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Biological variation estimates for prostate specific antigen from the European Biological Variation Study; consequences for diagnosis and monitoring of prostate cancer

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ABSTRACT

Background: Prostate-specific antigen (PSA) is central in the diagnosis of prostate cancer. However, high-quality biological variation (BV) estimates for PSA are scarce. Here BV estimates from the European Biological Variation Study (EuBIVAS) for total (tPSA), free (fPSA), conjugated PSA (cPSA), and percent free PSA (%fPSA) are provided.

Method: EuBIVAS samples were collected weekly from thirty-seven healthy males (22–59 years) for 10 weeks. All samples, stored at −80 °C, were measured in duplicate with a Roche Cobas e801. Outlier and homogeneity analysis were performed followed by CV-ANOVA to determine BV, analytical variation, analytical performance specifications (APS), reference change values (RCV) and the number of samples required to estimate the homeostatic set points.

Results: Within-subject BV estimates were for tPSA 6.8% (6.1–7.4); fPSA 7.1% (6.5–7.7) cPSA: 8.8% (8.0–9.7) and %fPSA 5.3% (4.8–5.8), delivering RCV for increase of 15–20% and indicating that one sample is sufficient to estimate the homeostatic set points within ± 15%. BV estimates for tPSA were lower than previously published estimates. Estimates for fPSA, cPSA and %fPSA have not previously been reported in healthy subjects.

Conclusions: Highly powered EuBIVAS BV estimates of tPSA, fPSA, cPSA and %fPSA provide updated APS and RCV for monitoring for prostate cancer.

List of abbreviations

APS	analytical performance specification
B_{APS}	analytical performance specification for bias
BIVAC	Biological Variation Data Critical Appraisal Checklist
BV	biological variation
CV_A	analytical variation
CV_{APS}	analytical performance specification for imprecision
CV_I	within-subject biological variation
CV_G	between-subject biological variation
EuBIVAS	European Biological Variation Study

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Table 1
Within-subject (CV_I) and between-subject (CV_G) biological variation (BV) estimates for total PSA (tPSA), free PSA (fPSA), conjugated PSA (cPSA) and % free PSA with 95% CIs, accompanied by the corresponding BV estimates in the online 2014 BV database.

	Number of individuals	Total number of results	Mean number of samples/ individual	Mean number of replicates/sample	Mean Value (95% CI)	CV_A % (95% CI)[a]	CV_I % (95% CI)	CV_G% (95% CI)	NHSP 15%	NHSP 10%	Online 2014 BV database	
											CV_I %	CV_G %
tPSA (μg/L)	35	599	8.80	1.89	0.85 (0.82–0.89)	3.3 (3.0–3.5)	6.8 (6.1–7.4)	42.0 (33.5–56.8)	1.0	2.2	18.1	72.4
fPSA (μg/L)	35	625	9.09	1.93	0.32 (0.31–0.33)	1.4 (1.2–1.5)	7.1 (6.5–7.7)	46.2 (36.3–62.0)	0.9	2.0	NA	NA
cPSA (μg/L)	34	600	9.03	1.91	0.53 (0.50–0.56)	4.7 (4.3–5.1)	8.8 (8.0–9.7)	57.7 (44.8–79.3)	1.7	3.8	NA	NA
% free PSA (%)	34	558	8.38	1.92	40.3 (39.0–41.6)	2.7 (2.5–2.9)	5.3 (4.8–5.8)	36.1 (30.5–51.4)	0.6	1.4	NA	NA

NA, Not Available.
NHSP: Number of samples required to estimate the homeostatic set points.
[a] Analytical variation (CV_A) estimates were based on CV-ANOVA of duplicate analysis of all study samples.

