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Hematological Profile of Serbian Youth National Soccer Teams

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Soccer is one of the most widely played and complex sports in the world, where players need technical, tactical, and physical skills to succeed. Technical and tactical skills in soccer are highly dependent on the player's physical capacity. The selection, development and professional guidance of young players is a priority for many top soccer clubs in order to maintain their sporting and financial status. The aim of the present study was to determine hematological profile of youth national soccer teams and to compare the values of fifteen hematological parameters between 3 Serbian youth national teams (under 14, 15 and 16 years old), as well as between soccer players and non-athletes. 80 young soccer players and 30 non-athletes participated in the study. 15 hematologic parameters (WBC, RBC, HGB, HCT, PLT, MCV, MCH, MCHC, PDW, LYM %, MON %, GRAN %, LYM, MON, GRAN) were measured. In order to determine the significance of differences between the groups on a multivariate level a multivariate analysis of variance (MANOVA) was administered, and to test the differences between the groups on an univariate level a univariate analysis of variance (ANOVA) was applied. It was concluded that there is no significant difference in all the variables (WBC, Ly, Mo, Gr, PLT, HGB, HCT, etc), except RBC, probably due to age, androgen affection on erythropoesis, field positoning and diet. From a practical point of view, the clinician has to take into account not only age, but also training status of individuals when evaluating their blood tests.

Key words: blood parameters, selection, differences, youth soccer

Introduction

Soccer is one of the most widely played and complex sports in the world, where players need technical, tactical, and physical skills to succeed. However, studies to improve soccer performance have often focused on technique and tactics at the expense of physical abilities such as endurance, strength, speed as well as physiological, mainly hematological parameters.

Technical and tactical skills in soccer are highly dependent on the player's physical capacity (Bangsbo, 1994; Hoff et al., 2002).

During the last two decades, there has been significant accumulation of scientific data regarding soccer physiology and medicine. Previous investigations have evaluated ideal physiological and anthropometric profile of successful soccer players, mostly from Europe and America (Rhodes et al., 1986; Mangine et al., 1990; Davies et al., 1992). Athletes are usually monitored by using biochemical and hema-

