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Yearly intrasubject variability of hematological biomarkers in elite athletes for the Athlete Biological Passport

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Abstract

Confounding factors including exercise and environments challenge the interpretation of individual Athlete Biological Passports (ABPs). This study aimed to investigate the natural variability of hematological ABP parameters over 1 year in elite athletes compared with healthy control subjects and the validity of a multiparametric model estimating plasma volume (PV) shifts to correct individual ABP thresholds. Blood samples were collected monthly with full blood counts performed by flow cytometry (Sysmex XN analyzers) in 20 elite xc-skiers (ELITE) and 20 moderately trained controls. Individual ABP profiles were generated through Anti-Doping Administration & Management System Training, a standalone version of the ABP's adaptive model developed by the World Anti-Doping Agency. Additionally, eight serum parameters were computed as volume-sensitive biomarkers to run a multiparametric model to estimate PV. Variability in ELITE compared with controls was significantly higher for the Abnormal Blood Profile Scores ($P = 0.003$). Among 12 Atypical Passport Findings (ATPF) initially reported, six could be removed after correction of PV shifts with the multiparametric modeling. However, several ATPF were additionally generated ($n = 19$). Our study outlines a larger intraindividual variability in elite athletes, likely explained by more frequent exposure to extrinsic factors altering hematological biomarkers. PV correction for individual ABP thresholds allowed to explain most of the atypical findings while generating multiple new ATPF occurrences in the elite population. Overall, accounting for PV shifts in elite athletes was shown to be paramount in this study outlining the opportunity to consider PV variations with novel approaches when interpreting individual ABP profiles.

KEYWORDS
Athlete Biological Passport, elite athletes, hematology, longitudinal monitoring, plasma volume

1 | INTRODUCTION

Longitudinal screening of hematological or steroidal variables has complemented the direct detection of prohibited substances to allow for both an indirect detection of doping in sports and targeted tests triggered by abnormal variations in individual variables over time.[1,2] Using an adaptive Bayesian framework, the hematological module of the Athlete Biological Passport (ABP) consists of individual and longitudinal monitoring of indirect markers of altered erythropoiesis that represent diagnostic biomarkers responsive to blood doping.[3] The ABP was hence developed to discriminate the effects of specific doping practices such as recombinant human erythropoietin use from natural physiological variation. This adaptive model therefore aims to identify nonphysiological variations that may correspond to different types of blood doping by using initial population reference values, before individual standards are incorporated to define expected ranges for single variables.[4]

Twelve hematological markers and two scores currently compose the blood module of the ABP.[5] Values for hemoglobin concentration ([Hb]) and the OFF score (OFFs) (as primary markers of the ABP's hematological module) beyond individual thresholds or in an atypical variation sequence will generate so-called Atypical Passport Findings (ATPF) and a mandatory review by an appointed expert who ultimately weights the likelihood of doping with any other causes. Designed to have a higher sensitivity than the same parameters taken independently, the two additional scores, namely, the OFFs and the Abnormal Blood Profile Score (ABPS), combine multiple biomarkers and are part of the longitudinal follow-up.[6]

In addition to its deterrent effect,[7] the significant impact of blood doping on ABP biomarkers has been outlined over time.[8] However, for experts to correctly identify artificial biomarker variation, the normal physiological variation must be established. Accordingly, several studies aimed to estimate this natural variation for general hematological parameters[9,10] or for specific markers such as reticulocytes.[11] Therefore, short-term (~4 weeks, i.e., the duration of an altitude training camp) effects of confounders such as transient plasma volume (PV) shifts and natural erythropoiesis alterations are well described.[12] However, although further investigated in recreational endurance athletes,[13] the evidence of long-term (>1 month) physiological variation in elite endurance athletes is limited.

To counteract the confounding effects of PV fluctuations inherent to the athlete's daily life on the concentration-based ABP parameters, a multiparametric approach based on serum markers has been developed to correct PV variations influencing concentration-based ABP parameters.[14] By correcting individual ABP thresholds, this model proved to be promising to support the interpretation of concentration-based variables[14] while strong confidence in PV estimates required a sufficient number of samples. First proposed in athletes during an altitude training camp[15] and a multiple-stage cycling race,[16] this model has recently been applied to active women taking oral contraception.[17] Despite some additional ATPF generated when the PV correction is applied,[15] this model allows adapting the limits to decrease the effect of PV shift on concentration-based ABP parameters. However, if the suggested multiparametric model seems to be accurate over a 6-month follow-up in healthy active males,[18] no study has yet evaluated the model over an extended period of time in elite athletes.

The present study aimed to determine the monthly variability of PV and hematological markers over 12 months in elite athletes compared with a healthy control group. It was first hypothesized that the within-subject variation would be larger in the elite group due to frequent exposure to confounders. In addition, this study addressed the validity of a multiparametric model to account for actual PV changes from additional serum biomarkers over a prolonged time frame in elite athletes. It was finally hypothesized that changes in the latter volume-sensitive markers would allow to adequately predict PV shifts for a better correction of potential ATPF generated.

