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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/