

## Variation of PSA value in men and risk of high-grade prostate cancer, analysis of the PLCO

### Study

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**Conflicts of Interest**

All authors of this manuscript have no conflict of interest to disclose.

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**Objective:** To investigate variations of PSA levels among men with an initial normal PSA level in the PLCO Study.

**Methods:** Data were extracted from the PLCO study dataset on all men in the intervention arm, with two tests performed in a period of less than two years and with an initial result of the first test below 4 ng/mL. The range of variation between first and second tests was computed. Risks of cancer stratified on Gleason score were computed using logistic regression.

**Results:** 31,286 men had two PSA tests within two years and with an initial value below 4 ng/mL. From the first to second test, the median variation of PSA levels was 3.4% (IQR: -15%; +26%). The variation in PSA value was not associated with the delay between first and second test ( $p=0.36$ ), with age ( $p=0.16$ ), BMI ( $p=0.41$ ) and race ( $p=0.12$ ).

2,781 prostate cancers were diagnosed during follow-up. Adjusting for age and initial PSA level, the risk of prostate cancer increased linearly with increasing PSA level at second test, with an odds ratio of 1.079 (95% CI (1.058, 1.101)) for each percent increase in PSA level. However, the variation in PSA was not associated with a higher Gleason score ( $p<0.95$  for level variations in cancer of Gleason score  $<7$  vs  $\geq 7$ ).

**Conclusions:** While an increase in PSA level over time is associated with increased risk of prostate cancer, this association is not related to more aggressive tumors.

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## **Introduction**

The risk of prostate cancer is directly related to the level of total prostate specific antigen (tPSA) measured in blood <sup>1</sup>. PSA is being considered the best biochemical marker for prostate cancer and is widely used for early detection, diagnosis and monitoring. But the evaluation of use of PSA in screening activities showed that PSA testing induced substantial over-diagnosis of prostate cancer, mainly due to poor specificity of the test.

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 <sup>2-4</sup>. This reflects an additional component of intra-individual or within-subject variation, known as biological variation.

Variation of PSA level could lead to unnecessary biopsies if based on a single measurement. It has been shown in the Polyp Prevention Trial that, following an initial abnormal PSA level, an important proportion of men returned to normal PSA level at the subsequent visit (26% for an initial value above 2.5 ng/ml and 30% for an initial value above 4 ng/ml) <sup>5</sup>.

The normal variation of PSA level has been evaluated in the past in few studies, and already an important variation in men has been reported. In a series of eight men with adenocarcinoma of the prostate, the maximum variation above and below the mean were of 19.3% and 17.7% <sup>6</sup>.

The biological variation of tPSA has implications for screening and diagnosis. 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.

However, subsequently there have been investigations of variation in tPSA from most recent sources (e.g. <sup>7-10</sup>) without any real conclusions being drawn and a lack of cohesion between findings and hypotheses investigated.

Therefore, variations of PSA levels were studied in men participating in the intervention arm of the PLCO Study <sup>11</sup> and with an initial PSA level below 4 ng/mL. This database has a major advantage of standardization of the follow-up of men and a good quality monitoring of patients and follow-up.

## **Material and methods**

Data were extracted from the PLCO study dataset of August 2012 on all of the 38,340 men in the intervention arm. These Cancer Data Access System (CDAS) datasets are slightly more up to date than the original report at 13 years of follow-up with reclassification of some events based on updated data and further review, and this could explain slight discrepancies in numbers from this report as compared to the article of Andriole et al (2012) <sup>11</sup>.

All potential PSA measurements were extracted for each man, so these could be part of the annual test in the intervention or any additional test performed during follow-up. We included men who received two valid PSA tests performed in a period of less than two years, thus excluding 576 individuals with greater delay between two tests. As our objective was the evaluation of PSA variability in men considered having an initial normal test, we excluded 2577 men who did have an initial abnormal with a PSA value of 4 ng/ml or above. Because the present study investigated change in PSA, 1597 individuals with only one PSA test and

2343 individuals with no PSA measurement were also excluded. Some of these exclusion criteria could be overlapping.

Unlike other reports on the subject <sup>5</sup>, the analysis was restricted to the first two tests and did not include any further tests. This decision was based on the fact that including more tests for men could introduce a bias in the analysis. For example, men with more tests could have a reduced global variation because of smoothing effect of combining more information. In addition, there could be a differential risk of cancer: men with more tests could be under more surveillance because more at risk of cancer (for example because of a family history); alternatively men with more tests could be at a lower risk of cancer because of prolonged latency, and men without cancer have necessarily more PSA tests than men with a cancer diagnosed few years after inclusion.

