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BIOLOGICAL VARIATION AND REFERENCE CHANGE VALUE (RCV) OF PROSTATE SPECIFIC ANTIGEN (PSA) LEVELS IN THE SERUM OF HEALTHY YOUNG INDIVIDUALS

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ABSTRACT:

Purpose: The objectives of this study were to provide data about the intra-individual biological variations in prostate specific antigen levels for young men and to evaluate the significance of changes in serial results from an individual.

Materials and Methods: The study group comprised 26 healthy male volunteers; median (range) age was 32.5 (22-49) years. Four blood samples were obtained from each subject, one at each 14-day interval. Serum PSA levels were measured by a chemiluminescent microparticle immunoassay (CMIA) on a random-access analyzer.

Results: The intra-individual biological variation for total serum prostate specific antigen was 20.7%. The reference change value (RCV) for tPSA was 49.4% ($p < 0.05$)

Conclusion: The estimates of intra-individual biological variation and RCV for total PSA in the group were similar to the published mean data on elderly subjects. For men under 50 years old, a change greater than 50% in tPSA levels in a 14-day interval indicates a significant change. The biological variation should be considered when evaluating PSA results for all age groups. Moreover, for the reliable use of the PSA assay in clinical practice determinations of CVA, CVI, and RCV are required. When screening and monitoring, especially familial forms of prostate cancer in younger men, our estimates of biological variation and RCV for total PSA may be considered.

Key words: Biological Variability, Total PSA

SAĞLIKLI GENÇ BİREYLERDE SERUM PROSTAT SPESİFİK ANTİJEN (PSA) DÜZEYLERİNİN BİYOLOJİK VARYASYONU VE REFERANS DEĞİŞİM DEĞERİ

ÖZ:

Amaç: Sağlıklı genç bireylerde prostat spesifik antijen (PSA) düzeylerinin birey-içi biyolojik varyasyon değerlerini saptamak ve bir bireyden ard arda yapılan iki ölçüm arasındaki değişimin anlamlılığını değerlendirebilmek.

Gereç ve Yöntem: Çalışmaya 26 sağlıklı gönüllü erkek [ortanca; yas aralığı,32,5 yıl (22-49)] alındı. Ondört günlük aralar ile her bireyden dörder tane numune toplandı. Serum PSA düzeyleri kemiluminesan mikropartikül immün yöntem (CMIA) ile otoanalizörde çalışıldı.

Bulgular: Total PSA'nın birey-içi biyolojik varyasyonu % 20,7 ve referans değişim değeri % 49,4 olarak bulundu ($p < 0,05$)

Sonuç: Sağlıklı genç bireyler için hesaplanan birey-içi biyolojik varyasyon değeri, yaşlı bireyler için bildirilmiş sonuçlar ile uyumlu idi. 50 yaşından küçük erkekler için;14 günlük ara ile ölçülmüş iki PSA sonucu arasında %50'den büyük bir değişim anlamlı bir farkı ifade etmektedir. Tüm yaş grupları için PSA sonuçları değerlendirilirken biyolojik varyasyon dikkate alınmalıdır. Klinik pratikte PSA testinin güvenilir şekilde kullanılabilmesi için analitik varyasyon (CVA), birey-içi varyasyon (CVI) ve RCV değerlerinin bilinmesi gerekir. Prostat kanserinin özellikle de ailesel formunun tarama ve takibi sırasında, elde etmiş olduğumuz verilerin faydalı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Biyolojik Varyasyon; Total PSA

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INTRODUCTION

The PSA test and the digital rectal examination (DRE) are used as primary screening tools in the early detection of prostate cancer. Transrectal ultrasound (TRUS) guided needle biopsies are performed to confirm the diagnosis following PSA and/or DRE testing[1]. Some countries recommend screening for prostate cancer annually beginning at age 50. In high risk populations, including men with a family history of prostate cancer or men of African ancestry, screening should start at the age of 45 years[2]. Eighty percent of prostate cancer cases are diagnosed in men older than 65 years of age[3]. However, with the advent of individual screening using the serum PSA test, diagnosis of localized cancer has become increasingly common in younger patients[4].

Total variation in the PSA test includes analytical and biological variation. Analytical variation depends on laboratory processing and measurement (assay performance) and sample handling[5]. Serial measurements of an individual's tPSA concentration, even when all results are obtained by a single method, may fluctuate with higher amplitude than can be explained by the assay's analytical variation[6]. This reflects intra-individual or within-subject variation (CVI), known as biological variation. Biological variation is defined as an inherent fluctuation that can be described as random variation around the mean concentration during steady-state periods[7]. The concentration of PSA is affected by considerable intra-individual variation[8].

