Eur J Appl Physiol (2011) 111:9–16
DOI 10.1007/s00421-010-1641-1

REVIEW ARTICLE

Seasonal variations of haematological parameters in athletes

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Accepted: 26 August 2010 / Published online: 15 September 2010

Abstract The influence of training and competition workloads is crucial for evaluation of longitudinal haematological data in athletes. There are only a few papers on the variation of haematological parameters during long-lasting periods and, especially, during an entire competitive season. We summarized that some haematological parameters can be influenced by long-term training and competition periods. Haemoglobin (Hb) and haematocrit (Ht) are decreased during the more intense periods of training, throughout the season. In different sport disciplines, the decline of Hb ranges from 3 to 8% during the competition season, while the range of reticulocytes (Ret%) varies from 5 to 21%. Reticulocytes are also decreased after long periods of training and competitions, but their variation is not necessarily associated with that of Hb. The qualitative variations (trend of modifications) of haematological parameters are roughly independent of the sport discipline, but quantitatively (amount of modifications) dependent on sport discipline. The modifications are more evident in cycling, running, swimming than they are in football and rugby. The variations of haematological parameters within the same sport discipline are qualitatively concordant and quantitatively different among separate but consecutive competitive seasons. These findings are described in aerobic and team sports sportsmen. The definition of reliable reference ranges in sportsmen would only be possible by following the best laboratory practices. For antidoping purposes more studies investigating haematological modifications during the season are advisable.

Keywords Athletes · Haematology · Season · Haemoglobin · Haematocrit · Reticulocytes · Sport · Biological passport · Antidoping

Communicated by Susan Ward.

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Introduction

The haematological passport for professional cyclists has been introduced by the Union Cycliste Internationale (UCI) and will also be adopted by other International Sports Federations for evaluating abnormal values and help identify the use of fraudulent and prohibited procedures aimed at boosting the red blood cell mass and endurance performance (Sottas et al. 2009). The passport is based on a Bayesian approach (Robinson et al. 2007), which makes it interesting and appreciable, because values are individualized and focused on the biological variability of the various parameters included. The Bayesian statistical approach is traditionally used for the determination of the probability of a test result conditioned by several variables and/or factors, and can be applied to analyze and classify a full sequence of individual blood parameters. The Bayesian approach is generally used in medicine when prevalence of a phenomenon, as blood doping, could not be calculated.

The method realises, on the basis of variations of haematological parameters over time, a plausibility of blood doping (Sottas et al. 2008). Some haematological experts are charged with the final decision about the prosecution of athletes who have very high probability of the use of substances or procedures which increase oxygen transport. It is however not completely clear how other variables, such as analytical variability, age, gender, and training status, might be introduced and appropriately evaluated by the software, before sending the athlete's data to the appointed experts who are in charge of analysis and interpretation. Since the interpretation may have serious consequences for the athlete, we here discuss the factors significantly influencing the variability of parameters included in the blood passport.

In the Bayesian approach six covariables were introduced (Sottas et al. 2008): gender, ethnic origin, altitude, age, sport discipline, and type of analyzer. The values of five covariables, except for type of analyzer, assumed as independent, were obtained using the Bayer Advia 120 instrument (Sharpe et al. 2002). The validation of the model was completed on 101 subjects, presumably "clean" athletes and erythropoietin-treated volunteers, by using Abbott Cell Dyn 4000, Beckman Coulter AcT and GEN-S, and Sysmex XT2000i (Robinson et al. 2007; Sottas et al. 2008). Within the Bayesian network, data concerning the differences between haematological analyzers derived from the study by Ashenden et al. (2004) and from another study by Robinson et al. (2005) were introduced. The other variables introduced in the network were derived from a wide cohort of professional athletes (Sharpe et al. 2002), but the possible modifications of haematological parameters due to competition season were not reported (Banfi et al. 2010).

The influence of training and competition is a crucial aspect because it might influence the evaluation of longitudinal haematological data in an athlete. There are only few papers on the variation of haematological parameters during long-lasting periods and, especially, during an entire competitive season. Moreover, the data accumulated by federations and agencies are incomplete: mean values of different seasons have been reported, but not including their changes in different parts of the season (Manfredini et al. 2003; Mørkeberg et al. 2009a).