The prostate cancer incidence data for this study were collected up to 31 December 2009, and any occurrence of prostate cancer was included. Data on Gleason score were extracted and classified with lesions less than 7 versus tumors with a Gleason score of 7 or more.

The variation of PSA level between the first and second test was expressed in percentage of variation relative to baseline value: [Variation = (Second PSA-First PSA)/ First PSA]. This computation allows for the baseline value to be taken into account. Hence a change from 1 ng/ml to 1.5 ng/ml would be a 50% variation similarly to a change from 2 ng/ml to 3 ng/ml would be a 50% variation. It then gives less variation for a change from 3 to 3.5 than from a change from 1 to 1.5. To describe the overall variation of PSA measured between first and second measurement, either positive or negative, the absolute value of variation was also reported as the total variation.

Test of the association between PSA level at baseline and other factors was conducted in a bivariate analysis with chi-square test.

The evaluation of the impact of individual factors, as well as baseline PSA value, on the total variation was performed with a multivariate linear regression. The impact of age, BMI, tobacco smoking, delay between first and second test, initial PSA value was estimated.

Risks of prostate cancer according to PSA level variations using logistic regression were also computed. This model was adjusted for age at entry in the trial, initial PSA value and variation in PSA between first and second test.

All analyses were conducted with SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA).

## **Results**

Among the 38,340 men participating to the intervention arm, 31,286 men had two PSA tests within two years and with an initial value below 4 ng/mL, a level widely taken as the upper limit of normal.

The PSA level measured in the first test was significantly ( $p < 0.0001$  chi-square test) associated with age (Table 1).

The association of baseline PSA value was also found for BMI ( $p < 0.0001$  chi-square test), for which lower PSA values were observed for obese patients (median 0.94 ng/ml among the 7,337 obese patients: BMI above  $30 \text{ kg/m}^2$ ) and for overweight men (median 1.06 ng/ml among the 15,392 overweight patients: BMI between 25 and  $30 \text{ kg/m}^2$ ), as compared to men with normal BMI (median 1.11 ng/ml among the 7,880 patients with normal weight: BMI between 18.5 and  $25 \text{ kg/m}^2$ ). Tobacco smoking was also significantly associated with a lower PSA level at baseline ( $p < 0.0001$  chi-square test): the median PSA for current smokers was 1.01 ng/ml, for former smokers the median PSA was 1.01 ng/ml, as compared to never

smokers which PSA level was 1.08 ng/ml. The effect of tobacco smoking was independent of BMI as in an linear regression of PSA value at baseline adjusted on BMI status, current smoker had on average 0.10 ng/ml (95%CI (-0.14, -0.07)) less than never smokers. Race was not significantly associated with PSA level at baseline ( $p=0.09$  when comparing the six different ethnic groups and  $p=0.25$  when comparing PSA levels between non-Hispanic white men versus non-Hispanic black men).

The median duration between first and second test was 344 days (interquartile range [IQR]: 324; 365). From the first to second test, the median variation of PSA levels was 3.4% (IQR: -15%; +26%) indicating that for a quarter of men the PSA level at second test was 15% lower than at first test, and that for another quarter of men the PSA level at second test was 26% higher than at first test (figure 1).

In other words, for half of men, the change in PSA level between the first and the second test exceeded 20%. The variation in PSA value was not significantly associated with the delay between first and second test ( $p=0.36$  GLM model), with age ( $p=0.16$ ), with BMI ( $p=0.41$ ) and with race ( $p=0.12$ ). In terms of total variation, the PSA value varied either positively or negatively by an average of 18% between first and second test (interquartile range: 9.2%, 36.8%).

In a multivariate model of the variation of PSA, older age was significantly associated with higher variation in PSA between first and second test. In addition the baseline value of PSA was strongly associated with total variation: lower initial value of PSA tended to be associated with greater total variation (table 2).

2,781 prostate cancers were diagnosed during follow-up. Adjusting for age and initial PSA level, the risk of prostate cancer increased linearly with increasing PSA level at second test, with an odds ratio (OR) of 1.079 (95% CI 1.058-1.101) for each % increase in PSA level.