Although there is little evidence that biological variation (for general analytes) differs in younger and older individuals[7], it is not so clear for PSA. Optimum interpretation of the significance of changes in serial PSA measurements requires knowing the analytical variation and biological variation[9]. The current European consensus is that quality specifications in laboratory medicine are best based on calculations involving biological variation[10].

Clinicians use changes in serum PSA concentrations to help make decisions. For this reason it is important that biological and analytical components of variation be defined to help determine what constitutes a significant change in serum PSA[11].

Several studies have described biological variation in PSA in different patient groups, number of individuals, number of samples taken per subject, and time interval between. In a previously published review Soletermos et al. reported a mean value of 20% intra-individual biological variation and 50% RCV for tPSA in

men over 50 years old[9]. Glenski et al.[12] studied (samples derived once or twice daily for 4 weeks) biological variation for tPSA in 20 healthy individuals mean (range) age 26 (20-29) years and estimated 16% intra-individual biological variation (CVI).

There have not been many reports specifically focusing on the evaluation of biological variability in PSA in young age groups.

In this study we evaluated the biological variation in total PSA levels in young men with no established diagnosis of prostate cancer, prostatitis, prostatic infarction, or any urinary tract infection in our population. We investigated how much a concentration should change to exceed the biological variation; in other words, what is the reference change value (RCV) to improve the test characteristics.

MATERIALS AND METHODS

We determined biological variation in serum PSA measurements by measuring total serum PSA on 4 occasions each 2 weeks apart and then calculating the coefficient of variation[13,14]. We studied 26 healthy volunteers from our laboratory staff. Men older than 49 years, and those with a history of prostate cancer, digital rectal examination within the previous week, transrectal prostatic ultrasonography and biopsy within the previous 2 months, documented urinary tract infection, or acute prostatitis were not included in the study. The data were analyzed for outliers. Outliers were defined as those exceeding ±3SD[15]. After the exclusion of any outliers, 20 subjects were included in the final statistical analysis; median (range) age was 32.5 (22-49) years. All of the men agreeing to participate in this study were white and none of them had a family history of prostate cancer. We did not examine or manipulate the prostate in any way during the study interval, and none of the men had symptoms of urinary tract infection or prostatitis during the protocol.

All serum samples were stored frozen at -20 °C until testing at the end of the collection period. Blood collection was performed in standardized conditions in order to minimize sources of pre-analytical variation. Venipuncture was performed after an overnight fast, between 8 and 10 a.m., in the antecubital vein of the subjects. All blood samples were collected from seated subjects by three experienced investigators (3 medical doctors) using a conventional venipuncture with minimal stasis. Blood sampling was always carried out by the same investigator in each subject. The samples were allowed to clot before separation by centrifugation at 3000 g for 10 min.

Each sample from one individual was assayed in duplicate and, after removing outliers, the average value of the individual's duplicate measurements was used for the statistical procedures.

Serum PSA levels were measured by a chemiluminescent microparticle immunoassay (CMIA) on a random-access analyzer (Architect i2000; Abbott). The within-run (intra-assay) analytical variation was minimized by assaying all of the samples with the same lots of reagent on the same day. In addition, all samples from a subject were analyzed in random order in the same assay to eliminate inter-assay variation. The intra-assay coefficient of variation (CVA) was 4.3% for the tPSA assay. We determined the coefficients of analytical variation (CVA) from analyses of several serum control pools running concurrently with the study group. For total PSA we used two lyophilized pools obtained commercially. For each pool we determined the mean and standard deviation and then calculated CVA with the following formula: CVA= (SD/mean) × 100. Because the CVA values for different quality control sera were similar, we used the average CVA for the control sera[7,11,13,14,16,17,18].

Informed written consent was obtained from all participants, and the study was approved by the local human ethics committee.

STATISTICAL ANALYSIS, VARIABILITY CALCULATION

Serum levels of PSA for the subjects were recorded and assessed. Biological variation was defined as the mean coefficient of variation for total serum PSA. The total variation and the coefficient of variation, expressed in terms of percent coefficient of variation, were calculated for each of the 20 men.

For each patient we calculated the mean, standard deviation (SD), and coefficient of variation in the tPSA values for the four visits by means of a commercially available statistics program (SPSS 10) and Microsoft Excel. From these data, the mean and SD for the entire population were calculated. For PSA, total intra-individual variance (SDtI2) was calculated from data for each subject and transformed into the total intra-individual CV (CVtI) by use of the homeostatic mean of each individual.