2 | METHODS

2.1 | Study participants and design

Forty subjects were recruited for this study subdivided into a group of 20 elite xc-skiers (ELITE) (16 men and 4 women) and a control group of 20 healthy and moderately trained sports students (CON) (11 men and 9 women). Anthropometric characteristics and laboratory data of the study population are reported in Table 1. All participants were informed of the study protocol and potential risks and signed a consent document as an agreement before the start of the study. The study protocol was approved by the regional research ethics committees (Lillehammer, Norway, #21/01894; Lausanne, Switzerland, #2018-01019; and Copenhagen, Denmark, #H-21022277) and conducted in strict compliance with the Declaration of Helsinki.

2.2 | Blood sampling and analysis

Participants visited the laboratory every month during the entire year of the protocol with an average of 30.8 ± 11.3 days between each visit for a total of 11.1 ± 1.1 visits over 12 months. Hematological sample analysis was performed in three distinct laboratories. In total, 441 samples were collected in Lausanne ($n = 155$), Copenhagen ($n = 66$), and Lillehammer ($n = 220$). Because the study investigates intrasubject variability, fresh blood samples were analyzed directly at each site of collection allowing all samples from the same subject to be analyzed on the same instrument. Venous blood samples were collected by experienced phlebotomists with very strict preanalytical and analytical procedures to minimize any effect on the latter result.[5] Consequently, strenuous exercise was avoided by the participants 2 h before the measurements, and each blood sample was taken after strictly observing 10 min of rest, in a sitting position. Visits were randomly distributed throughout the day to reproduce a real-life scenario. Whole blood was collected in one 3 mL ethylenediamine

TABLE 1 Demographic characteristics and laboratory data of the study population.

	ELITE ($n = 20$)		CONTROL ($n = 20$)	
	Men ($n = 16$)	Woman ($n = 4$)	Men ($n = 11$)	Woman ($n = 9$)
Average samples	10.9 ± 1.3	11.3 ± 1	11.4 ± 0.9	11 ± 0.7
Max/min samples	12/8	12/10	12/9	12/10
Age (years)	26 ± 5.8	25 ± 2.8	35 ± 9.0	23 ± 1.5
Weight (kg)	75 ± 5.6	65 ± 3.6	72 ± 7.5	62 ± 6.0
Height (cm)	181 ± 5.8	171 ± 3.9	178 ± 4.1	166 ± 4.8
$\dot{V}O_2$max (mL·min·kg^{-1})	71 ± 3.9	62 ± 5.6	—	—
Hbmass (g)	979 ± 122	715 ± 74	913 ± 97	585 ± 66
PV (mL)	3637 ± 487	3054 ± 297	3585 ± 66	2909 ± 363
[Hb] (g·dL^{-1})	15.0 ± 0.8	13.7 ± 0.9	14.7 ± 0.8	12.5 ± 0.6
Ret%	0.99 ± 0.2	1.07 ± 0.2	0.97 ± 0.2	1.21 ± 0.3
OFFs (a.u.)	91 ± 10	75 ± 11	88 ± 10	60 ± 9
ABPS (a.u.)	−0.73 ± 0.75	−1.19 ± 0.52	−1.17 ± 0.52	−1.43 ± 0.43

Note: Values reported as means ± standard deviations.
Abbreviations: ABPS, Abnormal Blood Profile Score; [Hb], hemoglobin concentration; Hbmass, total hemoglobin mass; OFFs, OFF score; PV, plasma volume; Ret%, reticulocyte percentage; $\dot{V}O_2$max, maximal oxygen uptake.

tetraacetic acid BD Vacutainer® tube (EU ref. 368.856) and two 5 mL serum BD Vacutainer® SST II Plus tubes (EU ref. 367.955). After homogenizing the ethylenediamine tetraacetic acid tubes at room temperature for 15–30 min, blood analyses were performed with an automated flow cytometer (Sysmex XN, Sysmex, Norderstedt, Germany). The following variables were recorded: hematocrit, [Hb], red blood cell count (RBC), reticulocyte percentage (Ret%) and reticulocyte count, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), mean corpuscular volume (MCV), immature reticulocyte fraction, platelets, red cell distribution width, white blood cells, and OFFs (calculated as $([Hb] \times 10) - 60 \times \sqrt{Ret\%}$). Samples from CON nos. 1–7 were processed on a Sysmex XN-350, CON nos. 8–20 on a Sysmex XN-1000, and ELITE using a Sysmex XN-450. [Hb] and Ret% were verified to produce two successive analyses with differences equal to or less than 0.1 g·dL^{-1} for [Hb%].[5] The first valid test result was then kept. Samples for each subject were analyzed on the same analyzer for the whole study period.

