Biological Variation of Total Prostate-Specific Antigen: A Survey of Published Estimates and Consequences for Clinical Practice

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Background: The objectives of this study were to determine whether a single result for total prostate-specific antigen (tPSA) can be used confidently to guide the need for prostate biopsy and by how much serial tPSA measurements must differ to be significant. tPSA measurements include both analytical and biological components of variation. The European Group on Tumor Markers conducted a literature survey to determine both the magnitude and impact of biological variation on single, the mean of replicate, and serial tPSA measurements.

Methods: The survey yielded 27 studies addressing the topic, and estimates for the biological variation of tPSA could be derived from 12 of these studies.

Results: The mean biological variation was 20% in the concentration range 0.1–20 μg/L for men over 50 years. The biological variation means that the one-sided 95% confidence interval (CI) of the dispersion for a single tPSA result is ~33%. Three replicate samples with one analysis on each narrow the one-sided 95% CI for the mean concentration to ~20% and facilitate decisions on prostate biopsy. During monitoring of serial measurements, the change needed for significance is ~50% (P <0.05).

Conclusions: The biological variation of tPSA has implications for screening, diagnosis, and monitoring. Single measurements may not be sufficiently precise for screening and diagnosis. Replicate samples and calculation of the mean concentration may improve precision by reducing the dispersion. Monitoring of tPSA requires an estimate of either the change needed for significance or, alternatively, of the significance of the change.

Prostate cancer is the most common cancer among men in many Western countries and is the second leading cause of male death from cancer in the United States. Total prostate-specific antigen (tPSA),11 currently the best biochemical marker for prostate cancer, is increasingly used for screening, diagnosis, and monitoring (1, 2). It is well documented that tPSA assays from different manufacturers may yield different results for the same blood specimen because of intermethod differences (3). In addition, it is known that 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 (4–6). This reflects an additional component of intraindividual or within-subject variation, known as biological variation. According to the homeostatic model for time

11 Nonstandard abbreviations: tPSA, total prostate-specific antigen; EGTM, European Group on Tumor Markers; DF, degrees of freedom; CI, confidence interval; and RCV, reference change value.
series analysis, which applies to most analytes, biological variation is defined as an inherent fluctuation that can be described as random variation around the mean concentration during steady-state periods (biological or homeostatic static set-point) (7–10). Biological variation has also been used as a term for diurnal or circadian variation, but this is not appropriate because biological variation is a global term for several different types of random variation, whereas diurnal and circadian variation properly designate systematic, rather than random variability. The increasing use of serial tPSA measurements in clinical decision-making has encouraged several research groups to study the biological variation of this analyte quantitatively. The published estimates disagree, however, up to a factor of 10, ranging from 5.4% to 56% (4–6).

In conducting this review, the goals of the European Group on Tumor Markers (EGTM) were (a) to determine whether a sound estimate for the biological variation of tPSA could be derived from a survey of published studies; (b) to illustrate, by examples, how biological variation can affect the interpretation of single, replicate, and serial tPSA results; and (c) to provide guidance and recommendations for interpretation of single, replicate, and serial tPSA measurements in urologic practice.

Methods

LITERATURE SURVEY

Literature was searched in (a) the Medline database, using the key words chronobiology OR biological variation OR biological variability OR physiological variation OR physiologic variability OR intraindividual variation OR intraindividual variability OR daily variation OR daily variability OR circadian rhythm OR diurnal rhythm AND prostate-specific antigen; (b) local literature databases at the involved institutions; and (c) recent reviews (4–6). All of the titles generated by the search and the abstracts, when available, were reviewed for relevance by at least 3 of the authors, and the full articles were obtained. The reference listings within these articles were also included. Each of the articles was then read by at least 3 of the authors, and those for subsequent inclusion in the data extraction stage were identified if information on the following criteria were provided: (a) characteristics of the study population, (b) length of monitoring period, (c) sampling interval, (d) samples per participant, (e) tPSA concentration, (f) analytical method, (g) total variation (CV_t) and analytical (CV_a) or biological variation (CV_b), and (h) sample analysis (within the same run or across different runs).