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Table 1

Basic statistical parameters of soccer players U-14, U-15 and U-16

Variables	Group/N		Mean	Min	Max	Range	SD	Error	Skew	Kurt
WBC	U-14	27	5.867	3.400	7.700	4.300	1.079	0.208	-0.205	-0.454
	U-15	28	5.800	3.800	9.100	5.300	1.449	0.274	0.394	-0.460
	U-16	25	5.840	3.800	9.100	5.300	1.146	0.229	0.935	1.567
RBC	U-14	27	4.759	4.210	5.510	1.300	0.329	0.063	0.173	-0.277
	U-15	28	4.984	3.960	5.550	1.590	0.392	0.074	-0.774	0.272
	U-16	25	4.993	4.470	5.710	1.240	0.322	0.064	0.437	0.006
HGB	U-14	27	129.074	112.000	147.000	35.000	9.675	1.862	0.158	-0.682
	U-15	28	133.000	107.000	156.000	49.000	11.106	2.099	-0.123	0.312
	U-16	25	135.560	113.000	152.000	39.000	8.377	1.675	-0.421	1.144
HCT	U-14	27	0.394	0.347	0.452	0.105	0.029	0.006	0.266	-0.635
	U-15	28	0.406	0.332	0.466	0.134	0.033	0.006	-0.329	-0.357
	U-16	25	0.414	0.358	0.460	0.102	0.024	0.005	-0.065	-0.056
PLT	U-14	27	292.778	170.000	379.000	209.000	57.162	11.001	-0.224	-0.525
	U-15	28	291.143	196.000	414.000	218.000	57.859	10.934	0.218	-0.912
	U-16	25	289.600	189.000	370.000	181.000	47.035	9.407	-0.447	-0.114
MCV	U-14	27	82.815	77.000	88.000	11.000	2.558	0.492	0.074	0.224
	U-15	28	81.643	77.000	87.000	10.000	2.683	0.507	0.412	-0.390
	U-16	25	83.000	71.000	89.000	18.000	4.082	0.816	-1.098	1.724
MCH	U-14	27	27.096	25.500	28.900	3.400	0.920	0.177	0.231	-0.680
	U-15	28	26.661	24.400	29.200	4.800	1.065	0.201	0.393	0.282
	U-16	25	27.232	22.500	29.300	6.800	1.495	0.299	-1.395	2.911
MCHC	U-14	27	327.296	319.000	335.000	16.000	3.750	0.722	0.043	-0.183
	U-15	28	327.000	317.000	336.000	19.000	4.830	0.913	-0.187	-0.405
	U-16	25	327.800	315.000	335.000	20.000	4.072	0.814	-1.300	3.003
PDW	U-14	27	14.807	13.700	17.600	3.900	0.850	0.164	1.424	3.245
	U-15	28	14.950	13.400	17.100	3.700	0.922	0.174	0.557	0.205
	U-16	25	14.376	12.900	15.600	2.700	0.831	0.166	-0.151	-1.299
LYM %	U-14	27	39.081	22.900	52.600	29.700	6.873	1.323	-0.444	0.656
	U-15	28	36.504	21.900	46.400	24.500	6.158	1.164	-0.556	-0.199
	U-16	25	37.212	24.500	49.700	25.200	6.553	1.311	-0.176	-0.643
MON %	U-14	27	7.911	6.000	9.600	3.600	1.071	0.206	-0.188	-1.033
	U-15	28	8.211	5.600	10.700	5.100	1.322	0.250	-0.020	-0.610
	U-16	25	8.420	6.000	11.300	5.300	1.138	0.228	0.136	0.883
GRAN %	U-14	27	53.007	38.800	69.400	30.600	7.358	1.416	0.403	0.445
	U-15	28	55.286	47.600	71.900	24.300	6.403	1.210	0.948	0.462
	U-16	25	54.368	42.200	68.400	26.200	6.905	1.381	0.308	-0.532
LYM	U-14	27	2.207	1.300	3.500	2.200	0.485	0.093	1.056	1.597
	U-15	28	2.004	1.200	2.800	1.600	0.361	0.068	-0.133	-0.049
	U-16	25	2.080	1.500	3.000	1.500	0.349	0.070	0.316	0.652
MON	U-14	27	0.407	0.200	0.700	0.500	0.100	0.019	0.597	1.763
	U-15	28	0.421	0.200	0.600	0.400	0.120	0.023	-0.445	-0.716
	U-16	25	0.444	0.300	0.600	0.300	0.096	0.019	0.185	-0.781
GRAN	U-14	27	3.252	1.800	5.300	3.500	0.846	0.163	0.312	0.100
	U-15	28	3.375	1.900	6.300	4.400	1.175	0.222	1.006	0.656
	U-16	25	3.308	1.800	6.000	4.200	0.978	0.196	1.144	1.460

tological indices for evaluating possible pathologies and performance status (Dolci et al., 2007).

The selection, development and professional guidance of young players is a priority for many top soccer clubs in order to maintain their sporting and financial status (Vaeyens et al., 2006). It is essential, however, to understand the key elements of talent identification and the development process for soccer (Martindale et al., 2005; Williams and Franks, 1998). Given a lack of discrete objective measures of performance, as in individual sports, identifying soccer talent is complex and requires a multivariate approach (Hoare and Warr, 2000; Williams and Franks, 1998; Reilly et al., 2000). Potential predictors of soccer talent include anthropometric, physiological, neuromotor, cognitive-perceptual and psychosocial variables (Williams and Franks, 1998).

Evaluation of youth players is complicated by individual differences in the timing and tempo of changes in body size, functional capacities and motor efficiency during puberty (Malina et al., 2004; Philippaerts et al., 2006).

The aim of the present study was to determine hematological profile of youth national soccer teams and to compare the values of fifteen hematological parameters between 3 Serbian national teams (under 14, 15 and 16 years old) as well as between soccer players and non-athletes (control group).

Material and methods

Subjects

Research was performed on a sample of 80 young soccer players from 3 Serbian national teams (under 14 – 27 players, under 15 – 28 players and under 16 – 25 players) and 30 non-athletes of the same age. To be included in the study, subjects had to meet the following criteria, which were assessed through the administration of a questionnaire: be in good health, with no known diseases, not use medications during the week preceding blood sampling, follow a regular diet, not use dietary supplements in excess of the recommended dietary allowances on a regular basis within the trimester preceding blood sampling, not use steroids or other banned substances. All participants were members of soccer clubs and had been training for the past three years or longer, at least 4 days per week, with training sessions lasting 1-1,5h.

