first and the third observations in order to identify a significant trend within the first three observations. This method, however, is based on a parametric estimation of the distribution of within-subject variances in the reference population and could not, therefore, be implemented to obtain a personal profile.

We estimated a subject-specific reference range based on an individual homeostatic set-point (the mean value of the determinations of an individual series) and a measure of dispersion estimated from the reference population (i.e. the 95th percentile of the within-subject coefficient of variation distribution). CV-based subject-specific reference ranges result in narrower intervals in subjects with lower mean values and wider intervals in subjects with the highest mean values.

This seems to be a desirable property, since all the critical hematologic parameters but hemoglobin showed a significant correlation between mean values and standard deviation. The distribution of the within-subject coefficients of variation of hemoglobin and hematocrit calculated in athletes for whom more than 3 repeated measures were available and who had erythropoietic indices within population reference ranges showed that the 95th percentile falls below 5% in both parameters. This value was assumed as a measure of dispersion; the subject-specific reference range, as defined above, resulted in a 10% fluctuation around the individual mean value.

As far as reticulocyte count, serum ferritin and soluble transferrin receptor levels are concerned, the within-subject CVs were much higher, probably because of a greater analytical variance which we were unable to estimate in this series of data. This observation persuaded us against defining individual intervals for these parameters.

Finally we defined two score systems based on the number of parameters falling out of range,

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Table 5. Potential benefits of blood cell counts and evaluation of body iron status for the individual athlete's health.

Parameter	*Potential benefits*
Hemoglobin	Determining Hb level in a young athlete is extremely important. In fact, the prevalence of anemia is high in the second decade of life: iron deficiency, thalassemia syndromes and hemoglobinopathies account for the vast majority of anemic conditions worldwide. For athletes coming from countries with limited financial resources, this may represent the first chance to have an inherited disorder diagnosed. Determining a subject-specific reference range for hemoglobin improves the physician's capability of monitoring the individual athlete. Aerobic training is expected to result in lower Hb levels, and values between 12 and 13 g/dL for males, and between 11.5 and 12.5 g/dL are likely the normal adaptation in most cases. On the other hand, Hb levels close to the lower limit of the subject-specific reference range may also indicate an anemic condition, whose cause(s) should be searched for.
Hematocrit	Hematocrit does not provide substantial additional information with respect to hemoglobin. Moreover, it involves a higher analytical error. Many physicians, however, still use this parameter, especially when facing an individual with increased red cell mass.
MCV	This erythroid parameter is extremely important for the differential diagnosis of anemia. Iron deficiency anemia and the anemia of thalassemic syndromes are typically microcytic (MCV < 80 fL), whereas megaloblastic anemias (folate or vitamin B12 deficiency) are typically macrocytic (MCV > 100 fL). An MCV between 60 and 70 fL in a young athlete with marginally low Hb levels is strongly suggestive of thalassemia trait. An MCV values around 80 fL may indicate iron-deficient erythropoiesis. Therefore, this parameter should be carefully considered when examining blood cell counts.
Reticulocyte count	This parameter is still ignored by the vast majority of physicians facing an anemic patient. This behavior is no longer tolerable since the reticulocyte can nowadays be determined routinely with automated cell counters. By definition, hemolytic anemias are associated with increased reticulocyte counts whereas hypoplastic anemias show low reticulocyte counts. A high reticulocyte count in a non-anemic athlete may indicate a congenital hemolytic disorder, e.g., hereditary spherocytosis.
Serum iron, transferrin saturation and serum ferritin	Full evaluation of body iron status is extremely important, since young athletes may have iron deficiency or iron overload. Iron deficiency is very common in the second decade of life, especially in women. Genetic hemochromatosis is one of the most prevalent genetic disorders worldwide,[21] and early diagnosis is extremely important to prevent irreversible organ damage. Evaluation of body iron status also allows iron overload secondary to IV iron abuse[22] to be detected. This malpractice was very popular in endurance sports in the '90s, and many athletes still have iatrogenic iron overload. Although intravenous iron is primarily taken up by the reticuloendothelial cells, it is later redistributed to parenchymal cells. Therefore, this type of iron overload will eventually produce organ damage comparable to that occurring in genetic hemochromatosis, including the risk of developing hepatic carcinoma.
Soluble transferrin receptor	The soluble transferrin receptor level has been shown to be closely related to the number of red cell precursors in the bone marrow and to provide a reliable quantitative assay of the rate of erythropoiesis.[23,24] Elevated values are found in individuals with thalassemic syndromes or hemoglobinopathies. In addition, the soluble transferrin receptor level can provide useful information about the adequacy of iron supply to the erythroid marrow. As such, it is very useful to diagnose iron-deficient erythropoiesis. Finally, the soluble transferrin receptor measurement is useful in diagnosing anemia of inflammation with concomitant iron deficiency

Table 6. Potential tools for deterring and preventing blood doping. A prospective study should be performed to define the best parameter combination that allows athletes with acquired abnormal patterns to be excluded from competition, while allowing individuals with constitutionally altered parameters to compete. Competition rules should clearly state that having normal hematologic parameters as documented by the hematologic passport is a prerequisite for participation.