NHSP	number of samples required to estimate the homeostatic set points
PCa	prostate cancer
cPSA	conjugated PSA (tPSA-fPSA)
PSA	prostate-specific antigen
tPSA	total PSA
fPSA	free PSA
%fPSA	fPSA/tPSA%
RCV	reference change value

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer and a frequent cause of cancer death in men worldwide [1]. Although a new wave of PCa biomarkers has recently emerged [2] total prostate-specific antigen (tPSA), its free fraction (fPSA), conjugated PSA (cPSA) and the percent ratio between fPSA and total PSA (%fPSA) remain of the utmost importance in the diagnosis and monitoring of this disease [3]. However, given its relatively low specificity, PSA may be elevated not only in men with PCa, but also in patients suffering from a number of benign conditions such as benign prostatic hyperplasia and prostatitis. When monitoring PSA concentrations in men over time and when comparing with a decision limit, knowledge on the expected total variation, including analytical and biological variation (BV), is essential. BV components comprise the within-subject BV (CV_I), defined as the fluctuation of a measurand around a homeostatic set point in a steady state condition, and the between-subject BV (CV_G), defined as the variability between the homeostatic set points between different subjects [4]. BV estimates for PSA may be influenced by individual factors such as PSA metabolism, renal function, BMI, physical and sexual activity [5]. Applications for BV data include assessment of significance of change in serial measurements observed within a subject (reference change value; RCV) [6], and the setting of analytical performance specifications (APS) [7–10]. Sound estimates for the BV components are necessary to understand how BV can affect the interpretation of single, replicate, and serial results of tPSA, fPSA, cPSA and %fPSA, and to provide guidance and recommendations for the interpretation of these markers in clinical practice. For example, such analyses would be key to understand if a single measurement of PSA may reliably be used in the assessment of the risk of PCa or if, conversely, repeated measurements should be performed over time to account for its natural fluctuations. Moreover, evaluating the reliability of a single measurement of PSA might also be key in the context of PCa screening programs, which are mainly based on single PSA measurements [2]. Concerns have been raised around the quality of existing BV studies [11, 12] and, consequently, around the robustness of data sets underpinning estimates of BV collated and made available in an online 2014 BV database [13]. Furthermore, available BV data is in the case of tPSA limited and for fPSA, cPSA and %fPSA scarce or not available. The aim of this study was to use data from the European Biological Variation Study (EuBIVAS) [14–18], a large-scale highly powered BV study including 37 healthy men from 5 different European countries to deliver well-characterized and updated BV data and associated measures for total, free, conjugated and %fPSA in serum.

2. Materials and methods

The health status and the inclusion/exclusion criteria of the individuals enrolled in the EuBIVAS and the protocol used to collect, process and store the samples have previously been reported in detail [14].

2.1. Sample collection and handling

Briefly, EuBIVAS involved six European laboratories from five different countries (Italy, Norway, Spain, The Netherlands, and Turkey) that enrolled 91 healthy volunteers, males and females [14, 16–18]. For our study, the subgroup of 37 healthy males was included (median age 35 years, range 22 to 59) (Supplemental Data Table 1). All involved laboratories followed the same protocol for the pre-analytical phase. All subjects compiled an enrolment questionnaire to verify their health status and to collect information regarding their lifestyle. None of the subjects performed cycling in the two days immediately preceding the blood drawing or underwent to digital rectal examination during the 10 weeks of blood collection. Further exclusion criteria were verified by a selection of laboratory tests performed during the first collection as previously described [14]. For each eligible individual, fasting blood samples were drawn weekly for 10 consecutive weeks (April–June 2015).

Serum samples collected from all the subjects by each laboratory, obtained after centrifugation at 3000*g* for ten minutes at room temperature, were aliquoted and sent, frozen in dry ice, to the coordinating center San Raffaele Hospital in Milan and stored in a freezer at −80 °C until analysis (December 2017–January 2018).

The EuBIVAS protocol was approved by the Institutional Ethical Review board of San Raffaele Hospital in agreement with the World Medical Association Declaration of Helsinki and by the Ethical Board/ Regional Ethics Committee for each center.