Table 3 shows the distribution of prostate cancer cases by variation of PSA level between first and second test.

However, the variation in PSA was not associated with a higher Gleason score ( $p=0.95$  for level variations in cancer of Gleason score  $<7$  vs  $\geq 7$  in a logistic regression model adjusting for age and baseline PSA value). Compared to men with decrease or no change in PSA level over time, men with variations in level of 50% or more had a 4.2 (95% CI 2.8-6.2) greater risk to be diagnosed with a lesion having a Gleason score of 5 or less, a 2.0 (95% CI 1.7-2.4) greater risk to be diagnosed with a cancer of Gleason score  $\geq 6$  but below 8, and a 2.5 (95% CI 1.7-3.7) greater risk to be diagnosed with a cancer of Gleason score  $\geq 8$ .

## **Discussion**

In one quarter of men with an initial PSA level less than 4 ng/mL, the level of a second PSA test performed on average one year later will be 20% lower, while for another quarter of men it will be 20% higher. While an increase in PSA level over time is associated with increased risk of prostate cancer, this association seems stronger for cancers of low Gleason score than for potentially more aggressive cancers.

Boddy et al (2004) found in a series of 14 patients with benign prostatic biopsy and an initial PSA level below 4 ng/ml that the coefficient of variation of PSA was 14.1%<sup>12</sup>. This figure is in line with our observation of an average coefficient of variation of 18.8%.

A study focusing on PSA in relation to prostate volume found a yearly increase of PSA associated with PSA baseline value<sup>13</sup>. While this report also found that the initial PSA level was an important factor, lower values were found to be associated with greatest variability. As Nichols et al (2012) used a different metric, the variation of PSA expressed as the difference in ng/ml per year, this metric was also applied to the PLCO dataset. However, the

findings remained unchanged with greater variation observed for the lower values of PSA at baseline.

The initial PSA level was lower among obese and overweight men as reported in previous studies <sup>14,15</sup>. In a study conducted in healthy men, free of prostate cancer, in Washington State, the geometric mean of PSA was 1.18, 1.12, and 0.94 for respectively normal, overweight and obese men <sup>14</sup>. These levels of PSA by obesity status were also observed in this report. Wright et al reported that this association was however partially confounded with use of aspirin and non-steroidal anti-inflammatory drugs (NSAID) <sup>14</sup> which we could not evaluate in these data. We found that further changes in PSA over time were not associated with BMI level.

Ethnicity is a factor associated with access to care and PSA testing is not spared from this inequality. In men with high PSA level, fewer non-Hispanic black men would conduct a repeated PSA test as compared to non-Hispanic white, in particular for those not in the age eligible for Medicare (less than 65) <sup>16</sup>. However, when adjusting for age, baseline PSA and other factors, we did not find an association between change in PSA level and ethnicity as we could have expected that if non-Hispanic black men had fewer tests. As the PLCO study was restricted to men with two tests within two years and in the intervention arm of the PLCO study, and because PLCO study is a randomized trial, these could have selected the populations and reduced the impact of inequality of access to care by ethnic group.

In the PCPT study, the level of PSA was further related with severity of the disease. Prostate cancer with a Gleason score of 7 or above were 40 % with a PSA above 4 ng/ml, as compared to 21 % for those with a Gleason score below 7. Even among those with a PSA below 4 ng/ml this association remained: 60 % of prostate cancer with a Gleason score of 7

or above being above 2 ng/ml as compared to 35% of those with a Gleason score less than 7<sup>1</sup>.

While we demonstrated that an elevated PSA provided an increasing risk of prostate cancer, the results from the PLCO study are in contradiction with the PCPT study, as we found that the initial level of PSA was unrelated to the severity of disease.

Our study has potential limitations. First, a strict selection was used with only men with two tests within two years and no initial value of PSA greater than 4ng/ml. However, this represented 81.6% of the initial sample and therefore a strong bias is unlikely. A contamination by previous PSA tests could have created a bias in the evaluation of PSA variation, however the PLCO study excluded men with more than one PSA blood test in the previous three years. The PLCO study design adds also some limitation but also bring major advantages for the present research. On one hand, participants to PLCO study constitutes, as for any prospective study, a selected population with higher levels of education, lower history of chronic disease. Hence, variations of PSA between two test could be slightly different in the general population. On the other hand, this design enable to investigate variation of PSA in an homogeneous population which received regular opportunity to take a PSA test. Other design conducted in population would have been confronted to greater biases such a self-selection for the number of tests, intervals between tests, all these related to individual's characteristics such as perception of risk, health status, socio-economic level, etc.