In order to respect and guarantee the strict reliability and comparison of the measurements between all analyzers, a correction of −0.22 of the Ret% was performed on all samples from a Sysmex XN-350 or 450 as recently advised[19] (XN-350 and 450 models being calibrated as XT instruments). Internal quality controls provided by the manufacturer (Sysmex XN-Checks, levels 2 and 3) were run through twice before the analysis of each batch of samples. According to the ABP guidelines provided by the World Anti-Doping Agency, two successive results with differences of ≤0.1 g·dL^{-1} for [Hb] and 0.15% (if inferior to 1%) or 0.25% (if superior to 1%) for Ret% were required before recording the first value in the analytical sequence. A semiautomated system was used to determine the PV by applying the carbon monoxide (CO) rebreathing method (OpCo: Detalo Health, Birkerod, Denmark) with a procedure fully detailed elsewhere.[20]

2.3 | ABP profiles

To determine individual limits considered as "natural physiological variation," the ABP model was implemented using a Bayesian adaptive algorithm. Starting with population averages as an initial reference, upper and lower thresholds were determined for each profile and then individualized according to the introduced results of the subject. New ABP profiles for each participant were generated on the Anti-Doping Administration & Management System (ADAMS) Training, the online ABP software developed by the World Anti-Doping Agency which includes the adaptative functionality. Afterward, results for each time point were recorded to generate adaptative longitudinal follow-up. In addition to [Hb] and Ret% recorded from the blood analyses, ABPS[21] and OFFs were reported by the software as compound scores for each measurement. [Hb] and OFFs are defined as the primary markers of the ABP, while Ret% and ABPS constitute the secondary markers. An ATPF can be generated by two scenarios: (a) [Hb] or OFFs values exceed the individual limits (specificity at 99%, i.e., at least 1:100 probability of this finding resulting from a natural physiological condition) or (b) if a sequence of [Hb] or OFFs levels deviates from a physiologically acceptable range then called "sequence ATPF" (specificity at 99.9%).[5] Initially based on population ranges, it is generally assumed that at least three blood samples are required to establish a basic ABP profile and provide individualized profiles.[5] Therefore, all ATPF generated during the first three points were not considered in the results.

2.4 | PV biomarkers

After blood sampling, serum samples were kept in a vertical position for 20 min before being centrifuged (Z326K, Hermle Labortechnik,

Wehingen, Germany) for 10 min at 2500 rpm. Finally, tubes were aliquoted in three 800 µL aliquots and immediately stored in a freezer at −20°C. All transport of serum samples to the laboratory was performed frozen (using dry ice to ensure the proper transport temperature) and analyzed within 24 h after thawing (single freezing and thawing cycle). Serological analyses were performed at the Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland). Transferrin (TFN), creatinine (CRE), calcium (CA), low-density lipoprotein (LDL), albumin (ALB), and total protein (TP) were analyzed by photometric immunochemistry technique (COBAS 8000, Roche Diagnostics, Switzerland). All samples from the same subject were analyzed in a single batch. The analytical results of eight volume-sensitive biomarkers ([Hb], platelets, TFN, CRE, CA, LDL, ALB, and TP) were used to run the multiparametric model and estimate the PV variations (*Z*-scores). Subsequently, individual reference upper and lower limits for [Hb] and OFFs were adjusted based on the PV estimation and predicted hemodilution or hemoconcentration. In addition to the corrected thresholds, the multiparametric model provides a weighting function to temper abnormal values of one or more biomarkers in the PV estimation. Therefore, if one marker shows an opposite trend compared with the other because of individual factors (e.g., pathology), the weight of the related marker in the PV estimation will be reduced. Based on this, a confidence level index normalized between 0% and 100% is determined for all estimation points, providing a further interpretation of the adjusted limits. A complete description of the applied methodology is detailed by Lobigs et al.[18]

2.5 | Statistical analyses

Data are reported as means ± standard deviations. The normality of the distributions was tested with the D'Agostino and Pearson tests. As part of the hematological parameters of the primary markers of the ABP, OFFs and ABPS metrics were generated by the ADAMS Training Software. Seasonal variations were quantified by calculating group averages during summer (June, July, and August), autumn (September, October, and November), winter (December, January, and February), and spring (March, April, and May). Differences between groups for annual hematological averages were determined using unpaired Student's *t*-tests. Mixed models for repeated measures with Tukey's multiple comparisons were performed to evaluate the differences between groups (ELITE vs. CON), sexes (male vs. female), and seasons on ABP variables (i.e., [Hb], Ret%, OFFs, and ABPS). Additionally, time × group interaction between ELITE and CON was investigated through linear mixed model for repeated measures with Šidák's multiple comparisons. Groups' variability (ELITE vs. CON) over time for hematological and volume-sensitive variables was calculated as coefficients of variation (CV, %) from the mean of each subject's CV over successive time points (CV_I). Finally, unpaired Student's *t*-tests were performed between groups to compare CV_I averages of each hematological variable. Linear regression analyses were conducted to compare PV shift estimated *Z*-scores (with volume-sensitive biomarkers) and PV shift measured *Z*-scores (with CO-rebreathing method) for both ELITE and CON (excluding the first three samples). The level of significance was set at $P < 0.05$. All statistical analyses and figures were generated using GraphPad PRISM® Version 9 (GraphPad Software Inc., La Jolla, CA, USA).