Parameter	*Use for deterring and preventing blood doping*
Hemoglobin subject-specific reference range	Can be checked before competition: values above the individual upper limit should represent a criterion leading to exclusion from competition.
Hematocrit subject-specific reference range	Can be checked before competition: values above the individual upper limit should represent a criterion leading to exclusion from competition.
Reticulocyte count	Can be checked before competition: values outside the normal reference range should represent a criterion leading to exclusion from competition (this would not apply to athletes with constitutionally high values as documented by the hematologic passport).
Reticulocyte hemoglobin or hematocrit	Can be checked before competition: values above the normal reference range should represent a criterion leading to exclusion from competition (this would not apply to athletes with constitutionally high values as documented by the hematologic passport).
Parameters of body iron status	Can be checked before competition. Evidence of iron overload should represent a criterion leading to exclusion from competition. This would not apply to athletes with constitutionally high values as documented by the hematologic passport: however, athletes with genetic hemochromatosis should be treated (phlebotomy therapy) in order to normalize their body iron status.
Soluble transferrin receptor	Can be checked before competition: values above the normal reference range should represent a criterion leading to exclusion from competition (this would not apply to athletes with constitutionally high values as documented by the hematologic passport).
Serum erythropoietin	Can be checked before competition: values outside the normal reference range should represent a criterion leading to exclusion from competition (this would not apply to athletes with constitutionally high values as documented by the hematologic passport)

using individual ranges for hemoglobin and hematocrit and population reference ranges for reticulocyte count, serum ferritin and soluble transferrin receptor. The three-variable score including hemoglobin, hematocrit and reticulocyte count can be quickly obtained with new generation cell counters and is, therefore, suitable for an evaluation on site before a competition.

This study provides a basis for the implementa-

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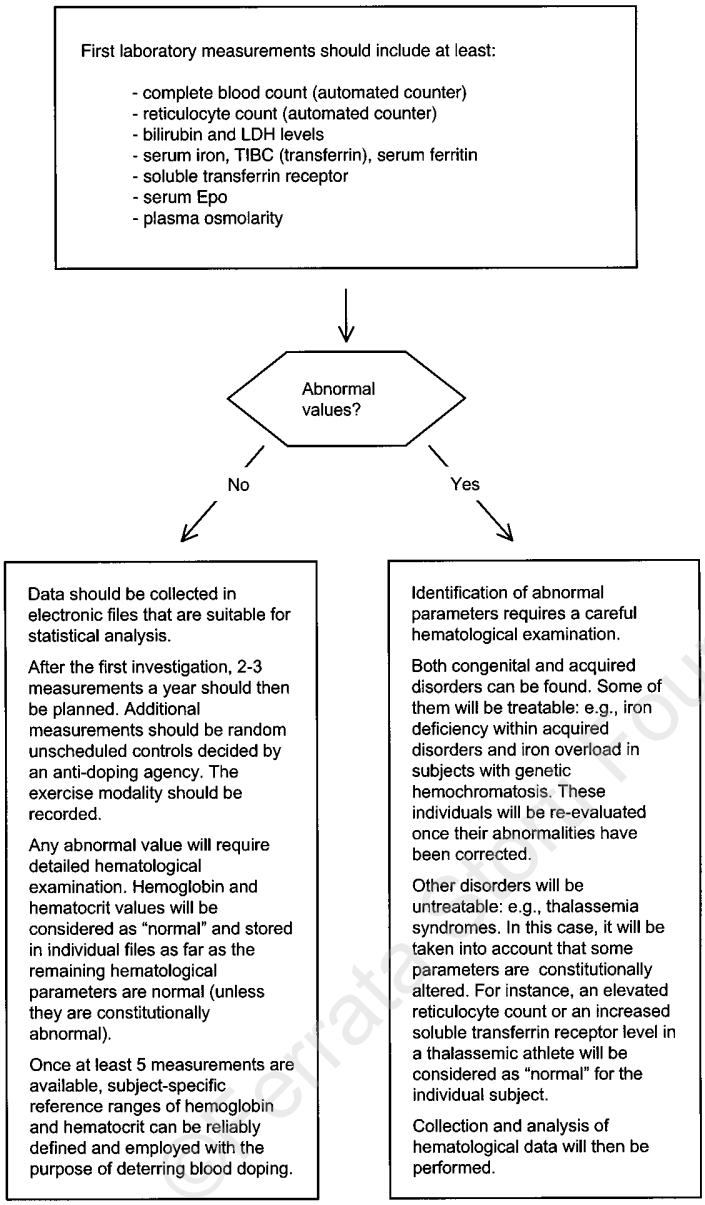

Figure 5. Guidelines for implementing the hematological passport for athletes competing in endurance sports.