2.2. Analytical methods

All analyses were performed on the Roche Cobas e801 at San Raffaele Hospital, Milan using the following Roche reagents/

Table 2
Analytical performance specification (APS) for imprecision (CV_{APS}) and bias (B_{APS}) and reference change values (RCV) based on the biological variation (BV) estimates as reported in Table 1, compared to the desirable APS reported in the online 2014 BV database.

	Current study			Online 2014 BV database	
	CV_{APS} (%)[a]	B_{APS} (%)[b]	RCV (%)[c] decrease; increase	CV_{APS} (%)	B_{APS} (%)
tPSA	3.4	10.6	−16.0; 19.0	9.1	18.7
fPSA	3.5	11.7	−15.4; 18.1	NA	NA
cPSA	4.45	14.8	−20.5; 25.8	NA	NA
%free PSA	2.6	9.1	−12.8; 14.7	NA	NA

NA: not available.
[a] $CV_{APS} = ½\ CV_I$.
[b] $B_{APS} = 0.25(CV_I^2 + CV_G^2)^{0.5}$.
[c] RCV were calculated as described in the text delivering asymmetric values for rise and fall at the probability level of 95% for significant unidirectional change, applying CV_A estimates based on duplicate measurement of all study samples.

calibrators:

Total PSA (code 7027966190), total PSA G2 CS Elecsys V2.1 Calibrator (code 4485220190), free PSA reagent (code 7027320190), free PSA CS Elecsys V2 calibrator (code 3289796190).

All samples from the same study participant were analyzed in duplicate within a single run. As internal quality control, PreciControl Universal level 1 and level 2 (code 11731416190) for tPSA and PreciControl Tumor Marker level 1 and level 2 (code 11776452122) for fPSA were analyzed in duplicate in each single run.

2.3. Data analysis

Assessment of outliers, variance homogeneity, normality and steady state were performed as detailed below, with outlier identification and removal performed for replicates and samples on CV-transformed data. Homogeneity of analytical CV (CV_A) (between-replicates) was examined using the Bartlett test [19] and of CV_I by the Cochran test [20]. The Shapiro-Wilk test was used to verify the normality of the residuals [21]. To examine if subjects were in steady-state, linear regression on the pooled mean group concentration for the whole study period was performed for each measurand. Subjects were considered in steady-state if the 95% confidence interval (CI) of the slope included the zero. Larger individual systematic changes were identified by the homogeneity test of the CV_I (Cochran test) [20]. Calculation of CV_I estimates was performed using CV-ANOVA, an ANOVA method where data first is transformed using the CV-transformation [22].

CV_G was estimated by ANOVA on natural log-transformed data after applying the Dixon q test [23] to detect outliers between individuals and the Shapiro-Wilk test to verify the normality assumption [21]. To evaluate differences between participants from the different countries, data were visually inspected.

95% CIs for BV estimates were calculated as described by Sahai [24]. APS for the analytical imprecision (CV_{APS}) and analytical bias (B_{APS}) were calculated according to:

$$CV_{APS} = ½\ CV_I$$

$$B_{APS} = 0.25\sqrt{CV_I^2 + CV_G^2}$$

RCV were estimated using the formulas below, applying CV_A estimates based on duplicate measurement of study samples from all subjects:

$$SD^2_{A,log} = \log_e(CV_A^2 + 1)$$

$$SD^2_{I,log} = \log_e(CV_I^2 + 1)$$

$$SD_{combined,log} = \sqrt{SD^2_{A,log} + SD^2_{I,log}}$$

$$RCV\% = 100\% \times e^{((\pm Z_\alpha \times \sqrt{2} \times SD_{combined,log}) - 1)}$$

where $Z_\alpha = 1.65$ for the probability level of significant change set at 95%.

The numbers of samples required to estimate the homeostatic set points (NHSP) [5] were calculated using the following equation:

$$NHSP = (Z * \sqrt{CV_A^2 + CV_I^2}/D)^2$$

where D is the allowed percentage deviation from the true homeostatic set point, and Z is 1.96 (for a p-value < .05). NHSPs were calculated associated with 15% and 10% deviations from the true homeostatic set points.