Our aim was to collect literature data concerning seasonal variations of haematological parameters in athletes.

Seasonal variations of haematological parameters in athletes

A few studies have documented the seasonal variations of haematological parameters in cycling. The first description of a decrease of haemoglobin (Hb), haematocrit (Ht) and red blood cells (RBC) in professional cyclists was by Guglielmini et al. (1989). In 40 professional cyclists followed for a period of 15 months RBC, Hb, and Ht decreased during the racing period. Conversely, ferritin and mean corpuscular haemoglobin (MCH), increased during the season. In this study, the athletes were continuously supplemented by intravenous iron therapy. Based on a series of 1,698 samples drawn from 45 female and 184 male elite German cyclists, Schumacher et al. (2002) confirmed that seasonal variations for haematological parameters occur. Winter and summer values were recorded for Hb, Ht, and RBC. The Hb values were lower in summer for both genders (from 141 ± 7 g/L in winter to 137 ± 8 in females, $p < 0.05$, and from 155 ± 8 to 150 ± 9 g/L in males, $p < 0.05$), and this trend was also evident for Ht and RBC. No significant differences could, however, be observed for MCH, mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) in females, whereas an increase of these parameters was evident in males. The different behaviour of MCV and MCH in males and females may be due to different iron supplementation pattern.

In 28 top-level male cyclists of a Danish cycling team the haematological values were repeatedly controlled during a year (from December 2006 to November 2007) by drawing 374 blood samples (Mørkeberg et al. 2009b). At the beginning of the study, when the cyclists were out of competition, the mean Ht was 45%, but it decreased to 42% during intense training and competitive season (February–August), reaching the nadir in September (40.7%). The Ht increased from September to November, at the end of competitions, to 44.7%. The same pattern of variation was observed for Hb, with an initial mean value of 152 g/L. The Hb decreased constantly during the competitive season, reaching a plateau during the June–August period (−14.8%), decreasing further until a nadir of 140 g/L in September. In the following months the value increased, approaching the baseline value, namely at 153 g/L. The reticulocyte (Ret) values were fairly stable during the entire season (0.8–1.1%), but values lower than the initial ones were recorded in July and September.

A trend for modifications of haematological parameters during the competitive season has also been confirmed in sport disciplines other than cycling, albeit with different degrees. Rjetjens et al. (2002), for example, observed that RBC decreased after the onset of the race season in four female and seven male elite triathletes followed for three seasons. Throughout the year, mean Hb values were close to the lower limit of the reference interval over the race season, although the differences during the off season, training season and race season were non-statistically significant. The training season started in January and

ended in mid March, whereas the race season ended in late October. During the training season, altitude training regimes at various elevations (i.e., 1,500–2,600 m) were included. Hb increased from 145 ± 5 to 147 ± 5 g/L after the training season, and decreased to 143 ± 4 g/L during race season in males (original values in mmol/L). Hb and Ht tended to be highest during the training season. Mean RBC was significantly ($p < 0.05$) reduced between the training and race season.

A decrease of haemoglobin during the season was also described in five male runners (149 ± 3–143 ± 3 g/L, $p < 0.05$) and 8 male swimmers (148 ± 2–139 ± 1 g/L) after a intense training period (Pizza et al. 1997).

Haematological changes were also monitored in 27 soccer players, tested for three consecutive years (Malcovati et al. 2003). The mean Hb concentration was 150 g/L in the initial phase of the season (July–September), 146 in the central part (October–January), and 147 in the final period (February–May). Ht did not change significantly (43.4 vs. 43.0%). The relative mean Ret count was 1.11% in the initial phase of the season (July–September), 0.97% in the central part (October–January) and 0.99% in the final period (February–May).