Blood sampling

Venous blood samples were collected into plain evacuated tubes from a forearm vein with minimal stasis after approximately 10 min of rest in a sitting position between 8 and 9 am, after an overnight fast and at least 24 hours from the last workout. An aliquot of each sample was immediately mixed with EDTA solution to prevent clotting for hematology. The rest of the sample was left to coagulate for 30 min at room temperature and was centrifuged at 1500 x g for 10 min in order to separate the serum for chemistry. The serum was stored at -20°C.

Assays

We measured 15 hematologic parameters WBC, RBC, HGB, HCT, PLT, MCV, MCH, MCHC, PDW, LYM %, MON %, GRAN %, LYM, MON, GRAN. The hematologic parameters were measured in a Sysmex K-1000 (Kobe, Japan) autoanalyzer. The hematologic measurements were generally performed within 3 hours.

Extraction of reference values

The value of a hematologic parameter pertaining to an individual will be referred to as a reference value, according to the terminology of the International Federation of Clinical Chemistry (Gräsbeck et al., 1978). Because some participants visited the laboratory more than once, they had more than one reference value for a certain parameter. In that case, we selected the median for statistical analysis.

Statistical analysis

Statistical methods applied were:

Descriptive statistics comprised: number of subjects (N), mean value (Mean), standard deviation (SD), minimum (Min) and maximum (Max) numerical results, range (Range) and standard error of the mean value (Error). Discriminative measurements were performed by two procedures:

Table 2

Basic statistical parameters of soccer players (all 3 groups)

Variables	N	Mean	Min	Max	Range	SD	Error	Skew	Kurt
WBC	80	5.835	3.400	9.100	5.700	1.225	0.137	0.369	0.008
RBC	80	4.911	3.960	5.710	1.750	0.363	0.041	-0.143	-0.271
HGB	80	132.475	107.000	156.000	49.000	10.067	1.126	-0.134	-0.099
HCT	80	0.405	0.332	0.466	0.134	0.030	0.003	-0.146	-0.509
PLT	80	291.213	170.000	414.000	244.000	53.789	6.014	-0.071	-0.626
MCV	80	82.463	71.000	89.000	18.000	3.170	0.354	-0.442	1.027
MCH	80	26.986	22.500	29.300	6.800	1.186	0.133	-0.547	1.426
MCHC	80	327.350	315.000	336.000	21.000	4.213	0.471	-0.450	0.309
PDW	80	14.723	12.900	17.600	4.700	0.893	0.100	0.595	0.883
LYM %	80	37.595	21.900	52.600	30.700	6.540	0.731	-0.317	-0.135
MON %	80	8.175	5.600	11.300	5.700	1.188	0.133	0.014	-0.276
GRAN %	80	54.230	38.800	71.900	33.100	6.872	0.768	0.446	0.077
LYM	80	2.096	1.200	3.500	2.300	0.408	0.046	0.762	1.826
MON	80	0.424	0.200	0.700	0.500	0.106	0.012	-0.034	-0.181
GRAN	80	3.313	1.800	6.300	4.500	1.000	0.112	0.939	0.917

Table 3

Basic statistical parameters of non-athletes

Variables	N	Mean	Min	Max	Range	SD	Error	Skew	Kurt
WBC	30	6.037	4.000	9.300	5.300	1.310	0.239	0.456	0.030
RBC	30	4.950	4.200	5.500	1.300	0.350	0.064	-0.453	-0.684
HGB	30	129.167	106.000	151.000	45.000	10.359	1.891	-0.021	-0.276
HCT	30	0.385	0.332	0.430	0.098	0.027	0.005	-0.001	-0.881
PLT	30	252.600	153.000	372.600	219.600	54.562	9.962	0.405	-0.610
MCV	30	81.300	76.000	86.000	10.000	2.731	0.499	0.130	-0.662
MCH	30	26.740	24.600	29.200	4.600	1.000	0.183	0.365	0.234
MCHC	30	324.433	318.000	333.000	15.000	3.588	0.655	0.129	-0.029
PDW	30	14.620	13.100	17.200	4.100	0.976	0.178	0.921	0.708
LYM %	30	33.367	19.700	45.200	25.500	6.285	1.147	-0.577	0.092
MON %	30	7.480	5.300	10.200	4.900	1.257	0.230	0.222	-0.885
GRAN %	30	53.953	40.500	70.500	30.000	7.109	1.298	0.764	0.456
LYM	30	2.050	1.200	3.100	1.900	0.461	0.084	0.605	0.085
MON	30	0.453	0.200	0.600	0.400	0.136	0.025	-0.474	-1.231
GRAN	30	3.033	1.700	5.700	4.000	1.001	0.183	1.149	1.453