3 | RESULTS

3.1 | Between-group variability

Average hematological values were significantly higher for men in ELITE compared with CON for [Hb] (15.0 ± 0.8 vs. 14.7 ± 0.8 g·dL^{-1}, $P = 0.006$) and ABPS (−0.73 ± 0.75 vs. −1.17 ± 0.52 a.u., $P < 0.0001$), while Ret% (0.99 ± 0.2% vs. 0.97 ± 0.2%, $P = 0.68$) and OFFs (91 ± 10 vs. 88 ± 10 a.u., $P = 0.08$) were not significantly different. For female subjects, differences were observed between ELITE and CON for [Hb] (13.7 ± 0.9 vs. 12.5 ± 0.6 g·dL^{-1}, $P < 0.0001$), Ret% (1.07 ± 0.2% vs. 1.21 ± 0.3%, $P = 0.02$), and OFFs (75 ± 11 vs. 60 ± 9 a.u., $P < 0.0001$), while ABPS was not statistically different (−1.19 ± 0.52 vs. −1.43 ± 0.43 a.u., $P = 0.13$) (Table 1). Although no significant variation over 12 months was observed for CON among the primary and secondary markers of the ABP, a significant effect of time was found for OFFs ($P = 0.025$) and ABPS ($P = 0.045$) in ELITE. However, no seasonal effect was reported for either group. Finally, when comparing ELITE and CON, a time × group interaction was found for [Hb] ($P = 0.015$), OFFs ($P = 0.009$), Ret% ($P = 0.022$), and ABPS ($P = 0.012$). Averaged hematological and serum variables for ELITE and CON over the 1-year monitoring are available in Tables S1 and S2.

3.2 | Within-subject variability

When comparing CV_I for each hematological variable, a greater variability for ELITE was observed for RBC ($P = 0.041$), MCV ($P = 0.005$), MCH ($P = 0.001$), MCHC ($P = 0.011$), and ABPS ($P = 0.002$) compared with CON (Figure 1). In addition, all serum biomarkers showed higher variability in the elite cohort, namely, TFN, CRE, CA, LDL, ALB, and TP (all at $P < 0.0001$). A significant correlation was observed between shifts in estimated and measured PVs in CON ($r = 0.56$, $R^2 = 0.31$, $P < 0.0001$) and ELITE ($r = 0.22$, $R^2 = 0.05$, $P = 0.024$) (Figure 3). Standard deviations and coefficients of variations for hematological and volume-sensitive variables are reported in Table 2.

3.3 | ABP profiles and multiparametric modeling of PV shifts

Once computed in ADAMS, 10 ATPF were generated in ELITE and two in CON, among 10 different individual subjects (eight ELITE and two CON). With specificity levels set at 99%, individual [Hb] thresholds were exceeded at six different time points in ELITE (6/168; 3.5%) and two in CON (2/241; 0.8%) (Table 3). In addition,