Also, Hb did not change during a season in 35 male Serbian professional soccer players, but Ht (mean ± standard deviation) was higher in the preseason period (43 ± 2%) than at start of the season (40 ± 3%) after the end of the conditioning period. Then, Ht increased to 42 ± 2% at mid season and to 44 ± 2% at the end of season (Ostojic and Ahmetovic 2009). Four blood samples were obtained from athletes of the Italian Rugby National team in the season 2004/2005 (Banfi et al. 2006a). The first sample was collected in August at the start of training period, the second in September after the training sessions and before the start of the championships, the third in January after the first part of championships and before the start of a six nation tournament and the fourth in May at the end of the national championship. The Hb values were 152 g/L at first and second collection, 147 g/L at the third, and 149 g/L at the end. The values of Ht had broader variations, from 45.4 and 45.7% at first and second collection, to 43.2 and 43.9%. The analysis of variance showed however no significant differences in any of the parameters evaluated. Ret measured by Abbott Cell Dyn 3500 were 1.08% at the baseline, 1.05% at the second blood collection, 0.90% at the third, and 0.85% in the final period. The variations of Ret during the season were significant when considering the first value as the baseline. In this study, variations of Ret were in accordance with those of Hb and RBC. Variations were observed during the first half, followed by decreasing trend in the second half of the season. However, Hb concentration remained within the physiological range.

As such, two considerations are relevant. Ret, Hb and RBC all show a nearly identical fluctuation during a season; Ret, however, undergo larger fluctuations as compared to the other variables. The values, as well as those of other RBC parameters, can be considered "physiological". In addition no significant variations were observed among different blood drawings for MCH, mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW). The fraction of immature reticulocytes (IRF%) was also constant in the four blood drawings, being 0.27 ± 0.08, 0.26 ± 0.04, 0.26 ± 0.05, and 0.26 ± 0.05%, respectively. Leukocytes and platelets were fairly stable during the entire season (Banfi et al. 2006a).

A study addressing the behaviour of Ret counts and IRF during a season in athletes from different disciplines was performed in a group of top-level sportsmen, including 13 rugby players from the Italian National Team, 12 alpine skiers from the Italian National Team, 19 professional cyclists from a ProTour team, and 19 football players from a team belonging to the First Division National Italian league (Banfi and Del Fabbro 2007). The data were collected from male athletes (19–36 years old) who were recruited to the sessions when blood samples were collected for monitoring their health status, during the entire season. These checkups generally took place 3–4 times per season: (a) before the start of the training period (pre-competitive setting); (b) at the beginning; (c) in the middle; (d) at the end of the period of competitive season. The athletes recruited had performed principally aerobic sports, which have some common aspects including an extended competitive season, a period of intense training before the season of competition, and high intensity and frequency of competitions. Ret counts in athletes of these various sport disciplines remained within the reference intervals throughout the competitive seasons.

In rugby players and skiers, the decrease of Ret during the season paralleled that of Hb, whereas this trend was not confirmed in cyclists and soccer players. Lack of correlation between the two parameters was observed in all sports during the season. The IRF increased in cyclists and skiers during the competitive season, whereas it was stable in rugby and soccer players. The behaviour of Ret and Hb in cyclists is furthermore similar to that described by Mørkeberg et al. (2009b).

The seasonal variations of Ret are often not statistically significant, although they have been reported in a team, during one season. The variations of Ret counts (%) and IRF measured with the Abbott Cell Dyn 4000 were not significantly modified during a competitive season (2003–2004) in alpine skiers from the Italian National team. Samples were obtained at rest in May, before the start of training (in July), during the aspecific training programme (in September), during the specific training programme (in November),

before the start of international competitions, and finally again in May. The males were enrolled from the national ski teams of downhill ($n = 4$), special ($n = 5$) and giant ($n = 8$) slalom, whereas females were recruited from the special slalom team ($n = 9$). The mean values of Ret varied from 1.11 to 0.91, 1.21, 1.09, 1.01%. The IRF values were more stable, being 0.30, 0.30, 0.33, 0.27, and 0.29%, respectively (Banfi et al. 2005). The individual variation of Ret during a competitive season in 96 elite athletes of various sports disciplines was found to be 21.3% and was considered not significant (Abellan et al. 2007).