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tion of the hematologic passport in endurance sports. Each young athlete should undergo blood cell counts and evaluation of body iron status as schematically indicated in Figure 5. The potential benefits of these tests for the individual athlete's health are listed in Table 5. By using proper sequential hematologic determinations it is possible to define subject-specific reference ranges for hemoglobin and hematocrit. Thus, the hematologic passport might be employed for excluding athletes with non-physiologic increases in hemoglobin and hematocrit from competition. The hematologic passport should be employed within a global strategy to deter blood doping, and should be combined with complementary interventions such as those listed in Table 6. This approach implies continuous monitoring of the athlete's health, providing a major means to deter blood doping. Analytical variance represents a major concern in this approach, both in the validation and the operative phase: efforts should be made to neutralize this component of variation.

References

1. Nelson M, Ashenden M, Langshaw M, Popp H. Detection of homologous blood transfusion by flow cytometry: a deterrent against blood doping. Haematologica 2002;87:881-2.
2. Berglund B, Hemmingsson P, Birgegard G. Detection of autologous blood transfusions in cross-country skiers. Int J Sports Med 1987;8:66-70.
3. Ashenden MJ. A strategy to deter blood doping in sport. Haematologica 2002;87:225-32.
4. D'Onofrio G, Zini G. Addendum to strategies to deter blood doping in sports. Haematologica 2002;87:ELT31.
5. Cazzola M. A global strategy for prevention and detection of blood doping with erythropoietin and related drugs. Haematologica 2000;85:561-3.
6. Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian JJ, Martin-Dupont P, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002;346:469-75.
7. Schmidt W, Biermann B, Winchenbach P, Lison S, Boning D. How valid is the determination of hematocrit values to detect blood manipulations? Int J Sports Med 2000;21:133-8.
8. Parisotto R, Gore CJ, Hahn AG, Ashenden MJ, Olds TS, Martin DT, et al. Reticulocyte parameters as potential discriminators of recombinant human erythropoietin abuse in elite athletes. Int J Sports Med 2000;21:471-9.
9. Parisotto R, Gore CJ, Emslie KR, Ashenden MJ, Brugnara C, Howe C, et al. A novel method utilizing markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. Haematologica 2000;85:564-72.
10. Parisotto R, Wu M, Ashenden MJ, Emslie KR, Gore CJ, Howe C, et al. Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica 2001;86:128-37.
11. Sharpe K, Hopkins W, Emslie KR, Howe C, Trout GJ, Kazlauskas R, et al. Development of reference ranges in elite athletes for markers of altered erythropoiesis. Haematologica 2002;87:1248-57.
12. Gore CJ, Parisotto R, Ashenden MJ, Stray-Gundersen J, Sharpe K, Hopkins W, et al. Second-generation blood tests to detect erythropoietin abuse by athletes. Haematologica 2003;88:333-44.
13. Progetto "Io non rischio la salute!" Roma: Commissione Scientifica Antidoping, CONI, 2000.
14. Fraser CG, E.K. H. Generation and application of data on biological variation in clinical chemistry. Critical Reviews in Clinical Laboratory Sciences 1989;27:409-437.
15. Harris EK. Some theory of reference values. I. Stratified (categorized) normal ranges and a method for following an individual's clinical laboratory values. Clin Chem 1975;21:1457-64.
16. Harris EK. Some theory of reference values. II. Comparison of some statistical models of intraindividual variation in blood constituents. Clin Chem 1976;22:1343-50.
17. Harris EK, Yasaka T. On the calculation of a "reference change" for comparing two consecutive measurements. Clin Chem 1983;29:25-30.
18. Harris EK, Brown SS. Temporal changes in the concentrations of serum constituents in healty men. Ann Clin Biochem 1979;16:69-78.
19. Shaskey DJ, Green GA. Sports haematology. Sports Med 2000;29:27-38.
20. Cook JD. The effect of endurance training on iron metabolism. Semin Hematol 1994;31:146-54.
21. Barosi G, Salvaneschi L, Grasso M, Martinetti M, Marchetti M, Bodini U, et al. High prevalence of a screening-detected, HFE-unrelated, mild idiopathic iron overload in Northern Italy. Haematologica 2002;87:472-8.
22. Deugnier Y, Loreal O, Carre F, Duvallet A, Zoulim F, Vinel JP, et al. Increased body iron stores in elite road cyclists. Med Sci Sports Exerc 2002;34:876-80.
23. R'zik S, Loo M, Beguin Y. Reticulocyte transferrin receptor (TfR) expression and contribution to soluble TfR levels. Haematologica 2001;86:244-51.
24. Ho CH. The differential diagnostic values of serum transferrin receptor, serum ferritin and related parameters in the patients with various causes of anemia. Haematologica 2001;86:206-7.

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Contributions

All authors gave substantial contributions to conception and design, or analysis and interpretation of data; to drafting the article or revising it critically for important intellectual content; and final approval of the version to be published. In addition, LM was primarily responsible for collection of data, CP for statistical analyses, and MC for the conception and design of the study.

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