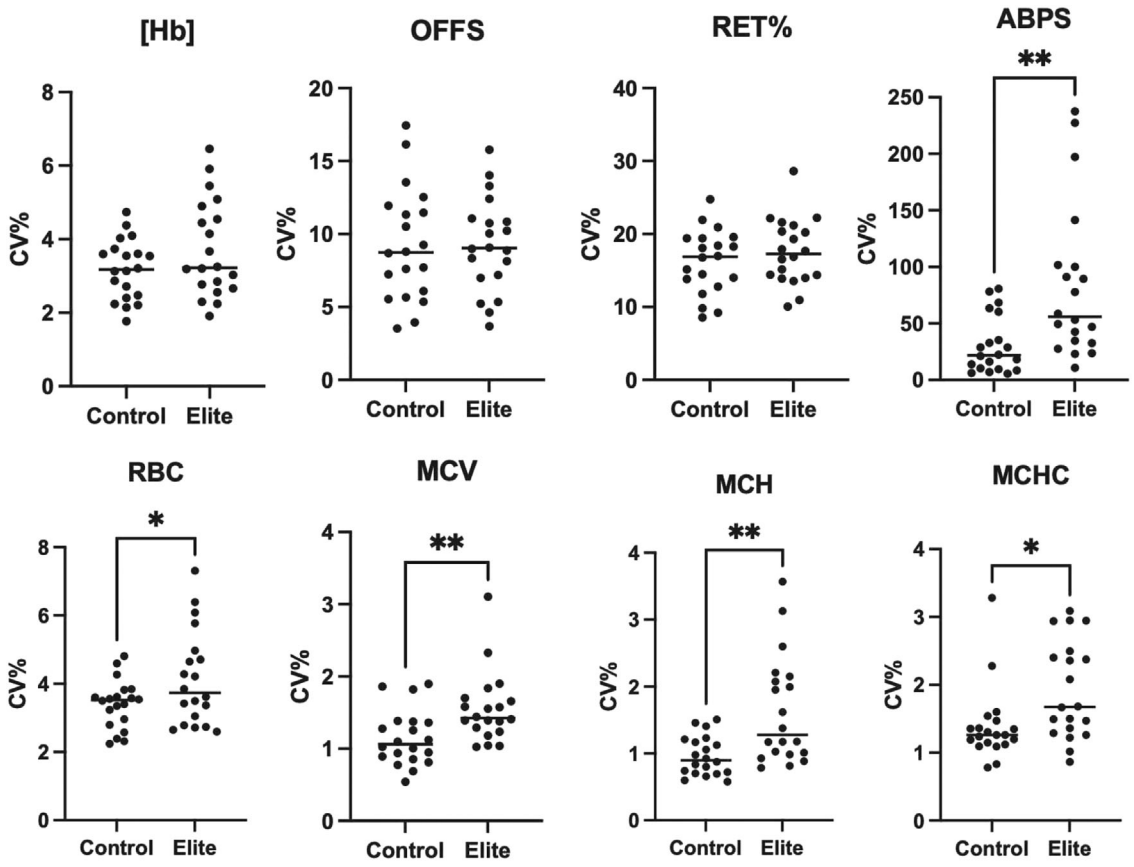

FIGURE 1 Within-subject coefficient of variation (CV%) for hematological variables in control and elite groups. Hemoglobin concentration [Hb], OFF score (OFFs), reticulocytes percentages (ret%), Abnormal Blood Profile Score (ABPS), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). *$P < 0.05$ and **$P < 0.01$ for the difference between groups.

the individual OFFs limits generated four ATPF in ELITE (4/168; 2.4%). RET% exceeded the individual limits on a single occasion for one ELITE subject. When applying the multiparametric model, six ATPF (out of 12 generated by [Hb] or OFFs) could be explained by variations in estimated PV (five for ELITE and one for CON) (Figure 2). However, multiple additional ATPF not initially reported by the traditional Bayesian limits were generated by the multiparametric model ($n = 19$; 18 for ELITE and 1 for CON). The mean confidence interval in PV estimation based on serum markers was significantly lower for ELITE (53%) compared with CON (71%) ($P < 0.0001$). All ATPF generated by traditional and corrected thresholds are reported in Table S3. Individual profiles with corrected thresholds for all subjects are available in Figures S1–S40.

4 | DISCUSSION

In the present study, the hematological variability between ELITE and CON was investigated over a 1-year period. Additionally, this is the first study to test the validity of the multiparametric model based on volume-sensitive variables over an extended period, therefore representative of the real-life situation. With an increased variability for few biomarkers only, the initial hypothesis suggesting greater variability in an athlete's population was only partially supported. Concomitantly, the number of ATPF reported in ELITE ($n = 10$) was higher than in CON ($n = 2$). However, the multiparametric model showed some limitations to systematically remove the influence of PV shift on the ABP concentration-based biomarkers in our elite population.

When comparing individual variability (men and women combined), several hematological variables showed significantly higher variability in ELITE compared with CON, namely, RBC, MCV, MCH, MCHC, and ABPS (Figure 1). These CV_I values seem higher compared with those recently observed in recreational endurance athletes,[13] which may be explained by the effect of exercise on concentration-based hematological variables (i.e., fluid shifts between intravascular and interstitial spaces leading to higher PV variations). Indeed, with slightly higher variability already observed in comparison with a general population, these results clearly illustrate the likely amplifying impact of training level on hematological variability. Therefore, by highlighting an elevated individual dispersion, these results highlight the within-subject variability specific to this elite population. This may have implications for research related to the ABP and the investigation of confounding factors. Therefore, to provide a representative overview of the target population, these findings reinforce the need

TABLE 2 Dispersion and variability for Athlete Biological Passport and serum biomarkers in ELITE and CONTROL groups.

	ELITE			CONTROL		
		Standard deviation	Coefficient of variation		Standard deviation	Coefficient of variation
Blood biomarkers	($n = 220$)			($n = 221$)		
[Hb] ($g \cdot dL^{-1}$)		0.54	3.72		0.43	3.17
Hct (%)		1.69	3.92		1.38	3.46
RBC ($10^6 \cdot \mu L^{-1}$)		0.19	4.13		0.15	3.40
Ret (%)		0.17	17.5		0.17	16.20
Ret# ($10^6 \cdot \mu L^{-1}$)		0.01	18.1		0.01	16.65
MCV (fL)		1.38	1.55		1.01	1.14
MCH (pg)		0.49	1.63		0.29	0.96
MCHC ($g \cdot dL^{-1}$)		0.65	1.92		0.47	1.38
RDW-SD (fL)		1.23	3.04		1.12	2.77
IRF (%)		1.40	29.4		1.45	31.76
PLT ($10^3 \cdot \mu L^{-1}$)		17.5	7.80		21.29	8.62
WBC ($10^3 \cdot \mu L^{-1}$)		0.85	17.0		1.13	18.03
OFFs (a.u.)		7.94	9.2		6.47	9.21
ABPS (a.u.)		0.54	83.3		0.32	30.77
Serum biomarkers	($n = 216$)			($n = 179$)		
TFN ($\mu mol \cdot L^{-1}$)		3.19	11.0		1.71	5.36
ALB (gL)		4.27	9.81		1.47	3.20
CA ($mmol \cdot L^{-1}$)		0.18	8.51		0.05	2.44
CRE ($\mu mol \cdot L^{-1}$)		8.10	11.0		4.15	5.57
TP ($g \cdot L^{-1}$)		6.49	9.93		2.13	3.11
LDL ($mmol \cdot L^{-1}$)		0.36	15.9		0.25	10.18