Sources of variations of haematological parameters during a season

Variation of haematological parameters during the season have different sources.

When training starts, a haemodilution occurs, mainly due to the increase of extracellular body liquids caused by the renin–aldosterone axis activation. Haemodilution has been considered the only source of low Hb (i.e. <140 g/L in males, <120 g/L in females) and ferritin values (Wright et al. 1992). The phenomenon contributes to generate the "sport anemia", although only in few cases clinical symptoms impairing athletic performances are evident (Mercer and Densmore 2005). Although the decrease of haemoglobin is dependent from blood dilution, an increase of RBC turnover is evident in athletes. However, important RBC destruction, often accompanied by impaired or insufficient iron intake or absorption may occur (Mercer and Densmore 2005).

It should also be considered that the measurement of total Hb mass (tHb) based on carbon monoxide re-breathing technique is unaffected by hydration status and haemodilution, and is therefore fairly stable (Eastwood et al. 2008), although it can be increased by training in association with altitude exposure (Brugniaux et al. 2006). The tHb shows a 1-year variation of 4.6, with a 6.9% maximal fluctuation (Prommer et al. 2008); the effectivity of tHb as a tool for antidoping purposes is still under discussion. The principal source of increased turnover of RBC is the intravascular haemolysis that is commonplace in some sports, due to the impact with the terrain (footstrike haemolysis), the mechanical damage of RBC during continuous muscle contractions (Telford et al. 2003; Peeling et al. 2008), and the continuous exposure to high-oxygen flux which causes oxidative damages, perturbation of osmotic homeostasis which might render the erythrocytes more susceptible to membrane damage during their transit through the microcirculation. The studies on sport haemolysis were mainly carried out with acute exercise (Miller et al. 1988), and it is thereby difficult to evaluate its influence during an entire competitive season. A study in top-level rugby players demonstrated however a correlation between the indexes of haemolysis [i.e., increase of bilirubin, decrease of haptoglobin, decrease of mean sphered cell volume (MSCV)], and Hb decrease by comparing the values before the start of training and competitions and those performed at the end of the competitions (Banfi et al. 2007). Altitude does also influence haematological parameters in athletes, as demonstrated in subjects who reside at high altitude (Vergouwen et al. 1999; Parisotto et al. 2000) and, partially, also in athletes training for a defined period of the entire season at altitude (Rjetjens et al. 2002). A study performed in a wide series of elite athletes belonging to various sport disciplines also showed that residence at a moderate altitude (1,730–2,220 m) is by far the most consistent effect with the largest influence on blood parameters. Acute altitude can modify plasma volume by 7–10% and, consequently, the measurement of several haematological parameters (Sharpe et al. 2002).

Anyway, the time elapsed at altitude, even artificially induced by normobaric tents, usually includes heavy training and, in some sport disciplines, also competitions. Thus, altitude in studies extended throughout an entire season might have a certain influence, but its effect might be limited over time.

It should also be mentioned that the data might contain some haematological results obtained from athletes who used of illicit substances and/or procedures. However, the data were obtained from teams and not from individuals.

Conclusions

We are persuaded that the variations of haematological parameters elicited by training and competitions should be taken into account when haematological values in athletes are compared with those obtained from a sedentary population. There are few papers including comparisons between athletes and non-athletes, showing contentious results. In some studies a difference is shown, while in others the variations are modest (Table 1). A relatively high number of athletes of both gender (3.9% of males, and 1.6% of females) demonstrating high Ht values (>51% for males, >47% for females) has been highlighted in the study of Johansson et al. (2009) and, on the contrary, a relatively high number of female athletes (8.6%) having low Hb values, though not significantly different from non-athletes, was reported by Di Santolo et al. (2008). Long-lasting low Hb concentration in athletes are likely due to iron deficiency, especially in females. Iron deficiency has two primary causes, blood loss and nutritional deficiencies due to poor intake or reduction of absorption of dietary iron (Mercer and Densmore 2005; Di Santolo et al. 2008). Also,