Data analyses were performed using Excel 2010 and IBM SPSS statistics, version 23.

3. Results

Median number of participants per center was 7 (range 3–9) (Supplemental Table 1). Participants were in general physically active, and about 16% were regular smokers. Median BMI was 24.4 (range 18.1–32.5), with Turkish participants having the highest median BMI at 27.5. No significant trends in the measurand concentrations for the whole study population were observed. The Shapiro-Wilk test, used to analyze data distribution, yielded a normal distribution for the residuals of the individuals' variation around their homeostatic set points for all measurands.

Two subjects were excluded from the study due to their increased pathological tPSA values: one Norwegian subject (negative trend; concentrations from the 1st collection at 8.05 µg/L to 4.83 µg/L at the 10th collection), and another from Spain (mean values of tPSA 6.22 µg/L).

The median number of results excluded due to outlier analysis was for tPSA, free PSA, cPSA and %fPSA 8.3%, 4.1%, 10.4% and 13.1% respectively (Supplemental Data Table 2).

To obtain variance homogeneity, one Italian subject was excluded for cPSA and %fPSA (Supplemental Table 2).

Figs. 1-4 show the distribution of results for the 10 samplings for tPSA, fPSA, cPSA and %fPSA for all study participants after exclusion of outliers for CV_I. For tPSA, a slight increase in mean concentration with increasing age was observed (Supplemental Fig. 1), while fPSA seemed not influenced by age (Supplemental Fig. 2), consequently cPSA increased and %fPSA decreased with increasing age with borderline significance (Fig. 5). No differences in mean concentrations between participants from the different countries were evident (data not shown). .)

tPSA in our population seemed not influenced by BMI (Supplemental Fig. 3).

BV estimates for tPSA (Table 1) were definitely lower than the estimates available in the online 2014 BV database [13], with implications for the corresponding APS for bias and imprecision and RCVs (Table 2). Only one sample is required to estimate the true homeostatic set points of tPSA, fPSA and %fPSA with ± 15% deviations (for a p-value < .05), while 2 samples are needed to reduce the deviation to 10% (Table 1).

CV_A estimates for tPSA and for fPSA delivered by CV-ANOVA based on results of duplicate measurements of study samples were CV_A 3.26% (3.0–3.5) and CV_A 1.35% (1.2–1.5) respectively (Table 1). No systematic trends were observed in the analyses during the study period based on assessment of internal quality control samples (data not shown).

4. Discussion

PSA is a central diagnostic marker for men's health with regard to

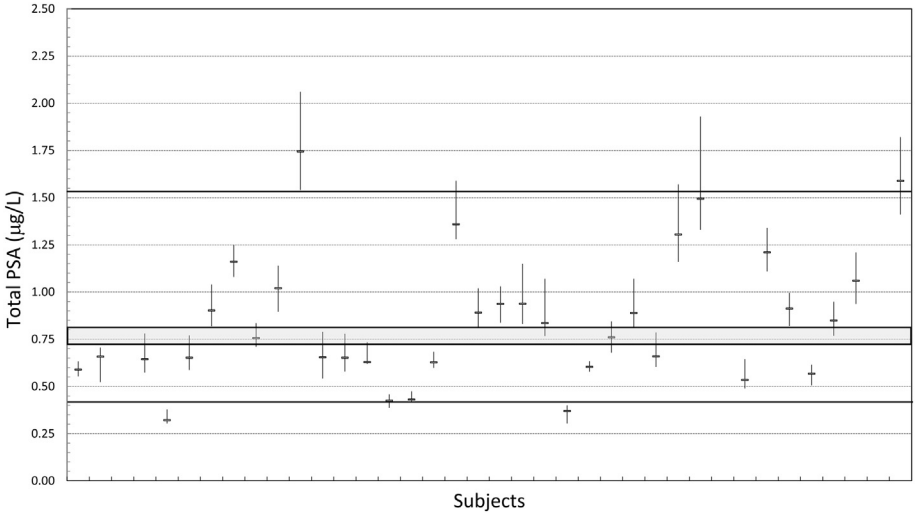

Fig. 1. Biological variation of total PSA.
Range minimum –maximum and median value (hyphen) for each subject after exclusion of outliers, ordered by age. Continuous lines point out the 5th and 95th percentiles and the median value (+ CI).