The total variation in the measurements for four specimens from one subject is composed of the biological variation and the analytical variation. Because CVtI includes analytical and biological components, the CVI (intra-individual biological variability) for PSA was obtained by subtraction using the general formula described by Fraser and Harris[7,13]:

$$CVI = \sqrt{(CVtI^2 - CVA^2)}$$

Thus, for serial results to be significantly different, the difference in numerical results must be greater than the combined variation inherent in the two results. This value is traditionally called the critical difference but is better called the reference change value (RCV). From the data generated, the RCV was calculated.

Calculation of RCV We can calculate RCVs using this formula:

$$RCV = \sqrt{2 \times Z \times} \sqrt{(CVA^2 + CVI^2)}$$

The number 2 in the formula is a constant for 2 measurements. RCV should be adjusted to local laboratory standards. In usual practice, we want to know about change in general and so we apply bidirectional Z-scores. Conventionally 95.5% probability is regarded as significant. As a consequence, gene-

rally 1.96 is the appropriate Z-score to use. The RCV provides the limit for a change attributable to random variation with probability specified by the Z value [7, 11, 13, 14, 16, 17].

RESULTS

The mean (median, range) age of the 20 study participants is given in Table 1. The mean (median) total PSA level for healthy young men, SD, range, and CV are given in Table 1. None of the men had any elevated (more than 4.0 ng/mL) total serum PSA. Parametric means and ±2SDs for serum total PSA measurements for the 20 subjects are shown in Figure 1.

The coefficient of analytical variation (CVA) was 4.3% for the tPSA assay. The intra-individual biological variation for total serum PSA was 20.7%. The RCV for total PSA for 2 measurements was calculated as 49.4%.

SI conversion for PSA: 1 ng/mL = 1 µg/L

Table 1 The patients' characteristics (n=20)

Age of patients, years	
Mean(median)	32.9(32.5)
Range	22-49
Mean(median) of first serum PSA levels, ng/mL	0.463(0.430)
SD	0.240
Range	0.120-0.910
The intra-individual biological variation for total PSA (CVI, %)	20.7

DISCUSSION

Single measurements for PSA may not be sufficiently informative. In clinical routines, repeated measurements of tPSA are used. To evaluate the significance of changes between two time points, we studied the biological variability in the PSA in a group of young and apparently healthy men.

The degree of variability in PSA measurements is important for interpreting test results in screening programs and particularly for interpreting the significance of changes between repeated tests[18]. Studies evaluating the biological variation in tPSA have yielded widely different results[9,14]. The key features of studies on biological variability are: definition of patient group, number of individuals, number of samples taken per subject, time interval between sampling, the statistical analysis of the data, and the suggested mode of use for calculating critical difference[19].

Biological variation in tPSA depends on the length of the monitoring period. The difference in CVI among the study groups with days, weeks, and months of monitoring was significant. The increasing CVI results are explained by the length of the monitoring period and the sampling interval[9]. Therefore, when comparing our results with the published studies we should consider the sampling interval.

CVI estimates, the summary of some of the previous studies and the present study, are listed in Table 2.

Table 2 Summary of the present study and some of the previous studies on biological variation in PSA

	Group (No. of subjects)	*Biological Variation (CV_I),%*	*Sampling Interval*	*Mean(range) age, years*	*Concentration, ng/mL (method)*
Nixon et al. (11)	PCa(6);BPH(11); healthy(7)	8.3	Daily	73 (67-83)	1.1-24.6(Tandem E;Hybritech)
Boddy et al. (17)	PCa(6)	2.1	7 days	64 (59-68)	8.3median (Centaur;Bayer)
Prestigiacomo and Stamey (21)	Healthy (78)	10.5	2 weeks	No information	4-10 (AIA;TOSOH)
Ornstein et al. (14)	Healthy (84)	14	2 weeks	65	<10(Tandem E;Hybritech)
Morote et al. (20)	PCa(44); BPH(63)	14.5	4-8 weeks	64 (47-83)	0.8-19.9(Tandem R;Hybritech)
Glenski et al. (12)	Healthy (20)	16.1	Once or twice daily	26 (20-29)	0.1-1.4(Tandem R;Hybritech)
Brow Browning and McFarlane (22)	Healthy (10)	18.1	Weeks-Months	Elderly	0.5-8.9(ELSA;CIS)
Bunting et al. (23)	PCa(66)	22.9	3-6 months	70(49-84)	<15(Hybritech;Roche Elecsys)
Present Study	Healthy (20)	20.7	2 weeks	32.5(22-49)	0.12-0.91(Architect;Abbott)