Skewniss (SKEW) pointing to the symetry of substance layout around arithmetic mean and Kurtosis (KURT) designating peakedness or flatness of distribution. In order to determine the significance of differences between the groups on a multivariate level a multivariate analysis of variance MANOVA was administered, and to test the differences between the groups on an univariate level univariate analysis of variance ANOVA was administered.

Statistica 8.0. software program was used to process data.

Results

Surveying Table 1 which shows the results of the central and dispersion parameters of the applied hematological variables of the selected soccer players up to 14 years of age (U-14), up to 15 years of age (U-15) and up to 16 years of age (U-16) it can be said that the distribution in the zones around arithmetic mean (Skew) is optimal in most variables. From Skewness one can also notice that there are somewhat higher results in LYM variable in U-14, and somewhat larger number of weaker results in variables MCV, MCH, MCHC, and somewhat larger number of stronger results in variable GRAN in U-16. However Kurtosis (Kurt.) whose value in almost all variables is significantly smaller than 2.75 points to the fact that distribution differs from the normal one (platikurtic distribution) which means that the test results are quite scattered. Normal distribution of data is in variables MCH and MCHC in U-16 and a little bit narrow in variable PDW in U-14.

Table 4

Multivariate differences between U-14, U-15 and U-16

Test	Value	F	Effect - df	Error - df	p
Wilks	0.51538	1.796	28	128	**0.0154**

Table 2 shows the results of the central and dispersive parameters of all football players) all three groups). By analysing it one can notice that the distribution of the data is symmetric (Skew.) and scattered (Kurt.)

Table 3 shows the results of the central and dispersive parameters of non-athletes (control group).By analysing it one can notice that the distribution of the data is symmetric (Skew.) in almost all variables except in the variable GRAN, which shows somewhat larger number of stronger results. As in the previous Table 2 the distribution is scattered.

Table 4 shows multivariate differences of the applied hematological variables between three groups of subjects (selections of soccer players up to 14, 15 and 16 years of age). Analysing it one can say that there are statistically significant differences in the applied variables between these groups on a multivariate level (p = 0.0154).

Analysing Table 5 which shows univariate differences of the applied hematological variables between three groups of subjects (U-14, U-15 and U-16) it can be concluded that statistically significant difference is present only in variable RBC. In all other variables there are no statistically significant differences (WBC, HGB, HCT, PLT, MCV, MCH, MCHC, PDW, LYM%, MON%, GRAN%, LYM, MON, GRAN).

Table 6 shows multivariate differences of the applied hematological variables between soccer players and non-athletes. Analysing it one can say that there are statistically significant differences in the applied variables between these groups on a multivariate level (p = 0.0000).