diagnosis and monitoring of PCa, though its role in screening is still controversial [25]. Our study provides updated BV estimates for PSA and its modifications, which may be used to set APS and aid in the interpretation of changes in test results when monitoring individuals over time. The BV estimates for tPSA delivered by our study are low (CV_I 6.8%, 95% CI; 6.1–7.4), being less than half the online BV estimate (18.1%), for which measures of uncertainty are not included. For tPSA, there are few studies published. Nixon [26] reports relatively similar estimates to ours for tPSA (median CV_I = 8.2%) but if evaluated according to the recently published Biological Variation Data Critical Appraisal Checklist (BIVAC) [27], the paper receives BIVAC grade C, related to statistical quality items. The publication of Panteghini et al. [28], also receiving a BIVAC grade C due to statistical issues, reports a higher CV_I (14%) but this is for five patients with untreated prostatic carcinoma with monitoring twice a day for 4 days, so that these data are not comparable with EuBIVAS data, being long-term BV estimates obtained from healthy subjects. The only previously published fPSA and %fPSA BV estimates were based on frequent measures (5 in two weeks) [26], or even daily [29] or on only 3 measurements [30], all having been performed in non-healthy subjects. PSA is an enzyme (kallikrein 3, a peptidase that liquefies the semen) and as most of the enzymes used for clinical diagnosis, it has no function in plasma where its presence depends on physiological "leakage" from the prostate gland (as typically occurs for also other enzymes, e.g. amylase) and its concentration is related to the mass of the gland. Information on parameters that may influence PSA such as sexual activity/ejaculations or presence of prostate or urinary infections was not available in our study and this may have influenced our estimates. However, to obtain variance homogeneity for CV_I only one subject was excluded for cPSA and %fPSA. A number of results were detected as outliers or excluded applying variance homogeneity tests, ranging from 4.1% (fPSA) to 13.1% (%fPSA) (Supplemental Table 2), which is in the higher range than for other measurands previously reported from the EuBIVAS [17, 18], but it is comparable with the situation reported for the enzymes [16].

The results of our study have important clinical implications. The low BV of PSA and %fPSA supports the reliability of a single as opposed to duplicate measurements to assess individual risk of PCa. Using the CV_A and CV_I estimates found in this study, just 1 blood sample is sufficient to estimate the homeostatic set point with a 15% approximation while 2 samples are required to obtain a 10% approximation (Table 1).