Our study supports the idea that PSA testing cannot be considered as a perfect marker of prostate cancer risk. This weakness is inherent to PSA as it measures a characteristic of the prostate which is the release of an antigen in the blood. PSA is also a good marker of other prostate features, such as prostatic volume<sup>17</sup> and inflammation<sup>18</sup>.

Variation in PSA was not associated with higher Gleason score. This observation is in agreement with the lack of effectiveness of PSA Velocity above PSA alone which was illustrated by several studies<sup>19</sup>.

PSA alone at low values does not seem to be a good indicator of risk of prostate cancer as it does not help to differentiate aggressive versus indolent tumors. The use of PSA density has been suggested to provide further discriminant properties<sup>20</sup>, but this measure is still not demonstrated as capable of identifying aggressive tumors.

## References

1. Thompson IM, Ankerst DP, Chi C, et al.: Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 2006; 98: 529-534.
2. Bunting PS: A Guide to the Interpretation of Serum Prostate Specific Antigen Levels. *Clin Biochem* 1995; 28: 221-241.
3. Price CP, Allard J, Davies G, et al.: Pre- and post-analytical factors that may influence use of serum prostate specific antigen and its isoforms in a screening programme for prostate cancer. *Ann Clin Biochem* 2001; 38: 188-216.
4. Yan Y: Intraindividual variation of prostate specific antigen measurement and implications for early detection of prostate carcinoma. *Cancer* 2001; 92: 776-780.
5. Eastham JA, Riedel E, Scardino PT, et al.: Variation of serum prostate-specific antigen levels: an evaluation of year-to-year fluctuations. *JAMA* 2003; 289: 2695-2700.
6. Maatman TJ: The role of prostate specific antigen as a tumor marker in men with advanced adenocarcinoma of the prostate. *J Urol* 1989; 141: 1378-1380.
7. Down L, Metcalfe C, Martin RM, et al.: Seasonal variation in prostate-specific antigen levels: a large cross-sectional study of men in the UK. *BJU Int* 2011; 108: 1409-1414.
8. Connolly D, van Leeuwen PJ, Bailie J, et al.: Daily, monthly and seasonal variation in PSA levels and the association with weather parameters. *Prostate Cancer Prostatic Dis* 2011; 14: 58-62.
9. Inman BA, Zhang J, Shah ND, et al.: An examination of the dynamic changes in prostate-specific antigen occurring in a population-based cohort of men over time. *BJU Int* 2012; 110: 375-381.
10. Wallner LP, Frencher SK, Hsu JW, et al.: Changes in serum prostate-specific antigen levels and the identification of prostate cancer in a large managed care population. *BJU Int* 2013; 111: 1245-1252.

11. Andriole GL, Crawford ED, Grubb RL, 3rd, et al.: Prostate cancer screening in the randomized Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial: mortality results after 13 years of follow-up. *J Natl Cancer Inst* 2012; 104: 125-132.
12. Boddy JL, Dev S, Pike DJ, et al.: Intra-individual variation of serum prostate specific antigen levels in men with benign prostate biopsies. *BJU Int* 2004; 93: 735-738.
13. Nichols JH, Loeb S, Metter EJ, et al.: The relationship between prostate volume and prostate-specific antigen variability: data from the Baltimore Longitudinal Study of Aging and the Johns Hopkins Active Surveillance Program. *BJU Int* 2012; 109: 1304-1308.
14. Wright JL, Lin DW and Stanford JL: The effect of demographic and clinical factors on the relationship between BMI and PSA levels. *Prostate* 2011; 71: 1631-1637.
15. Culp S and Porter M: The effect of obesity and lower serum prostate-specific antigen levels on prostate-cancer screening results in American men. *BJU Int* 2009; 104: 1457-1461.
16. Barocas DA, Grubb R, 3rd, Black A, et al.: Association between race and follow-up diagnostic care after a positive prostate cancer screening test in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer* 2013; 119: 2223-2229.
17. Roehrborn CG, Boyle P, Gould AL, et al.: Serum prostate-specific antigen as a predictor of prostate volume in men with benign prostatic hyperplasia. *Urology* 1999; 53: 581-589.
18. Nadler RB, Humphrey PA, Smith DS, et al.: Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *J Urol* 1995; 154: 407-413.
19. Loughlin KR. PSA velocity: a systematic review of clinical applications. *Urol Oncol.* 2014;32(8):1116-25.
20. Stephan C, Stroebel G, Heinau M, et al.: The ratio of prostate-specific antigen (PSA) to prostate volume (PSA density) as a parameter to improve the detection of prostate carcinoma in PSA values in the range of < 4 ng/mL. *Cancer* 2005; 104: 993-1003.