Abbreviations: ABPS, Abnormal Blood Profile Score; ALB, albumin; CA, calcium; CRE, creatinine; [Hb], hemoglobin concentration; Hct, hematocrit; IRF, immature reticulocyte fraction; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; OFFs, OFF score; PLT, platelet; RBC, red blood cell count; RDW-SD, red cell distribution width; Ret% and Ret#, reticulocyte percentage and count; TFN, transferrin; TP, total protein; WBC, white blood cell.

TABLE 3 Frequency of atypical passport findings (with specificity levels set at 99% and 99.9%) for hemoglobin concentration ([Hb]) and OFF score (OFFs) with and without the corrective model based on plasma volume estimation for ELITE and CONTROL subjects.

[Hb]		ELITE		CONTROL	
Without correction	99%	6/168	3.5%	2/241	0.8%
	99.9%	0/168	0%	0/241	0%
With correction	99%	16/168	9.5%	1/241	0.4%
	99.9%	0/168	0%	0/241	0%
OFFs					
Without correction	99%	4/168	2.4%	0/241	0%
	99.9%	0/168	0%	0/241	0%
With correction	99%	7/168	4.2%	1/241	0.4%
	99.9%	0/168	0%	0/241	0%

for more statistically significant elite athlete cohorts in antidoping research.

Considering individual ABP profiles, 10 ATPF in ELITE and 2 in CON were generated by the ADAMS software, mainly due to exceeding the individual threshold for [Hb] (Figure 2). Despite the inclusion of a larger number of subjects, the percentage of ATPF is higher than previously observed in longitudinal studies with both recreational[22] and elite athletes.[23] Moreover, in contrast to our hypotheses, some ATPF could not be corrected despite using the multiparametric model based on volume-sensitive markers. In