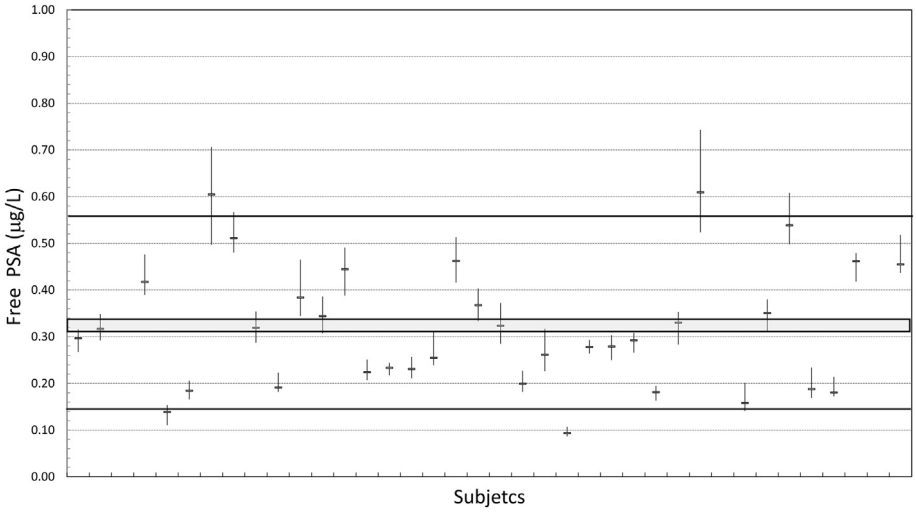

Fig. 2. Biological variation of free PSA.
Range minimum –maximum and median value (hyphen) for each subject after exclusion of outliers, ordered by age. Continuous lines point out the 5th and 95th percentiles and the median value (+ CI).

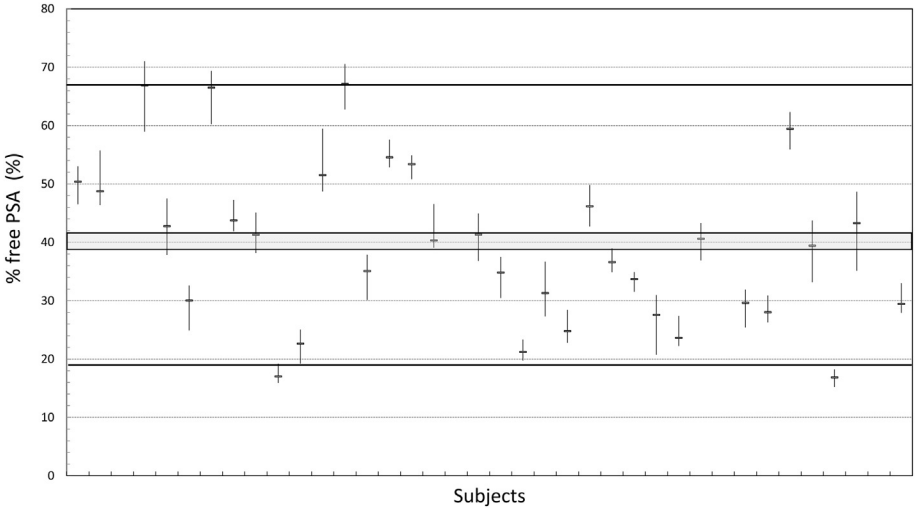

Fig. 3. Biological variation of % free PSA.
Range minimum –maximum and median value (hyphen) for each subject after exclusion of outliers, ordered by age. Continuous lines point out the 5th and 95th percentiles and the median value (+ CI).

This is of importance in particular in the context of PCa screening programs, which are mainly based on single PSA measurements [2]. Furthermore, appropriate BV data on %fPSA have been lacking, even though %fPSA has been shown to improve specificity in the detection of PCa [31].

Although the population included in our study was on average younger than the population typically assessed for PCa, there are no indications that CV_I may increase with age [29]. Furthermore, it is noteworthy that the role of PSA in younger men is gaining increasing importance as a triage test to tailor and optimize PCa screening [32]. Increased PSA concentrations at a young age are associated with the risk of developing PCa over time [32]. For this reason, measurement of PSA at age 40 is currently recommended by the European Association of Urology (EAU) guidelines to identify those men who may need more intense screening programs over time [33]. Therefore, our results are relevant in the context of screening and early detection of PCa.

A review of the BV of PSA published in 2005 by the European Group of Tumor Markers [34], evaluated 27 studies including BV estimates for tPSA and calculated the weighted average BV estimate from 12 that reported relevant BV estimates. Nine out of these 12 studies reported the total variation as the sum of analytical and BV, thus delivering a CV_I estimate calculated by subtracting an estimate of CV_A. The authors separated the studies into three different groups depending on the length of the study period (days, weeks and months) and reported that the calculated BV estimate was lower for "short term" studies i.e. study period in days, (CV_I 10%; range, 2.1%–19.6%) and in weeks (CV_I 15%; range, 14%–16.1%) than when performed in a study period over months (CV_I 20%; range, 18.1%–22.9%).