## Figure legends

Figure 1. Distribution of the percentage variation of PSA level between first and second measurement in the PLCO study. The blue curve is a kernel density estimation with bandwidth=0.6.

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## **Introduction**

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test and 2343 individuals with no PSA measurement were also excluded. Some of these exclusion criteria could be overlapping.

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In other words, for half of men, the change in PSA level between the first and the second test exceeded 20%. The variation in PSA value was not significantly associated with the delay between first and second test ( $p=0.36$  GLM model), with age ( $p=0.16$ ), with BMI ( $p=0.41$ ) and with race ( $p=0.12$ ). In terms of total variation, the PSA value varied either positively or negatively by an average of 18% between first and second test (interquartile range: 9.2%, 36.8%).

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Table 3 shows the distribution of prostate cancer cases by variation of PSA level between first and second test.

However, the variation in PSA was not associated with a higher Gleason score ( $p=0.95$  for level variations in cancer of Gleason score  $<7$  vs  $\geq 7$  in a logistic regression model adjusting for age and baseline PSA value). Compared to men with decrease or no change in PSA level over time, men with variations in level of 50% or more had a 4.2 (95% CI 2.8-6.2) greater risk to be diagnosed with a lesion having a Gleason score of 5 or less, a 2.0 (95% CI 1.7-2.4) greater risk to be diagnosed with a cancer of Gleason score  $\geq 6$  but below 8, and a 2.5 (95% CI 1.7-3.7) greater risk to be diagnosed with a cancer of Gleason score  $\geq 8$ .

## Discussion

In one quarter of men with an initial PSA level less than 4 ng/mL, the level of a second PSA test performed on average one year later will be 20% lower, while for another quarter of men it will be 20% higher. While an increase in PSA level over time is associated with increased risk of prostate cancer, this association seems stronger for cancers of low Gleason score than for potentially more aggressive cancers.

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findings remained unchanged with greater variation observed for the lower values of PSA at baseline.

The initial PSA level was lower among obese and overweight men as reported in previous studies <sup>14,15</sup>. In a study conducted in healthy men, free of prostate cancer, in Washington State, the geometric mean of PSA was 1.18, 1.12, and 0.94 for respectively normal, overweight and obese men <sup>14</sup>. These levels of PSA by obesity status were also observed in this report. Wright et al reported that this association was however partially confounded with use of aspirin and non-steroidal anti-inflammatory drugs (NSAID) <sup>14</sup> which we could not evaluate in these data. We found that further changes in PSA over time were not associated with BMI level.

Ethnicity is a factor associated with access to care and PSA testing is not spared from this inequality. In men with high PSA level, fewer non-Hispanic black men would conduct a repeated PSA test as compared to non-Hispanic white, in particular for those not in the age eligible for Medicare (less than 65) <sup>16</sup>. However, when adjusting for age, baseline PSA and other factors, we did not find an association between change in PSA level and ethnicity as we could have expected that if non-Hispanic black men had fewer tests. As the PLCO study was restricted to men with two tests within two years and in the intervention arm of the PLCO study, and because PLCO study is a randomized trial, these could have selected the populations and reduced the impact of inequality of access to care by ethnic group.

In the PCPT study, the level of PSA was further related with severity of the disease. Prostate cancer with a Gleason score of 7 or above were 40 % with a PSA above 4 ng/ml, as compared to 21 % for those with a Gleason score below 7. Even among those with a PSA below 4 ng/ml this association remained: 60 % of prostate cancer with a Gleason score of 7

or above being above 2 ng/ml as compared to 35% of those with a Gleason score less than 7<sup>1</sup>.

While we demonstrated that an elevated PSA provided an increasing risk of prostate cancer, the results from the PLCO study are in contradiction with the PCPT study, as we found that the initial level of PSA was unrelated to the severity of disease.