STATISTICS

The statistical definitions and calculation procedures to estimate the impact of CV_a and CV_b on tPSA results are mentioned in the text when appropriate and are based on a recent review (7). The arithmetic mean CV_b values compiled in different groups were compared with ANOVA supplied with the 2-sided t-test for further comparison. The weighted average CV_b for a group was weighted by the degrees of freedom (DF) calculated from the number of persons in each subgroup as well as the number of samples per person. For example, where 3 subgroups are considered, the formula used is:

\[
\text{Weighted average } CV_b = \sqrt{CV_{b1}^2 \times DF_1 + CV_{b2}^2 \times DF_2 + CV_{b3}^2 \times DF_3 / DF_1 + DF_2 + DF_3}
\]

Results

DIURNAL AND CIRCADIAN VARIATION

Before discussing the details of biological variation and interpretation of tPSA results during screening, diagnosis, and monitoring, it is important to address the conflicting information concerning the existence of a systematic physiologic variation of tPSA, also known as diurnal variation (sleep/wake) or circadian variation (a 24-h period). The survey identified 11 studies addressing this issue (12–22). One study reported a systematic circadian variation of tPSA with a nadir value in the early morning hours and a peak value at midafternoon; however, the changes only slightly exceeded what could be explained by analytical variation (12). Ten studies reported that the variations of tPSA during a 24-h period were random without any diurnal or circadian pattern (13–22). Consequently, it is unlikely that there is an optimal time point during a 24-h period to determine tPSA, and standardization of the sampling time is not recommended by the EGTM.

BIOLOGICAL VARIATION ESTIMATES

The literature survey identified 27 original articles addressing the biological variation concept of tPSA (21–47). Three studies reported data for biological variation directly, rendering further calculations unnecessary (23–25). Nine studies reported the CV_t for tPSA, i.e., the sum of the analytical and biological variation, as well as the CV_a (21, 22, 26–32). We subsequently back-calculated the CV_b by subtracting CV_a from CV_t; CV_b = √CV_t^2 − CV_a^2 (7). Fifteen studies reported considerable variability of serial tPSA concentrations, but it was impossible to derive the CV_b because of sufficient information on CV_t and CV_a was lacking (33–47).