Only three out of the 12 studies reported BV data directly [26, 28, 35]. Two of these [28, 35] are cited in the online 2014 BV database [13] as the papers used to calculate the tPSA BV data. However, the BV data reported in the database are identical to those from just one of these papers, i.e. that published by Browning MCK et al. reporting a CV_I of 18.1%. The mean tPSA concentration was only given as ranging from 0.5–8.9 (μg/L) [35]. If applying BIVAC [27] on this publication, a number of issues are apparent; trend and outlier analyses were not performed, and variance homogeneity was not assessed. As reported in [27], lack of adherence to these three quality items may lead to an overestimation of the CV_I which may contribute to their finding of a higher CV_I estimate, as compared to the EuBIVAS estimate of 6.8%

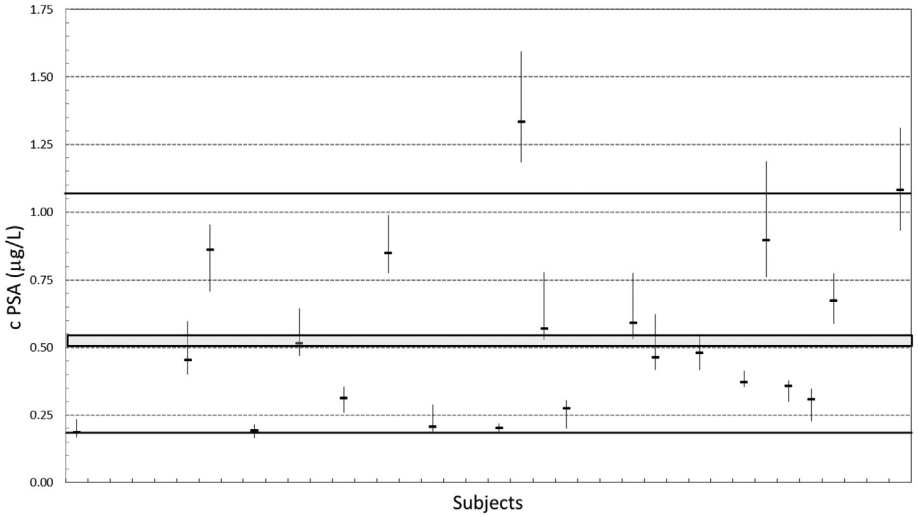

Fig. 4. Biological variation of c PSA.
Range minimum –maximum and median value (hyphen) for each subject after exclusion of outliers, ordered by age. Continuous lines point out the 5th and 95th percentiles and the median value (+ CI).