Our study has potential limitations. First, a strict selection was used with only men with two tests within two years and no initial value of PSA greater than 4ng/ml. However, this represented 81.6% of the initial sample and therefore a strong bias is unlikely. A contamination by previous PSA tests could have created a bias in the evaluation of PSA variation, however the PLCO study excluded men with more than one PSA blood test in the previous three years. The PLCO study design adds also some limitation but brings major advantages for the present research. On one hand, participants to the PLCO study constitutes, as for any prospective study, a selected population with higher levels of education, lower history of chronic disease. Hence, variations of PSA between two tests could be slightly different in the general population. On the other hand, this design enables to investigate variation of PSA in a homogeneous population which received regular opportunity to take a PSA test. Other design conducted in the population would have been confronted to greater biases such a self-selection for the number of tests, intervals between tests, all these related to individual's characteristics such as perception of risk, health status, socio-economic level, etc.

Our study supports the idea that PSA testing cannot be considered as a perfect marker of prostate cancer risk. This weakness is inherent to PSA as it measures a characteristic of the prostate which is the release of an antigen in the blood. PSA is also a good marker of other prostate features, such as prostatic volume<sup>17</sup> and inflammation<sup>18</sup>.

Variation in PSA was not associated with higher Gleason score. This observation is in agreement with the lack of effectiveness of PSA Velocity above PSA alone which was illustrated by several studies<sup>19</sup>.

PSA alone at low values does not seem to be a good indicator of risk of prostate cancer as it does not help to differentiate aggressive versus indolent tumors. The use of PSA density has been suggested to provide further discriminant properties<sup>20</sup>, but this measure is still not demonstrated as capable of identifying aggressive tumors.