Table 1 Comparison between athletes and sedentary people

		System, sport, season phase and reference
Athletes ($n = 98$; 62 males, 36 females)	Controls ($n = 349$; 110 sports students, 227 blood donors, 12 elite Norwegian swimmers; number of males and females not declared)	Method not specified; track and field; during competition season (Birkeland et al. 1997)
Hb (g/L) (mean)		
149 ± 10 (males)	152 ± 9*	
135 ± 10 (females)	136 ± 8	
Athletes ($n = 74$; 36 males, 38 females; lowlanders)	Controls ($n = 278$; 134 males, 144 females)	Abbott CD4000; highlanders: endurance athletes living between 2,000 and 3,000 m; lowlanders: 55 endurance athletes, 29 game and explosive sports; season phase not specified, but probably during competition season (Vergouwen et al. 1999)
Ht (%) (mean ± SD)		
0.44 ± 0.03 (lowlanders, males)	0.45 ± 0.03 (males)	
0.40 ± 0.02 (lowlanders, females)	0.41 ± 0.04 (females)	
Ht (%)		
0.47 ± 0.03 (highlanders, males)[a]		
0.44 ± 0.01 highlanders, (females)[a]		
Athletes ($n = 155$; gender not specified) Interval	Controls ($n = 23$; gender not specified) interval	Bayer H*3; cycling, swimming, rowing, track and field, boxing, cross country ski; before the start of competition season (Parisotto et al. 2000)
Ret ($\times 10^9$/L)		
46–51	40–52	
CHr (pg)		
30.7–31.2	30.4–31.4	
RetHb (g/L)		
1.14–1.59	1.24–1.61	
Athletes ($n = 106$) males	Controls ($n = 73$)	Coulter LH750; rugby, soccer, alpine ski; before the start of competition season (Banfi et al. 2006b)
Hb (g/L) (mean ± SD)		
158 (141–174)	151 (128–174)**	
Ht (%)		
46 (41–51)	45 (38–51)**	
RBC ($\times 10^{12}$/L)		
5.2 (4.6–5.9)	5.0 (4.2–5.7)**	
MCV (fL)		
88.4 (81.8–95.1)	89.1 (82.1–96.2) **	
MSCV (fL)		
85.7 (76.8–94.5)	85.9 (77.2–94.5)	
Ret (%) Median (2.5th–97.5th percentile)		
0.81 (0.30–1.54)	0.84 (0.26–1.79)	
MRV (fL)		
104.0 (93.1–114.8)	103.6 (93.0–117.8)	
IRF (%)		
0.29 (0.18–0.39)	0.30 (0.19–0.42)	
Athletes ($n = 60$) males[b]	Controls ($n = 14$)[b]	Bayer Advia120; speed skating; during competition season (Mayr et al. 2006)

Table 1 continued

		System, sport, season phase and reference
Hb (g/L) (mean ± SD)		
157.7 ± 8.9[c]	157.9 ± 9.6[c]	
Ht (%)		
46.37 ± 2.42[c]	46.16 ± 2.00[c]	
RBC ($\times 10^{12}$/L)		
5.21 ± 0.28[c]	5.36 ± 0.23**[c]	
MCV (fL)		
89.11 ± 3.35	86.20 ± 3.56**	
Ret ($\times 10^{9}$/L)		
63.44 ± 18.15	70.64 ± 14.17	
MCVr (fL)		
108.12 ± 3.17	103.81 ± 2.58**	
CHr (pg)		
30.22 ± 1.25	29.63 ± 1.79	
Athletes ($n = 56$) females[b]	Controls ($n = 17$)[b]	
Hb (g/L) (mean ± SD)		
139.8 ± 7.8[c]	143.2 ± 8[c]	
Ht (%)		
41.55 ± 2.07[c]	42.43 ± 2.23[c]	
RBC ($\times 10^{12}$/L)		
4.61 ± 0.28[c]	4.91 ± 0.30**[c]	
MCV (fL)		
90.25 ± 3.17	86.55 ± 3.59**	
Ret ($\times 10^{9}$/L)		
58.99 ± 14.88	59.64 ± 15.14	
MCVr (fL)		
109.22 ± 3.00	105.31 ± 3.68	
CHr (pg)		
29.89 ± 1.02	29.53 ± 1.11	
Athletes ($n = 70$; females, non-professional)	Controls ($n = 121$; females)	Cell Dyn Sapphire Abbott; volleyball, soccer, martial arts, skiing, cycling; before the start of competition season (Di Santolo et al. 2008)
Hb (g/L) (mean ± SD)		
127 ± 7.4	129 ± 8.6	
Ht (%)		
37.8 ± 2.42	38.1 ± 2.46	
RBC ($\times 10^{12}$/L)		
4.33 ± 0.34	4.38 ± 0.36	
MCV (fL)		
87.6 ± 3.97	87.3 ± 4.54	
MCH (pg)		
29.5 ± 1.46	29.6 ± 1.76	
RDW (%)		
13.1 ± 1.47	13.0 ± 1.56	
Ret (%)		
1.2 ± 0.42	1.2 ± 0.38	
Athletes ($n = 1{,}406$; 1,116 males, 290 females)	Controls ($n = 85{,}846$; 36,962 males, 48,884 females, blood donors)	Hemocue; rowing; season phase not specified (Johansson et al. 2009)