The CV_b estimates are listed in Table 1 in descending order by length of monitoring period. The monitoring periods clustered into 3 main groups: days, weeks, and months. For days monitoring, the arithmetic mean CV_b was 10% (range, 2.1%–19.6%) and derived from 303 individuals. For weeks monitoring, the arithmetic mean CV_b was 15% (range, 14%–16.1%) and derived from 131 individuals. For months monitoring, the arithmetic mean CV_b was 20% (range, 18.1%–22.9%) and derived from 890 individuals. All 3 groups included patients with prostate cancer as well as persons without malignant prostatic disease.
Table 1. Biological variation of tPSA depends on the length of the monitoring period.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Monitoring period</th>
<th>Biological variation (CV\textsubscript{b})</th>
<th>Monitoring period</th>
<th>Sampling interval</th>
<th>Samples/ person</th>
<th>Group (no. of persons)</th>
<th>Mean (range) age, years</th>
<th>Concentration, (\mu)g/L (method)</th>
<th>Reference(s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 19.6\textsuperscript{c}</td>
<td>1 day</td>
<td>30 min–6 h</td>
<td>4–18</td>
<td>PCa\textsuperscript{d,e} (32)</td>
<td>72 (55–81)</td>
<td>&gt;5.6 (IRMA PSA; DPC)</td>
<td>Mannini et al. (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>4 days</td>
<td>Twice daily</td>
<td>8</td>
<td>PCa\textsuperscript{c} (5)</td>
<td>NI</td>
<td>NI (Tandem E; Hybritech)</td>
<td>Panteghini et al. (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.3\textsuperscript{c}</td>
<td>10 days</td>
<td>Daily</td>
<td>10</td>
<td>PCa\textsuperscript{c} (6); BPH (11); healthy (7)</td>
<td>73 (67–83)</td>
<td>1.1–24.6 (Tandem E; Hybritech)</td>
<td>Nixon et al. (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td>14 days</td>
<td>Days</td>
<td>5</td>
<td>PCa\textsuperscript{c} (4); BPH (3); PI (1); healthy (1)</td>
<td>60 (48–69)</td>
<td>0.49–10.2 (LIA PSA; DPC)</td>
<td>Nixon et al. (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5\textsuperscript{c}</td>
<td>14 days</td>
<td>14 days</td>
<td>2</td>
<td>Healthy (78)</td>
<td>NI</td>
<td>4–10 (AIA PSA; TOSOH)</td>
<td>Prestigiacomo and Stamey (32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.1\textsuperscript{e}</td>
<td>21 days</td>
<td>7 days</td>
<td>4</td>
<td>BPH (58)</td>
<td>67 (59–80)</td>
<td>6.3\textsuperscript{f} (Centaur PSA; Bayer)</td>
<td>Bodd et al. (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1\textsuperscript{c}</td>
<td>21 days</td>
<td>7 days</td>
<td>4</td>
<td>PCa\textsuperscript{c} (6)</td>
<td>64 (59–88)</td>
<td>8.3\textsuperscript{f} (Centaur PSA; Bayer)</td>
<td>Bodd et al. (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.6\textsuperscript{c}</td>
<td>22 days</td>
<td>22 days</td>
<td>2</td>
<td>Healthy (91)</td>
<td>NI</td>
<td>4–10 (Tandem R; Hybritech)</td>
<td>Prestigiacomo and Stamey (32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 14\textsuperscript{e}</td>
<td>4 weeks</td>
<td>2 weeks</td>
<td>3</td>
<td>Healthy (84)</td>
<td>65</td>
<td>&lt;10 (Tandem E; Hybritech)</td>
<td>Omstein et al. (31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.5\textsuperscript{e}</td>
<td>4–8 weeks</td>
<td>4–8 weeks</td>
<td>2</td>
<td>PCa\textsuperscript{c} (44); BPH (63)</td>
<td>64 (47–83)</td>
<td>0.8–19.9 (Tandem R; Hybritech)</td>
<td>Morote et al. (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.1\textsuperscript{e}</td>
<td>4 weeks</td>
<td>Once or twice daily</td>
<td>25</td>
<td>Healthy (20)</td>
<td>26 (20–29)</td>
<td>0.1–1.4 (Tandem R; Hybritech)</td>
<td>Glenski et al. (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months 18.6\textsuperscript{c}</td>
<td>3 months</td>
<td>3 months</td>
<td>2</td>
<td>Healthy (814)</td>
<td>63 (50–79)</td>
<td>0.13–18 (ACS PSA; Ciba Corning)</td>
<td>Komatsu et al. (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.1</td>
<td>2–10 months</td>
<td>Weeks–months</td>
<td>6–10</td>
<td>Healthy (10)</td>
<td>Elderly</td>
<td>0.5–8.9 (ELSA PSA; CTS)</td>
<td>Browning and McFarlane (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.9\textsuperscript{c}</td>
<td>6–32 months</td>
<td>3–6 months</td>
<td>2–7</td>
<td>PCa\textsuperscript{c} (66)</td>
<td>70 (49–84)</td>
<td>≤15 (Hybritech; Roche Elecsys)</td>
<td>Bunting et al. (27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}The data are grouped in ascending order of monitoring period: days, weeks, and months.
\textsuperscript{b}Bodd et al. (26) and Prestigiacomo and Stamey (32) reported data from 2 different populations.
\textsuperscript{c}The biological variation (CV\textsubscript{b}) was calculated from the information on total variation (CV\textsubscript{t}) and analytical variation (CV\textsubscript{a}) reported in the referenced reports.
\textsuperscript{d}PCa, prostate cancer; NI, no information; BPH, benign prostate hypertrophy; PI, prostate inflammation.
\textsuperscript{e}Different stages.
\textsuperscript{f}Stages unspecified.
\textsuperscript{g}Median.
The difference in CV\textsubscript{b} among study groups with days, weeks, and months monitoring was significant (ANOVA, \(P < 0.05\)). Supplementary 2-sided \(t\)-tests showed a significant increment of CV\textsubscript{b} among all 3 groups \((P < 0.05)\). The increasing CV\textsubscript{b} results cannot be explained solely by population heterogeneity of the compared groups. For example, the CV\textsubscript{b} from similar subpopulations increased with the length of the monitoring periods, e.g., for healthy persons, the mean CV\textsubscript{b} was 9.6\% (range, 8.6\%–10.5\%) for days monitoring \((32)\) \((1\) study, \(2\) populations), 15\% (range, 14\%–16.1\%) for weeks monitoring \((21, 31)\), and 18.4\% (range, 18.1\%–18.6\%) for months monitoring \((23, 28)\). In addition, the CV\textsubscript{b} from different subpopulations overlapped within the monitoring periods, e.g., for days monitoring the mean CV\textsubscript{b} was 11.9\% (range, 2.1\%–19.6\%) for prostate cancer patients \((22, 25, 26)\) and 9\% (range, 8.6\%–10.5\%) for healthy persons \((32)\) \((1\) study, \(2\) populations; Table 1). More likely, the increasing CV\textsubscript{b} results are explained by the length of the monitoring period and the sampling interval. This relationship between CV\textsubscript{b} and monitoring interval is a general characteristic of biochemical compounds controlled by homeostatic regulation. If tests are performed frequently, serial results will not be random but will be auto-correlated \((7)\). Accordingly, 2 sets of measurements, each consisting of \((for\ example\) 3 consecutive results obtained from the same individual, may show relatively little variation within each set but quite a large variation between the 2 sets. Auto-correlation is considered eliminated and the CV\textsubscript{b} unfolded when CV\textsubscript{b} remains constant at increasing monitoring period or increasing sampling intervals \((48, 49)\). However, this may be impossible to achieve for tPSA because the concentration increases with age as a result of the physiologically increased volume of the prostate \((50)\). Additionally, CV\textsubscript{b} data derived from patients with prostate cancer may not represent steady-state estimates if the monitoring period or sampling interval is unduly long.