## References

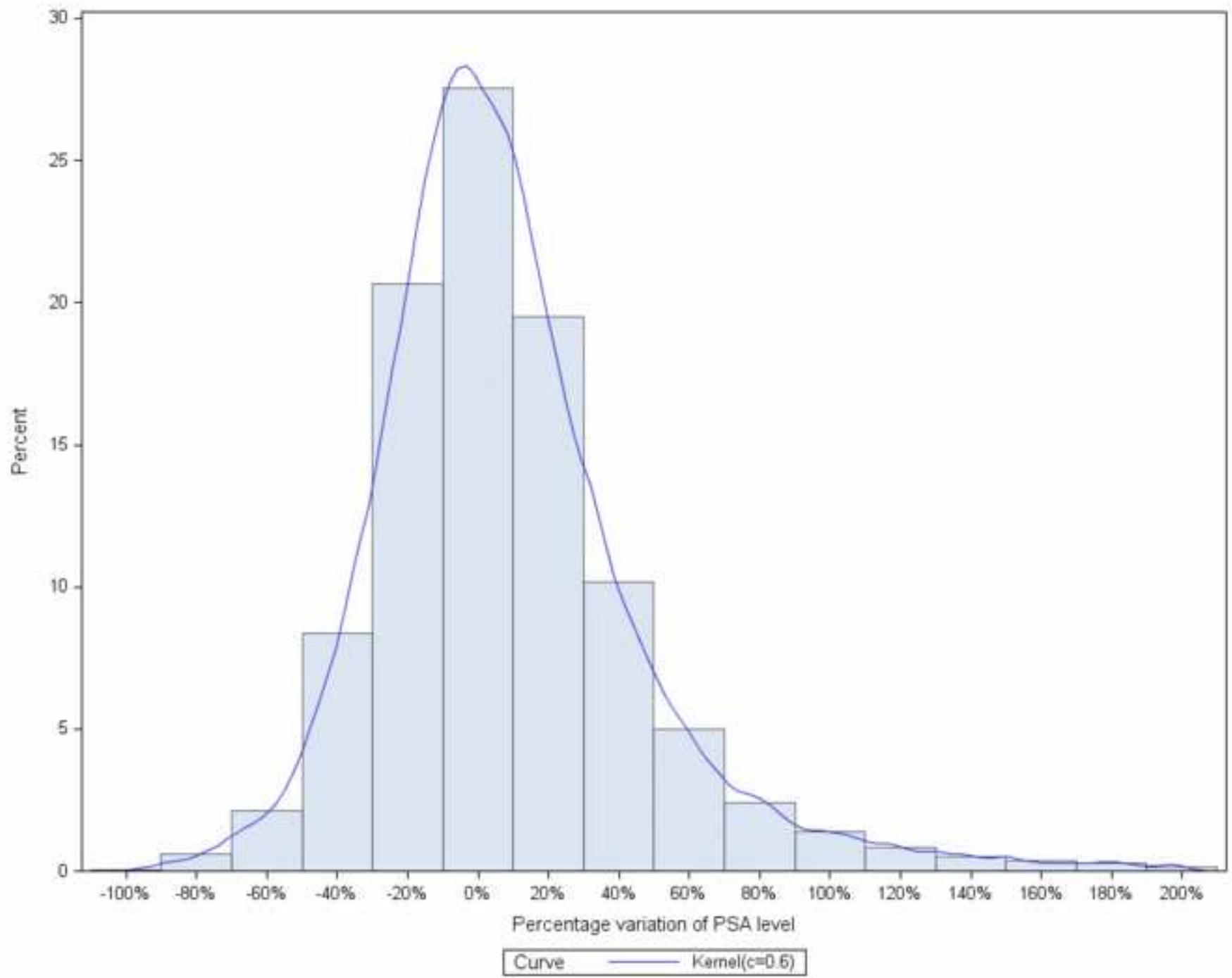
1. Thompson IM, Ankerst DP, Chi C, et al.: Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 2006; 98: 529-534.
2. Bunting PS: A Guide to the Interpretation of Serum Prostate Specific Antigen Levels. *Clin Biochem* 1995; 28: 221-241.
3. Price CP, Allard J, Davies G, et al.: Pre- and post-analytical factors that may influence use of serum prostate specific antigen and its isoforms in a screening programme for prostate cancer. *Ann Clin Biochem* 2001; 38: 188-216.
4. Yan Y: Intraindividual variation of prostate specific antigen measurement and implications for early detection of prostate carcinoma. *Cancer* 2001; 92: 776-780.
5. Eastham JA, Riedel E, Scardino PT, et al.: Variation of serum prostate-specific antigen levels: an evaluation of year-to-year fluctuations. *JAMA* 2003; 289: 2695-2700.
6. Maatman TJ: The role of prostate specific antigen as a tumor marker in men with advanced adenocarcinoma of the prostate. *J Urol* 1989; 141: 1378-1380.
7. Down L, Metcalfe C, Martin RM, et al.: Seasonal variation in prostate-specific antigen levels: a large cross-sectional study of men in the UK. *BJU Int* 2011; 108: 1409-1414.
8. Connolly D, van Leeuwen PJ, Bailie J, et al.: Daily, monthly and seasonal variation in PSA levels and the association with weather parameters. *Prostate Cancer Prostatic Dis* 2011; 14: 58-62.
9. Inman BA, Zhang J, Shah ND, et al.: An examination of the dynamic changes in prostate-specific antigen occurring in a population-based cohort of men over time. *BJU Int* 2012; 110: 375-381.
10. Wallner LP, Frencher SK, Hsu JW, et al.: Changes in serum prostate-specific antigen levels and the identification of prostate cancer in a large managed care population. *BJU Int* 2013; 111: 1245-1252.

11. Andriole GL, Crawford ED, Grubb RL, 3rd, et al.: Prostate cancer screening in the randomized Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial: mortality results after 13 years of follow-up. *J Natl Cancer Inst* 2012; 104: 125-132.
12. Boddy JL, Dev S, Pike DJ, et al.: Intra-individual variation of serum prostate specific antigen levels in men with benign prostate biopsies. *BJU Int* 2004; 93: 735-738.
13. Nichols JH, Loeb S, Metter EJ, et al.: The relationship between prostate volume and prostate-specific antigen variability: data from the Baltimore Longitudinal Study of Aging and the Johns Hopkins Active Surveillance Program. *BJU Int* 2012; 109: 1304-1308.
14. Wright JL, Lin DW and Stanford JL: The effect of demographic and clinical factors on the relationship between BMI and PSA levels. *Prostate* 2011; 71: 1631-1637.
15. Culp S and Porter M: The effect of obesity and lower serum prostate-specific antigen levels on prostate-cancer screening results in American men. *BJU Int* 2009; 104: 1457-1461.
16. Barocas DA, Grubb R, 3rd, Black A, et al.: Association between race and follow-up diagnostic care after a positive prostate cancer screening test in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer* 2013; 119: 2223-2229.
17. Roehrborn CG, Boyle P, Gould AL, et al.: Serum prostate-specific antigen as a predictor of prostate volume in men with benign prostatic hyperplasia. *Urology* 1999; 53: 581-589.
18. Nadler RB, Humphrey PA, Smith DS, et al.: Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *J Urol* 1995; 154: 407-413.
19. Loughlin KR. PSA velocity: a systematic review of clinical applications. *Urol Oncol*. 2014;32(8):1116-1125.
20. Stephan C, Stroebel G, Heinau M, et al.: The ratio of prostate-specific antigen (PSA) to prostate volume (PSA density) as a parameter to improve the detection of prostate carcinoma in PSA values in the range of < 4 ng/mL. *Cancer* 2005; 104: 993-1003.