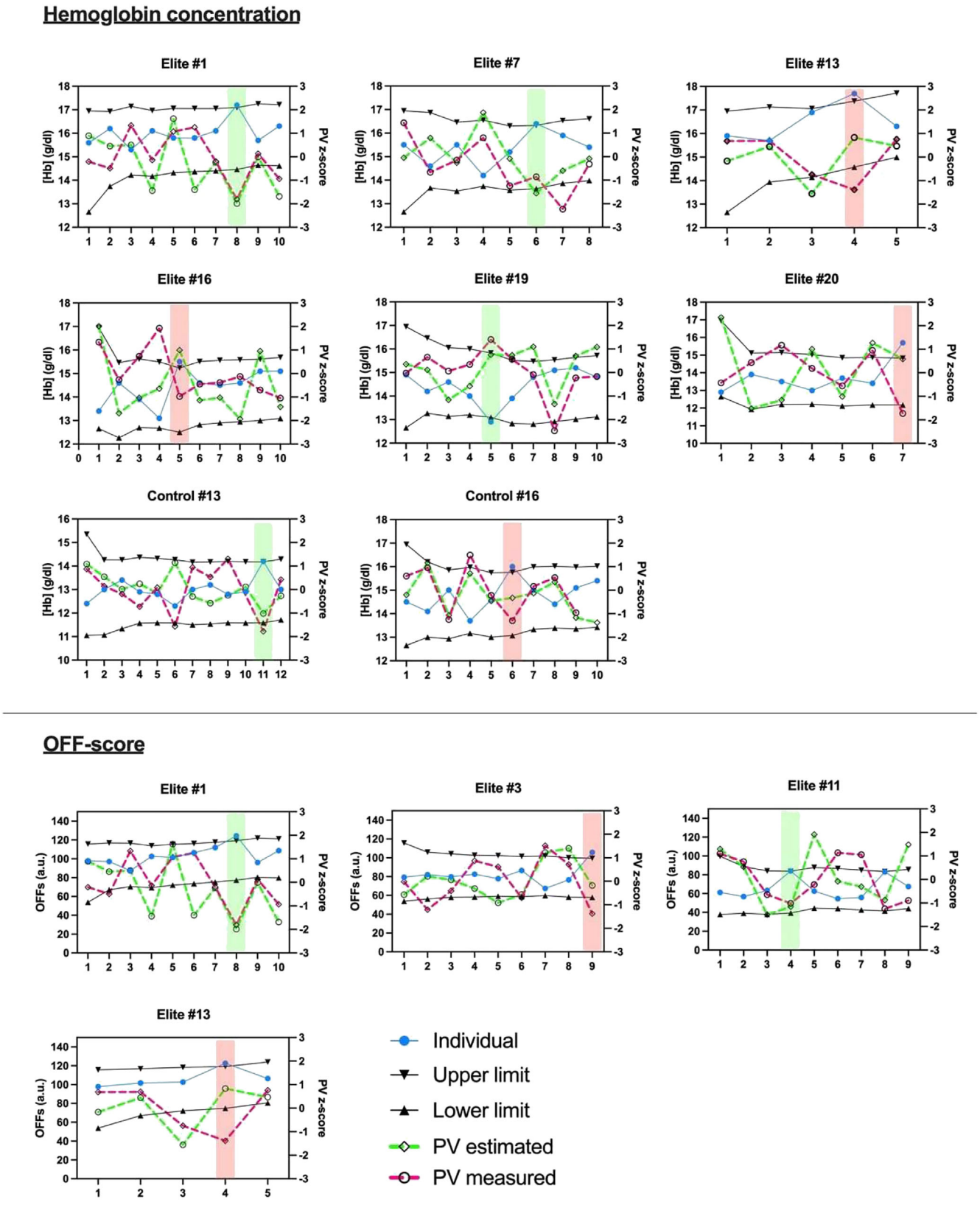

FIGURE 2 Illustration of all atypical passport findings (ATPF) generated by hemoglobin concentration ([Hb]) and OFF score (OFFs) for elite and control subjects. Black lines for ABP individual thresholds (upper and lower) set at a specificity of 99%, blue line for individual values, green line for estimated plasma volume (PV) *Z*-score (based on serum biomarkers), and violet line for measured PV *Z*-score (though CO-rebreathing method). Green bands correspond to ATPF rectified by the PV estimation model and red bands to uncorrected ATPF.

addition, the correction of individual limits with the multiparametric model subsequently generated a higher number of ATPF among ELITE ($n = 18$), suggesting considering such correction in athletes with care. This may be illustrated by the lower correlation reported between measured (CO-rebreathing method) and estimated (serum markers) PVs in ELITE ($R^2 = 0.05$) compared with CON ($R^2 = 0.31$) (Figure 3). These findings contrast with previous studies supporting the robustness of the model to consider PV fluctuations in the ABP context with a stronger correlation observed between measured and estimated PV shifts in elite road cyclists ($R^2 = 0.66$) or in healthy women ($R^2 = 0.26$).[16,17]

The weaker predictive ability for PV shifts in ELITE was likely explained by the higher variability of the biomarkers included in the multiparametric model with monthly measurements. While individual coefficients of variation observed in CON for PV variables were all in close agreement to or even lower than those previously reported,[18] a larger variability was systematically observed on average in ELITE. Considering this variability, it is therefore not surprising to observe a lower average confidence level (of the corrective model) for ELITE (53%) compared with CON (71%) (the confidence level for each additional ATPF is reported in Table S3). With a similar level obtained in healthy female subjects (59%),[17] the confidence level in elite athletes therefore seems slightly reduced. These results suggest that the model may be valid if sufficient samples are analyzed within a shorter time frame where large changes in PV may be expected (e.g., descent from altitude, tapering period, or stage race),[16,17] but not systematically,[15] while volume-sensitive blood biomarkers may be more altered when considering cumulated effects of several confounders that may occur with monthly samples.

The increased variability in PV markers may also be related to larger fluctuations in PV in an elite athlete population having higher absolute PV initial levels. Indeed, it is well-known that PV is largely influenced by lifestyle and therefore impacted by the level of physical activity.[24] In this context, many studies demonstrated the impact of training load variations on PV changes.[9,23,25,26] In addition, despite no physical activity for 2 h prior to each visit as required by ABP guidelines,[5] it is not possible to rule out an impact of prolonged exercise[27] before these 2 h as well as the cumulative effects of multiday racing (or strenuous training) on PV expansion.[28] Overall, our results, therefore, support the importance of considering acute variations in training volume when interpreting individual ABP profiles.[23]

Similarly, more prevalent exposure to extreme environmental conditions to benefit from additional physiological stimuli may also explain the increased variability of PV-related markers in ELITE. In this context, hematological adaptations during and after altitude training protocols have been extensively studied,[29] and many studies have shown a significant impact of hypoxia on ABP variables.[30,31] Consequently, hemoconcentration constitutes one of the first hematological adaptations in the hours following reaching altitude prior to later erythropoiesis stimulation,[29] resulting in an impact on [Hb] and Ret%. Besides, training in a hot environment is becoming more common among athletes,[32] which increases PV[33] and possibly accelerates erythropoiesis,[34] which will have a substantial influence on the hematological variables.