The CV\textsubscript{b} estimates in Table 1 derived from days monitoring are too inconsistent to be of real value. The biological variation estimates derived from weeks monitoring are consistent but based on a relatively small population \((n = 131)\). The CV\textsubscript{b} estimates derived from monitoring for 2–32 months and a sampling interval of weeks to months are consistent and based on \((a)\) a large population \((n = 890), (b)\) healthy individuals as well as prostate cancer patients managed conservatively with watchful observation, \((c)\) various study designs, and \((d)\) different assay methods. The weighted average CV\textsubscript{b} is 19.6\%, compared with the arithmetic mean CV\textsubscript{b} of 19.9\%. Both estimates provide a rounded up CV\textsubscript{b} of 20\%. Although a CV\textsubscript{b} estimate based on previous measurements for the patient in question is theoretically superior to a population-based average, the number of specimens needed to determine a reliable subject-specific CV\textsubscript{b} may not be available.

The following EGTM conclusions concerning the biological variation of tPSA apply to men above 50 years with a tPSA within the range 0.1–20 \(\mu g/L\) and are based on the data provided in Table 1:

- A fair estimate of the mean biological variation is 20\%.
- A sampling interval \(\geq 1\) month may be necessary when estimating biological variation.
- Biological variation is independent of assay method.

**Consequences for Clinical Practice**

**INTERPRETATION OF SINGLE AND REPLICATE MEASUREMENTS DURING SCREENING OR DIAGNOSIS**

The common urologic practice is \((a)\) to omit prostate biopsy if the tPSA concentration is below the applied cutoff, frequently 4 \(\mu g/L\), without clinical signs of prostate disease; and \((b)\) to perform prostate biopsy if the tPSA concentration is above 4 \(\mu g/L\) even without clinical signs of prostate disease and if the biopsy result has therapeutic consequences. The drawback of this single-sample–guided practice is a low predictive value of tPSA to exclude and to identify prostate cancer \((3)\).

It is appropriate to estimate the dispersion of tPSA measurements expressed as the confidence interval \((CI)\) in more detail. Three questions should be addressed: What is the CI of the tPSA result? Does the CI include the cutoff concentration? Is the reliability of the tPSA determination improved by replicate sampling? The 1-sided CI for a single sample analyzed once is calculated as:

\[
CI = tPSA \text{ concentration} \pm Z \times \sqrt{\text{CV}_a^2 + \text{CV}_b^2} \quad (7)
\]

The CI is calculated as 1-sided because it is compared only with the cutoff concentration, 4 \(\mu g/L\). Consequently, \(+\) is used for below cutoff and \(-\) for above cutoff concentrations. \(Z\) is the number of standard deviations appropriate to the chosen probability. The 1-sided \(Z\) values in the examples below are 0.52 (probability, 70\%), 1.64 (probability, 95\%), and 2.33 (probability, 99\%) \((51)\). The assumed corresponding values for tPSA concentration and CV\textsubscript{a} in the examples are for illustrative purposes only and should be adjusted to local laboratory standards. The CV\textsubscript{b} was set to 20\% as defined in the section on biological variation. The tPSA concentration lies within the CI limit with a probability specified by the \(Z\) value. The 1-sided CI for the mean of replicate samples each analyzed once is calculated as:

\[
CI = \text{mean tPSA concentration} \pm Z \times \sqrt{(\text{CV}_a^2 + \text{CV}_b^2)/n} \quad (7)
\]

where \(n\) is the number of measurements used \((7)\).

Interpretation of 1-sided CI limits for tPSA results below the cutoff concentration 4 \(\mu g/L\) are illustrated in the examples provided in Fig. 1, A and B. For tPSA results above the cutoff, results interpretation is illustrated in Fig. 1, C and D. In Fig. 1A, the single result is 3.3 \(\mu g/L\) \((\text{CV}_a = 5\%)\). According to the 70\% CI limit \((3.7 \mu g/L), >70\%\) of the possible tPSA concentrations are below the cutoff. However, the 95\% and 99\% CI limits \((4.4 \text{ and } 4.9 \mu g/L, \text{ respectively})\) include the cutoff, and the probability of an above cutoff tPSA concentration exceeds 5\% \((P > 0.05)\). In
Fig. 1B, the mean concentration (3.3 μg/L) is based on 3 samples. The 70%, 95%, and 99% CIs are 3.7, 3.9, and 4.2 μg/L, respectively. In Fig. 1C, the mean concentration (3.3 μg/L) is based on 3 replicate samples, each analyzed once. The 70%, 95%, and 99% CIs are 3.5, 3.9, and 4.2 μg/L, respectively. In Fig. 1D, the mean concentration (3.7 μg/L) is based on 3 replicate samples, each analyzed once. The 70%, 95%, and 99% CIs are 3.5, 3.9, and 4.2 μg/L, respectively.