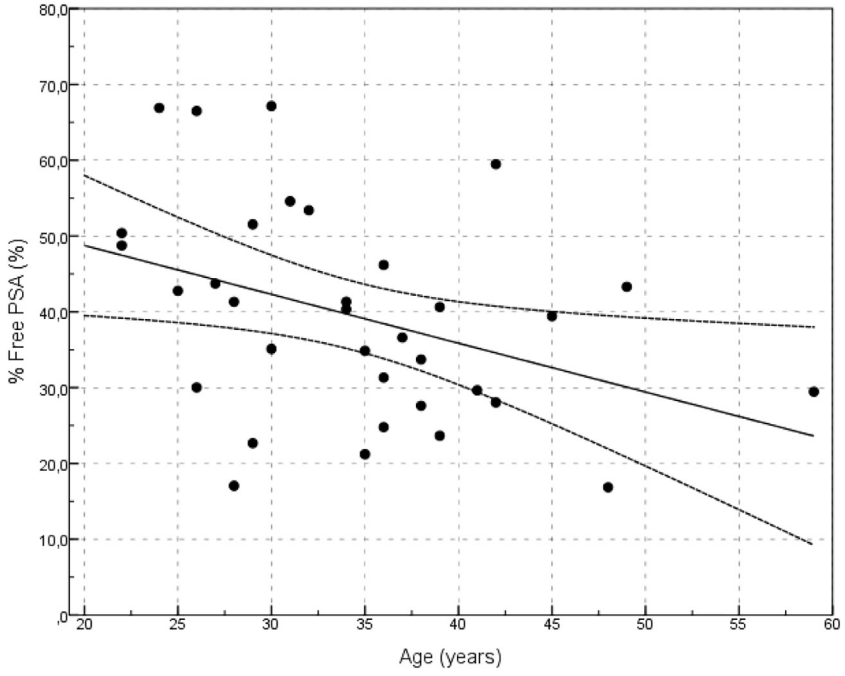

Fig. 5. x/y plot for % free PSA mean values and age.
Linear regression equation after exclusion of outliers: % free PSA = −0.64age (−1.2 - -0.1) + 61.6 (42.2–81.4); Pearson's correlation coefficient r = 0.385

[6.1–7.4]. The EuBIVAS study is both BIVAC compliant and highly powered, and in this context, represents an important update of PSA-related data.

After 2005, only a few papers with BV estimates for PSA have been published [36–38] Christensson et al. [36] performed a short-term study, collecting samples from subjects with benign disease or PCa for 2 weeks only, and reported a CV_I estimate of 11%. Bruun L et al. [37] and Nichols JH et al. [38] presented BV estimates based on data retrospectively obtained from a longitudinal study with collection every 2 years. Brunn et al. reported a CV_I estimate of 11.4% [37], while Nichols JH et al. presented the PSA variability only as absolute difference of concentration per year [38]. As for fPSA, cPSA and %fPSA there are no previously published studies performed in healthy individuals and the online 2014 BV database does not include estimates for these markers.

The CV_I estimates for tPSA obtained in our population are significantly lower than the three calculated CV_I values reported for the different study periods and used in the calculations of Solétormos et al. [34] to evaluate the significance of increase of tPSA in comparison with a decision limit for prostate biopsy. Based on estimates from the monthly monitoring period, Solétormos et al. report a RCV of 50% [34]. If using the EuBIVAS estimates, the RCV for increase is, however, only about 20% (Table 2). Our population is significantly younger (mean age 35 years) and with lower tPSA mean concentration (0.85 µg/L) compared with the previous studies [34, 36–38], but there are no indications that CV_I may increase with tPSA concentration [29]. It is thus possible that the main reason for the observed differences in CV_I might be the tight study design of the EuBIVAS with strict control of the pre-analytical phase and the statistical approach.

5. Study limitations

The analyses were performed using only 1 manufacturer's reagents. Reagents from different manufacturers may perform differently, but it is unlikely that this will affect the BV estimates.

Samples were stored for 30 months prior to analysis. There is conflicting data on prolonged storage of samples causing degradation of fPSA [39, 40]. This is, however, unlikely to affect our BV results as all the samples of the same subject were measured in the same run and those of all subjects within few days.

Finally, the study was not conducted specifically for PSA. Thus, our population is relatively young and we do not have information about sexual activity/ejaculations or possible urinary infections, however the outliers elimination due to variance heterogeneity should have eliminated any outlying samples possibly due to such events.

6. Conclusions

In conclusion, the fluctuation of tPSA and fPSA concentrations in serum in a group of young healthy subjects appears quite low even if a clear homeostatic control of PSA concentrations probably does not exist. However, it is difficult to say if these results will be appropriate to apply to patients with PCa or benign prostatic hyperplasia. Nonetheless, in view of the increasing importance of PSA assessment in young patients to tailor subsequent PCa screening programs, our results support the validity of a single PSA assessment as a reliable measurement (homeostatic set point within ± 15%), and are of particular relevance in view of the increasing importance of PSA assessment in young patients to tailor subsequent PCa screening programs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.07.043.

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