Table 1 continued

	System, sport, season phase and reference
Hb (mean ± SD)	
159.3 ± 11.6 (males)[d]	152.4 ± 9.8[**d]
Hb	
143.8 ± 10.4 (females)[d]	134.3 ± 9.5[**d]

The studies are chronologically listed

CHr haemoglobin content of reticulocytes, *RetHb* haemoglobin content of reticulocytes, *MRV* and *MCVr* mean volume of reticulocytes

* $p < 0.05$; ** $p < 0.01$

[a] $p < 0.01$ between highlanders and lowlanders athletes

[b] Age range, 14–18 years

[c] Significant difference ($p < 0.01$) for both athletes and non-athletes between gender for Hb, Ht, RBC

[d] Original values expressed in mmol/L

all the studies investigating the variations of haematological parameters during the competition season were performed on teams. Therefore, the potential iron dietary deficiency that occurred in some athletes has little influence on mean haematological values, as well as on their variations associated with training and competitions.

An effective comparison between athlete and non-athlete populations should hence be carried out standardizing the period of season when such assessment is carried out. The Bayesian approach encompasses the adoption of specific reference values because reference intervals derived from general population should be used cautiously in athletic populations, especially during intense training and competition periods and permanence in different locations (e.g., altitude training).

In conclusion we can summarize that:

1. Some haematological parameters (i.e., Ht, Hb and RBC) might be influenced by long-term training and competition periods.
2. Hb and Ht are decreased during the intense periods of training, throughout the season, in various sport disciplines, with the exception of soccer.
3. On average, in the published studies in different sport disciplines, the decline of Hb ranges from 3 to 8% during the competition season, while the range of Ret% varies from 5 to 21%.
4. Reticulocytes are also decreased after long periods of training and competitions; their variations are not necessarily associated with that of Hb.
5. The qualitative variations of haematological parameters are mainly independent of the sport discipline, but quantitatively dependent on sport discipline.
6. The variations of haematological parameters within the same sport discipline are qualitatively concordant and quantitatively different among separate but consecutive competitive seasons.

The variations of haematological parameters over a season have concrete implications for the Bayesian procedure. In fact, the ranges defined by statistical procedure calculated by a specific software are determined on the individual values of each athlete. If these parameters are modified by training and competition in some sport disciplines, the ranges are modified. Thus, some variations and (or) fluctuations of the parameters, even within the predefined ranges, could erroneously or contentiously interpreted.

We conclude that for antidoping purposes more studies investigating haematological change during the season might be advisable. We recommend that the evaluation of haematological parameters over time should be performed in many different sport disciplines and in wider population to guarantee a correct interpretation. The characteristics of training and competitions should collected as "athlete's whereabouts information", as already defined by World Antidoping Agency for athlete's location.

We also expect to stimulate a meaningful debate on this topic, hoping that a greater amount of data might be available to sports scientists and physicians.

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