Fig. 1. Dispersion of assumed tPSA results.

The bell-shaped curves indicating the gaussian dispersion of possible tPSA concentrations for the assumed tPSA results were generated with a Microsoft Excel spreadsheet. The dashed vertical lines indicate the applied cutoff concentration, 4 μg/L. (A), the assumed single result is 3.3 μg/L. The 1-sided limits for the 70%, 95%, and 99% CIs are 3.7, 4.4, and 4.9 μg/L, respectively. (B), the assumed mean concentration, 3.3 μg/L, is based on 3 replicate samples, each analyzed once. The 1-sided limits for the 70%, 95%, and 99% CIs are 3.5, 3.9, and 4.2 μg/L, respectively. (C), the assumed single result is 5.1 μg/L. The 1-sided limits for the 70%, 95%, and 99% CIs are 4.6, 3.4, and 2.7 μg/L, respectively. (D), the assumed mean concentration, 5.1 μg/L, is based on 3 replicate samples, each analyzed once. The 1-sided limits for the 70%, 95%, and 99% CIs are 4.8, 4.1, and 3.7 μg/L, respectively.

<5% of the possible tPSA concentrations are below the cutoff (P < 0.05). Figs. 1 demonstrates that (a) the higher the percentage CI, the wider the limits for tPSA concentrations attributable to random variation, (b) the probability that the cutoff concentration will be included in the CI is increased by a higher CI, and (c) the precision of the tPSA concentration estimate is improved by replicate measurements because the dispersion is reduced.

As the decision for prostate biopsy frequently depends on whether the tPSA result is below or above the cutoff concentration, 4 μg/L, the probability for the cutoff being within the CI is addressed in Table 2. The corresponding values for tPSA concentration and analytical variation are...
as follows: 2.9 μg/L (CVa = 5.3%), 6.1 μg/L (CVa = 3.9%), 3.3 μg/L (CVa = 5%), and 5.1 μg/L (CVa = 4%). In examples 1–4, the cutoff concentration, 4 μg/L, lies within the 1-sided 70% CI limits of the assumed concentrations with a probability <30% (P < 0.3) and within the 1-sided 95% CI limits with a probability <5% (P < 0.05), whereas the cutoff concentration lies within the 1-sided 99% CI limit with a probability >1% (P > 0.01). Adopting a probability of <5% (P < 0.05) for the cutoff lying within in the 1-sided 95% CI, the highest below-cutoff single concentration justifying biopsy is 2.9 μg/L and the lowest above cutoff concentration justifying omission is 6.1 μg/L (examples 1 and 2 in Table 2). Between 3 and 6 μg/L, a single tPSA result is too uncertain as a basis for biopsy decision because the cutoff concentration, 4 μg/L, will lie within the 1-sided 95% CI with a probability exceeding 5% (P > 0.05).

The utility of replicate sampling before deciding on prostate biopsy is shown in examples 3 and 4 of Table 2. Between 3.0 and 3.3 μg/L, up to 3 measurements may be necessary to obtain a probability <5% for the cutoff being within the 1-sided 95% CI limit and thus justifying omission of biopsy. Between 3.4 and 5.0 μg/L, 3 measurements are not enough to decide on biopsy because the cutoff will lie within the 1-sided 95% CI limit. Between 5.1 and 6.0 μg/L, up to 3 measurements may be necessary to obtain a probability <5% for the cutoff being within the 1-sided 95% CI limit and thus justifying biopsy.

The intention of the examples in Table 2 is not to suggest a new cutoff value for tPSA but to illustrate that CIs can be helpful for decisions on prostate biopsy. For single measurements, the 1-sided 95% CI limit is ~33%, and for the mean of 3 measurements, the 1-sided 95% CI limit is ~20% of the result (Table 2). Consequently, replicate measurements allow biopsy decisions based on concentrations closer to the cutoff compared with a single result. Below cutoff, the decision limit is 3.3 μg/L for the mean of 3 measurements and 2.9 μg/L for a single result. Above cutoff the values are 5.1 and 6.1 μg/L, respectively. Helpful calculators to determine the CI limit of a single result, the CI limit of the mean of replicate measurements as well as the number of samples needed to obtain an estimate of and individual’s tPSA concentration at a predetermined probability are available on the internet (52). The sampling interval, however, is important because, if short, replicate measurements may be auto-correlated, influencing their mean value and the range of the CI. To reduce the influence of auto-correlation, the interval between replicate samples should be ≥1 month (Table 1). Application of CI limits may also be useful to consider in studies involving age-dependent cutoff concentrations as well as fixed cutoffs lower or higher than 4 μg/L (50, 53).