PCa, prostate cancer; BPH, benign prostate hypertrophy

Ornstein et al. used a similar strategy by taking three PSA values 2 weeks apart in 84 healthy men from a prostate screening program of mean age 65 years and estimated 14% CVI [14]. All patients were aged >50 years and had an initial PSA level of <10 ng/mL. Ornstein et al. reported that biological variation in PSA was not significantly affected by age, baseline total serum PSA, or recent ejaculation among individuals of age range 53-84 years. Our study covered a younger age group and we estimated 20% CVI. This result may indicate a greater biological variation when considering the results of patients younger than 50 years old.

Considering the similarity for the age group, Glenski et al.[12] studied (samples derived once or twice daily for 4 weeks) CVI for tPSA in 20 healthy individuals of mean (range) age 26 (20-29) years and estimated 16.1% CVI. Their concentration range was 0.1-1.4 µg/mL (method, Tandem R; Hybritech), which comes closest to ours. We observed a higher level of biological variation in tPSA for the study group.

However, our results are consistent with a review reporting a mean biological variation of 20% in the tPSA, concentration range 0.1-20 µg/L, for men over 50 years old. It was a survey of published estimates. Estimates for the biological variation in tPSA could be derived from 12 studies[9].

Morote et al.[20] studied the biological variation in PSA in a group of patients (age range 47-83 years) with normal digital rectal examination but had either benign prostatic hyperplasia or prostatic adenocarcinoma. The sampling interval and monitoring period were 4-8 weeks (2 samples/person); 14.5% CVI was estimated. They suggested that individual variability in total PSA and percent free PSA values are such as to change the indications for prostate biopsy in 15% of patients when PSA evaluation is repeated in a period of less than 3 months[27].

Total biological variability in total PSA determines the critical difference. It is better to call the critical difference the reference change value (RCV). The RCV defines the minimum percent change between two consecutive PSA measurements that suggest a significant change beyond the normal biological and analytical variation. In this study group, an increase or a decrease in tPSA of approximately 50% indicates a true increase at the 95% confidence level and may reflect a clinically

significant change. We calculated the RCV for total PSA for 2 measurements to be 49.41%. This result was very similar to Soletormos et al.'s published mean estimate of ~50% ($p<0.05$) for men over 50 years old[9].

An important question is whether biological variation differs among healthy individuals compared with patients with prostate cancer. The mean biological variation for prostate cancer patients and screened men is of the same order[9]. Even if the analyte is actually affected by the disease, if the illness is chronic and the disease state is stable again there is evidence that intra-individual biological variation is of the same magnitude as in healthy people where expressed in CV[7]. We studied healthy individuals. Therefore, the results of the present study may be adopted for patients with prostate cancer or disease for young age groups.

Generally, estimates of intra-individual biological variation in particular are constant, irrespective of the number of subjects and the methodology[7]. Therefore, the small number of subjects was not a limitation for this study.

Several clinical and analytical factors may have a potential influence on tPSA concentrations. Ejaculation and exercise have been studied. As a result of these studies, it is reported that the changes were too small to be of clinical significance[9]. Therefore, in our study group we did not ask about the recent ejaculation time.

There is a need for validation of the results of this study with further research on such younger age groups.

In conclusion, the intra-individual biological variation in tPSA was 20.7% among healthy men younger than 50 years old. During monitoring, it may be considered as indicative of recurrent, responsive, or progressive disease, which means a change of ~50%.

The biological variation should be considered when evaluating PSA results for all age groups. Moreover, for the reliable use of PSA assays in clinical practice determinations of CVA, CVI, and RCV are required. When screening and monitoring, especially familial forms of prostate cancer in younger men, our estimates of biological variation and RCV for total PSA may be considered.

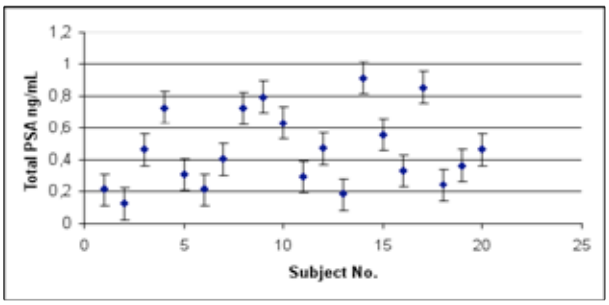

Figure 1 Parametric means and ± 2 SD for serum total PSA

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