Table 5

Univariate differences between U-14, U-15 and U-16

Variables	U	N	Mean	SD	F	p
WBC	U-14	27	5.867	1.079	0.020	0.9801
	U-15	28	5.800	1.449		
	U-16	25	5.840	1.146		
RBC	U-14	27	4.759	0.329	3.822	**0.0262**
	U-15	28	4.984	0.392		
	U-16	25	4.993	0.322		
HGB	U-14	27	129.074	9.675	2.884	0.0620
	U-15	28	133.000	11.106		
	U-16	25	135.560	8.377		
HCT	U-14	27	0.394	0.029	2.988	0.0563
	U-15	28	0.406	0.033		
	U-16	25	0.414	0.024		
PLT	U-14	27	292.778	57.162	0.022	0.9781
	U-15	28	291.143	57.859		
	U-16	25	289.600	47.035		
MCV	U-14	27	82.815	2.558	1.480	0.2341
	U-15	28	81.643	2.683		
	U-16	25	83.000	4.082		
MCH	U-14	27	27.096	0.920	1.740	0.1823
	U-15	28	26.661	1.065		
	U-16	25	27.232	1.495		
MCHC	U-14	27	327.296	3.750	0.237	0.7898
	U-15	28	327.000	4.830		
	U-16	25	327.800	4.072		
PDW	U-14	27	14.807	0.850	3.067	0.0523
	U-15	28	14.950	0.922		
	U-16	25	14.376	0.831		
LYM %	U-14	27	39.081	6.873	1.134	0.3271
	U-15	28	36.504	6.158		
	U-16	25	37.212	6.553		
MON %	U-14	27	7.911	1.071	1.217	0.3017
	U-15	28	8.211	1.322		
	U-16	25	8.420	1.138		
GRAN %	U-14	27	53.007	7.358	0.758	0.4721
	U-15	28	55.286	6.403		
	U-16	25	54.368	6.905		
LYM	U-14	27	2.207	0.485	1.782	0.1752
	U-15	28	2.004	0.361		
	U-16	25	2.080	0.349		
MON	U-14	27	0.407	0.100	0.782	0.4611
	U-15	28	0.421	0.120		
	U-16	25	0.444	0.096		
GRAN	U-14	27	3.252	0.846	0.102	0.9030
	U-15	28	3.375	1.175		
	U-16	25	3.308	0.978		

Table 6

Multivariate differences between soccer players and non-athletes

Test	Value	F	Effect - df	Error - df	p
Wilks	0.005386	1157	15	94	**0.0000**

Analysing Table 7 which shows univariate differences of the applied hematological variables between soccer players and non-athletes) it can be concluded that statistically significant difference is present in variables HCT, PLT, MCHC, LYM% and MON%. In all other variables there are no statistically significant differences.

Discussion

Sport and exercise scientists engaged in soccer research are interested in a multitude of factors that determine the performance of a player as well as the related underlying phenomena that explain how each factor influences that performance. Hematological and biochemical tests are used widely to access health and fitness of the intensively training athlete (Drust et al., 2007; Nikolaidis et al., 2003).

Leukocyte counts in athletes are usually similar to those of the general population. The number of leukocytes increases in response to stressful stimuli including exercise. Their source is in the marginated pool that is located along vessel walls. In addition, the leukocyte number is affected by demarginated leukocytes from the pulmonary micro-vascular pool in response to ventilation (Gurcan et al., 1998). Additionaly intense exercise causes tissue damage, production of stress hormones, and alterations in the circulating quantity and function of various immune cells (Natale et al., 2003). Our findings show there is no statisticaly significant difference in WBC in all three study groups according to reference range and

Table 7

Univariate differences between soccer players and non-athletes

Variables	SP./Cont.	N	Mean	SD	F	p
WBC	Soccer plrs.	80	5.835	1.225	0.569	0.4521
	Control gr.	30	6.037	1.310		
RBC	Soccer plrs.	80	4.911	0.363	0.257	0.6134
	Control gr.	30	4.950	0.350		
HGB	Soccer plrs.	80	132.475	10.067	2.320	0.1307
	Control gr.	30	129.167	10.359		
HCT	Soccer plrs.	80	0.405	0.030	10.256	**0.0018**
	Control gr.	30	0.385	0.027		
PLT	Soccer plrs.	80	291.213	53.789	11.156	**0.0012**
	Control gr.	30	252.600	54.562		
MCV	Soccer plrs.	80	82.463	3.170	3.152	0.0786
	Control gr.	30	81.300	2.731		
MCH	Soccer plrs.	80	26.986	1.186	1.020	0.3148
	Control gr.	30	26.740	1.000		
MCHC	Soccer plrs.	80	327.350	4.213	11.290	**0.0011**
	Control gr.	30	324.433	3.588		
PDW	Soccer plrs.	80	14.723	0.893	0.273	0.6022
	Control gr.	30	14.620	0.976		
LYM %	Soccer plrs.	80	37.595	6.540	9.311	**0.0029**
	Control gr.	30	33.367	6.285		
MON %	Soccer plrs.	80	8.175	1.188	7.234	**0.0083**
	Control gr.	30	7.480	1.257		
GRAN %	Soccer plrs.	80	54.230	6.872	0.035	0.8526
	Control gr.	30	53.953	7.109		
LYM	Soccer plrs.	80	2.096	0.408	0.261	0.6102
	Control gr.	30	2.050	0.461		
MON	Soccer plrs.	80	0.424	0.106	1.453	0.2307
	Control gr.	30	0.453	0.136		
GRAN	Soccer plrs.	80	3.313	1.000	1.699	0.1952
	Control gr.	30	3.033	1.001		

control group.