The following EGTM recommendations for screening and diagnosis of prostate cancer apply to men above 50 years with a tPSA within the concentration range 0.1–20 μg/L:

- A single tPSA concentration ought to be interpreted cautiously if the 1-sided 95% CI includes the applied decision cutoff.
- The CI can be narrowed by replicate sampling.
- The interval between replicate samples should be ≥1 month.
- Replicate measurements should be performed with the same assay type and at the same laboratory.
- Prostate biopsy should not be performed if the 1-sided 95% CI limit of the tPSA result is below the applied decision cutoff without clinical signs of prostate disease.
- Prostate biopsy should be performed if the 1-sided 95% CI limit of the tPSA result is above the applied decision cutoff and the biopsy result has therapeutic consequences.

### Interpretation of Serial Measurements during Monitoring

tPSA monitoring may provide early information about recurrent, responsive, and progressive prostate cancer, influencing treatment decisions. It is frequently asked how much a concentration should change to exceed the random variation—or to put it in other words, what is the reference change value (RCV)? The formula to use is (7):

$$ RCV = \sqrt{2} \times Z \times \sqrt{(CV_a^2 + CV_b^2)} $$

The number 2 in the formula is a constant for 2 measurements. The assumed corresponding values for tPSA concentration and CVa in the examples are for illustrative purposes only and should be adjusted to local laboratory standards. The CVb was set to 20% as defined in the

### Table 2. CIs for a single tPSA determination and the mean of replicate measurements.

<table>
<thead>
<tr>
<th>Example</th>
<th>n</th>
<th>Assumed tPSA concentration, μg/L</th>
<th>One-sided 70% CI limit, % of assumed concentration</th>
<th>One-sided 95% CI limit, % of assumed concentration</th>
<th>One-sided 99% CI limit, % of assumed concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2.9</td>
<td>+10% (up to 3.2 μg/L)</td>
<td>+34% (up to 3.9 μg/L)</td>
<td>+48% (up to 4.3 μg/L)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6.1</td>
<td>−11% (down to 5.4 μg/L)</td>
<td>−33% (down to 4.1 μg/L)</td>
<td>−48% (down to 3.2 μg/L)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3.3</td>
<td>+6% (up to 3.5 μg/L)</td>
<td>+18% (up to 3.9 μg/L)</td>
<td>+27% (up to 4.2 μg/L)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>5.1</td>
<td>−6% (down to 4.8 μg/L)</td>
<td>−20% (down to 4.1 μg/L)</td>
<td>−27% (down to 3.7 μg/L)</td>
</tr>
</tbody>
</table>

a Number of samples per person.
b The assumed tPSA concentration lies within the specified limit with a probability of 70%.
c The assumed tPSA concentration lies within the specified limit with a probability of 95%.
d The assumed tPSA concentration lies within the specified limit with a probability of 99%.
e Mean concentration based on 3 replicate samples, each analyzed once, same as in Fig. 1, B and D.
section on biological variation. The RCV provides the limit for a change attributable to random variation with a probability specified by the Z value (54, 55).

Example 5 in Table 3 provides the 1-sided RCV for a postoperative nadir tPSA concentration of 1.5 μg/L (CV_a = 8%) and demonstrates how much the nadir result should increase to exceed random variation with a specified probability. Accordingly, the probability that the increase is attributable to random variation is (a) <30% if a following concentration exceeds 1.7 μg/L (Z = 0.52; P < 0.30), (b) <5% if it exceeds 2.3 μg/L (Z = 1.64; P < 0.05), and (c) <1% if it exceeds 2.6 μg/L (Z = 2.33; P < 0.01). The Z values are 1-sided because the concentration is only expected to increase. Example 6 in Table 3 provides the 2-sided RCV for a baseline tPSA concentration of 20 μg/L (CV_a = 4%) and demonstrates how much the baseline result should decrease or increase after initiation of therapy to exceed random variation with a specified probability. Accordingly, the probability that the change is attributable to random variation is (a) <30% if a subsequent concentration exceeds 26 μg/L or decreases <14 μg/L (Z = 1.04; P < 0.30), (b) <5% if the concentration exceeds 31 μg/L or decreases <9 μg/L (Z = 1.96; P < 0.05), and (c) <1% if the concentration exceeds 35 μg/L or decreases <5 μg/L (Z = 2.58; P < 0.01). The Z-values are 2-sided because the concentration may either increase or decrease. The calculation procedures in both examples provide objective limits for concentration changes. Once determined, subsequent concentrations can easily be referenced as being nonsignificantly (e.g., P < 0.30), significantly (P < 0.05), or highly significantly (P < 0.01) increased compared with the test concentrations of, e.g., 1.5 and 20 μg/L. The importance of the procedure is that the new tPSA result is compared with a previous result instead of a traditional or age-adjusted cutoff concentration. The sampling interval in the 2 specified monitoring situations has minor importance for the RCV once the CV_b is adjusted to 20%. A helpful calculator to determine the RCV is available on the internet (56).