Besides higher variability of the volume-sensitive biomarkers, a disparity between the priors applied in the corrective model and the averages measured in the present population was systematically reported (independently of gender or athletic level) and could possibly explain some of the additional ATPF as well. Indeed, the average of many variables was significantly lower in comparison with the ones applied in the model, especially for LDL and CRE with differences of −20% and −27%, respectively. In addition, without recommendations related to the analyzer to be used, analytical variability between different measuring instruments needs to be considered. The analyzer used in the present study may not necessarily result in a lesser analytical variability compared with other models from the same manufacturer[15] or from different companies[17,18] previously used to measure serum variables. Consequently, a thorough calibration of the volume-sensitive biomarkers priors associated with specific guidelines related to the optimal analytical instrument to be used shall be considered a potential improvement in the future development of the model.

Therefore, if the corrective model seems to be suitable for moderately trained subjects, the daily-life PV fluctuations observed in elite athletes across the year seem to be a reasonable argument to explain the more mixed results in this cohort. However, despite apparently optimal preanalytical conditions, it should be mentioned

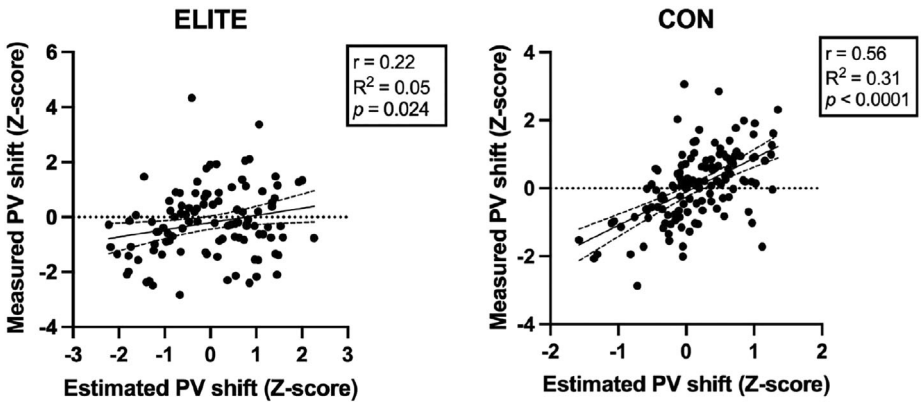

FIGURE 3 Measured changes in plasma volume (PV) with CO-rebreathing method versus predicted changes in PV applying the eight volume sensitive biomarkers. Changes (*Z*-scores) are determined by the deviations from an individual mean as computed by an adaptive Bayesian model.

that preanalytical conditions that might have affected the results of elite subjects cannot be completely excluded (e.g., putative alteration of frozen samples during transfer to the laboratory). Thus, considering the promising outcomes of previous studies that applied the corrective model,[16,17] these results must be carefully interpreted. The long-term applicability of this model as a major step forward in the interpretation of hematological profiles shall be considered for further investigations. Nevertheless, our results outline the opportunity to investigate novel approaches to obtain accurate estimates of PV to support the interpretation of individual longitudinal fluctuations of blood markers. In this context, the estimation of PV through a machine-learning approach requiring solely hematological variables collected for the ABP as recently proposed by our research group[35] could represent a particularly robust and easily applicable approach to discriminate confounding factors related to PV shifts.

5 | STRENGTHS AND LIMITATIONS

Investigating a cohort of elite subjects monitored longitudinally over 1 year represented a true strength of this study. The principal limitation lays however in the unequal distribution between men and women subjects, especially for the elite group (16 vs. 4). A more homogeneous distribution in male and female athletes may have helped further address any putative sex differences that were however not highlighted in the current study. In addition, although the hematology analyzers were from the same series (Sysmex XN series) with quality controls from identical batches, the use of distinct instruments between the two groups remains a limitation worth mentioning. Besides, the potential influence of preanalytical conditions on the model's performance in elite athletes cannot be completely excluded. Finally, despite informed consent describing the aims of the study with a link to antidoping, resort to illicit doping practices during the protocol cannot be fully excluded.

6 | CONCLUSION

The results of this study suggested a higher variability of multiple variables included in the hematological module of the ABP (e.g., ABPS) in elite athletes compared with healthy control subjects. Illustrated by a high within-subject variability despite within-group stability over 12 months, these findings reinforce the need to carefully consider several concomitant confounders that affect blood variables and their variations in individual elite athletes. In addition, increased occurrences of atypical variations in blood parameters (such as [Hb]) were observed in elite athletes, likely caused by PV shifts. In this context, a better assessment of confounding factors and the development of innovative approaches to estimate PV variations should be pursued in the later development of the ABP to improve the interpretation of hematological variations by experts in individual athletes.

AUTHOR CONTRIBUTIONS

R. F., C. L. and J. B. designed the study. B. K., R. F., J. S., J. H., H. S., C. L. and J. B. contributed to data collection. T. E. performed the multivariate analysis with the adaptive model. B. K. drafted the first version of the manuscript, and all authors revised it critically. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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