During exercise, the activated sympathetic nervous system increases blood flow to muscle as blood flow to splanchnic organs decreases. After exercise, sympathetic tone and blood pressure becomes reduced. The spleen contains lymphocytes and blood resides in gut vessels. A change in blood flow to these organs could affect the number of circulating lymphocytes (Nielsen, 2003). Also the number of granulocytes and monocites could be affected. Our study shows an increased number of lymphocytes and monocytes in athletes, compared to the non–athlete group($p<0.005$), though there is no significant difference in absolute number of lymphocytes, monocytes and granulocytes, in all three study groups, according to reference range. Also no pathological trends in studied athletes and non-athletes were found and the fluctuations of the number of total leukocytes and subpopulations seemed to be physiological (Dolci et al., 2007).

A high oxygen uptake is a prerequisite for athletic success in endurance sports. The oxygen transport capacity and consequently maximal oxygen uptake can be increased by increasing the red cell mass and consequently the hemoglobin concentration in the blood (Kuipers et al., 2007). However, the influence of physical activity on the levels of many routinely measured blood variables seem to be ambiogous (Nikolaidis et al., 2003). Athletes have several risk factors for anaemia and iron depletion due to poor nutritional intake of iron, haemolysis caused by repeated foot strikes, blood and iron loss through gastrointestinal, urinary tract and sweating (Dubnov and Constantini, 2004). Our study shows normal reference range in RBC in all three study groups. Statisticaly significant difference of RBC is shown in univariant differences ($p<0.005$), probably due to the age, testosterone level (Hero et al., 2005), field positioning, diet (Deakin, 2000; Unt et al., 2008; Herbert, 1996). The effects of strength training on red blood cell variables might be related to the length of the training term and it may become prominent in contrast to seasonal variation in the summer (Hu et al., 2008). Other studies show significant difference of RBC and HGB in higher value of males compared to females (Nikolaidis et al., 2003, Boyadjiev and Taralov, 2000).

Hct (Packed cell volumen) increment is usually associated with enhancement of the oxygen carrying capacity of the blood and aerobic performance, yet the consequence of Hct increment might be increased viscosity of the blood (Hu et al., 2008). In our study, Hct shows no increase beyond the normal range in all three study groups, but we find significant difference comparing athletes to the control goup ($p<0.005$) (Nikolaidis, M.G). Kuipers et al. show that the hematological profile in elite junior and senior long track speed skaters from 2000 to 2005 did not change significantly (Kuipers et al., 2007). Values for Hb, Hct in the study by Ostojic (Ostojic, 2002) were within the normal range of the male population and not significantly different between squads or from values reported from studies of soccer players by other investigators (Biancotti et al., 1992).

MCV, MCH, and MCHC values are in reference range in all three study groups. Comparing athletes and non-athletes MCHC shows significan differences ($p<0.005$) due to accelerated haemopoesis, as consequence of above mentioned mechanisms of anemia (Dubnov and Constantini, 2004). (Hu M. et al. point out on Hct, Hb and MCHC significant seasonal variations (Hu et al., 2008).

Physical exercise and training induce changes in the hemostasis of healthy people (Boyadjiev, 2004). Formation of a stable haemostatic white thrombus is certainly one of the most important functions of the blood platelets. They play a major role in the blood-clotting process through adhesion to the site of vessel injury immediately after vessel damage (El-Sayed et al., 2004). Although it is evidence-based that physical exercise alters platelet count and platelet functionality (Hilberg et al., 2003), comparing athletes and non-athletes in our study ($p<0.005$), we have found no significant increase of PLT count beyond the normal range in all three groups of youth soccer players.

Conclusion

In conclusion, it could be stated that there is no significant difference in all the variables (WBC, Ly, Mo, Gr, PLT, HGB, HCT, etc), except RBC, probably due to age, androgen affection on erythropoesis, field positoning and diet.

From a practical point of view, the clinician has to take into account not only the age, but also the training status of individuals when evaluating their blood tests.

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