It has been advocated that a tPSA velocity (change in concentration per year) exceeding 0.75 μg/L may be useful to identify patients with carcinoma of the prostate (57-59). Example 7 demonstrates that it may be advantageous to estimate the significance of the change in concentrations by calculating the Z-value with the formula (7):

\[ Z = \frac{PC}{\sqrt{2 \cdot CV_a^2 + CV_b^2}} \]

where PC is the percentage change between 2 tested concentrations. During 1 year, assumed tPSA concentrations for a man are 3.8 μg/L (CV_a = 4.5%), 4.1 μg/L, and 4.7 μg/L. The total increase is 24% with a tPSA velocity of 0.9 μg/L, suggesting emerging prostate cancer. However, this may be an invalid conclusion because the calculated 1-sided Z-value is 0.8, demonstrating that the change is nonsignificant (P > 0.2) (51). The example supports a recent clinical study questioning the diagnostic value of a tPSA velocity of 0.75 μg/L per year (60).

Example 8 suggests how to optimize the use of tPSA velocity in differentiating between random fluctuations and prostate cancer–induced increments. At the baseline concentration of 3.8 μg/L (CV_a = 4.5%), the increment limits attributable to analytical and biological variation (1-sided RCV) are 15%, 47%, and 67% for the 1-sided Z-values 0.52, 1.64, and 2.33, respectively. The probability that the change in concentration per year is attributable to random variation is (a) <30% if the tPSA velocity exceeds 0.6 μg/L, (b) <5% if it exceeds 1.8 μg/L, and (c) <1% if it exceeds 2.5 μg/L. Consequently, the tPSA velocity should exceed 1.8 μg/L per year to be significant (P < 0.05) and 2.5 μg/L per year to be highly significant (P < 0.01).

The following EGTM recommendations for monitoring of patients with prostate cancer apply to men above 50 years with a tPSA within the concentration range 0.1-20 μg/L:

- The tPSA change needed for significance and the significance of a tPSA change are required for objective interpretation of serial tPSA measurements.
- Serial measurements of tPSA should be performed with the same assay type and at the same laboratory.
- The probability that a tPSA change is attributable to random variation should be <5% before the change is interpreted as indicative of recurrent, responsive, or progressive disease, which means a change of ~50%.

<table>
<thead>
<tr>
<th>Example</th>
<th>Assumed baseline tPSA concentration, μg/L</th>
<th>70% RCV limit, (^a)</th>
<th>% of baseline concentration</th>
<th>95% RCV limit, (^b)</th>
<th>% of baseline concentration</th>
<th>99% RCV limit, (^c)</th>
<th>% of baseline concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.5</td>
<td>+16% (^d)</td>
<td>(up to 1.7 μg/L)</td>
<td>+51% (^d)</td>
<td>(up to 2.3 μg/L)</td>
<td>+72% (^d)</td>
<td>(up to 2.6 μg/L)</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>±30% (^e)</td>
<td>(up to 26 and down to 14 μg/L)</td>
<td>±57% (^e)</td>
<td>(up to 31 and down to 9 μg/L)</td>
<td>±75% (^e)</td>
<td>(up to 35 and down to 5 μg/L)</td>
</tr>
</tbody>
</table>

\(^a\) Within the specified limit, the probability is 70% that the change is attributable to random variation.

\(^b\) Within the specified limit, the probability is 95% that the change is attributable to random variation.

\(^c\) Within the specified limit, the probability is 99% that the change is attributable to random variation.

\(^d\) One-sided RCV limit.

\(^e\) Two-sided RCV limit.
Discussion

It remains a paradox that tPSA measurements are recommended by regulatory agencies, manufacturers, scientific societies, and several research groups but relevant guidelines to interpret single, replicate, and serial measurements that recognize the influence of both analytical and biological variation have not been provided. Additionally, there are no generally accepted recommendations describing how clinical tPSA trials should be designed, conducted, evaluated, and presented. The need, however, has been recognized, and helpful information on these issues is emerging (61–66).

Several clinical and analytical factors may have a potential influence on tPSA concentrations. Ejaculation and exercise have been debated intensively in this context. The issues have been reviewed recently, but no clear effect on tPSA concentrations has been demonstrated (4, 5). By comparing before and after values, some studies reported a slight increase, others found no change, and some studies reported a decrease in concentrations. The changes were too small to be of clinical significance.

It may be relevant to ask why the values for analytical variation were assumed instead of obtained from a specified method? The values were used for illustrative purposes to show that analytical variation depends on concentration, a characteristic that applies to all tPSA assay systems. On a general basis, the values are representative; however, they should be adjusted to local laboratory standards.

Another important question is whether biological variation differs among healthy individuals compared with patients with prostate cancer. A systematic difference in biological variation would cause concern. The mean biological variation for prostate cancer patients and non-prostate cancer is not of the same order, and the ranges overlap within each of the monitoring periods days, weeks, and months (Table 1). Accordingly, a difference in biological variation between prostate cancer and non-prostate cancer is not likely among men above 50 years with a tPSA concentration within the range 0.1–20 \( \mu g/L \).

The major determinant for biological variation is the time period of the study (Table 1). With the slow elimination rate of 0.8 \( \mu g/L \) per day for the major tPSA fraction, complexed PSA, it is hardly surprising that there may be auto-correlation of serial concentrations taken over short time periods (1). Similarly, auto-correlation has been reported for other tumor markers, including CA 15-3, CA 125, carcinoembryonic antigen (CEA), and tissue polypeptide antigen (TPA) (67, 68). Apparently, it is a general characteristic of several tumor markers that minimizing the effect of auto-correlation and unfolding of the biological variation may require a monitoring period longer than 1 month.

A tPSA-guided biopsy decision is preceded by informed consent addressing the pros and cons of this investigation. The decision may, however, be difficult if based on a single tPSA result and a traditional cutoff value of 4 \( \mu g/L \). For a man with a single tPSA result of, e.g., 3.3 \( \mu g/L \), the 1-sided 95% CI limit is up to 4.4 \( \mu g/L \), and it can be argued that prostate biopsy has been avoided by chance (Fig. 1A). Conversely, for a man with a single tPSA result of, e.g., 5.1 \( \mu g/L \), the 1-sided 95% CI limit is down to 3.4 \( \mu g/L \), and it can be argued that the biopsy has been performed by chance (Fig. 1B). Compared with a single measurement, the mean of 3 replicate samples with 1 analysis on each narrows the 1-sided 95% CI limit from 33% to 20% of the result and may facilitate a decision on prostate biopsy, particularly for tPSA concentrations close to the cutoff value (Table 2). The number of replicate samples and the probability for the chosen CI depend on the clinical need for reliability in the specified situation. Calculation of the mean concentration from replicate samples improves the reliability of the measurement, and if CVa > CVb, repeated analysis of 1 sample will reduce the variability further (52). It is a general characteristic of tPSA assay systems that the analytical variation, except for very low concentrations, is well below the biological variation of 20% (21, 22, 26–32).

The purpose of monitoring tPSA is to obtain reliable information about changes in prostate cancer activity in terms of recurrence, response, and progression. The provided assessment procedures enable calculation of the limits for a change attributable to random variation with a specified probability. Accordingly, a change should be ~50% to be significant (\( P < 0.05 \)) and ~70% to be highly significant (\( P < 0.01; \) Table 3). The procedures also enable estimation of the significance of a change as illustrated in examples 7 and 8 (see the Results section). The information may be used to decide whether to continue or end a therapy or to initiate a new treatment. Tumor marker assessment adjusted to the random analytical and biological variation has been suggested as a relevant monitoring tool for surveillance of breast and ovarian cancer (69, 70).

At present, there are no reports from clinical trials on the practicability of this method in prostate cancer, probably because the procedure requires considerable effort if performed manually. Monitoring of tPSA concentrations may, however, be facilitated by use of graphical software programs designed for these specific purposes (71).

In conclusion, the typical biological variation of tPSA is 20% and is the main contributor of tPSA variability in the concentration range 0.1–20 \( \mu g/L \) for men over 50 years. For the decision to omit or perform tPSA-guided prostate biopsy, the probability should be <5% for an above- and a below-cutoff concentration, respectively. During monitoring, the probability should be <5% for the change being the result of random variation before it is considered as indicative of recurrent, responsive, or progressive disease, which means a change of ~50%. It is the opinion of the EGTM that the process to establish a consensus on how to interpret single, replicate, and serial tPSA concentrations requires collaboration of competences among urology, laboratory medicine, and industry to a higher degree than previously recognized.
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References