### Figure legends

Figure 1. Distribution of the percentage variation of PSA level between first and second measurement in the PLCO study. The blue curve is a kernel density estimation with bandwidth=0.6.

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**Table 1. Distribution of men with an initial PSA value below 4 ng/ml by age and PSA value at entry in the intervention arm of the PLCO study.**

<b>Age group</b>		<b>ref: ≤ 59</b>	<b>60-64</b>	<b>65-69</b>	<b>≥ 70</b>	<b>Total</b>
<b>PSA value</b>						
<b>PSA &lt;0.5</b>	n	1,790	1,527	911	461	4,689
	%	17.0%	15.3%	13.0%	12.4%	
<b>PSA 0.5-1</b>	n	3,943	3,259	2,008	996	10,206
	%	37.4%	32.6%	28.6%	26.7%	
<b>PSA 1-1.5</b>	n	2,228	2,076	1,387	698	6,389
	%	21.1%	20.8%	19.8%	18.7%	
<b>PSA 1.5-2</b>	n	1,161	1,212	1,005	527	3,905
	%	11.0%	12.1%	14.3%	14.1%	
<b>PSA 2-3</b>	n	983	1,266	1,098	668	4,015
	%	9.3%	12.7%	15.6%	17.9%	
<b>PSA 3-4</b>	n	441	647	612	382	2,082
	%	4.2%	6.5%	8.7%	10.2%	
<b>Total</b>	n	10,546	9,987	7,021	3,732	31,286

**Table 2. Predictors of the percentage variation in PSA level between first and second test in the PLCO study. Results from a multivariate linear model adjusting for all variables**

Parameter		Estimate	95% confidence limits	p-value
Intercept		0.021	-0.109, 0.151	0.752
Age	≤ 59	Reference	-	-
	60 - 64	0.092	0.040, 0.144	0.0005
	65 - 69	0.103	0.046, 0.161	0.0004
	≥ 70	0.145	0.074, 0.216	<0.0001
BMI (kg/m <sup>2</sup> )	0 - 18.5	0.105	-0.262, 0.471	0.575
	18.5 - 25	Reference	-	-
	25 - 30	0.018	-0.033, 0.069	0.489
	≥ 30	-0.004	-0.065, 0.056	0.895
Smoking status	Never smoked	Reference	-	-
	Former smoker	-0.0008	-0.046, 0.044	0.972
	Current smoker	0.048	-0.024, 0.120	0.194
Race	White, Non-Hispanic	Reference	-	-
	American Indian	0.446	-0.008, 0.900	0.054
	Asian	0.006	-0.100, 0.112	0.912
	Black, Non-Hispanic	0.018	-0.089, 0.124	0.747
	Hispanic	-0.009	-0.158, 0.140	0.903
	Pacific Islander	0.263	-0.016, 0.542	0.065
	Missing	-0.077	-1.054, 0.901	0.878
Delay (per 1 year) between first and second PSA		0.050	-0.069, 0.169	0.412
Level of first PSA	< 0.5	0.524	0.459, 0.589	<0.0001

(ng/ml)

Ref: 0.5 - 1

Reference

-

-

1 - 1.5

-0.060

-0.119, -0.001

0.045

1.5 - 2

-0.114

-0.184, -0.045

0.001

2 - 3

-0.128

-0.197, -0.059

0.0003

3 - 4

-0.136

-0.225, -0.047

<0.0001

**Table 3. Distribution of prostate cancer cases according to PSA levels**

<b>PSA (ng/mL)</b>	<b>Men without prostate cancer (percentage )</b>	<b>Gleason &lt; 7 (percentage)</b>	<b>Gleason ≥ 7 (percentage)</b>	<b>Total</b>
<b>&lt; 0.5</b>	4, 643 (16.29)	19 (1.22)	26 (2.17)	4,688
<b>ref: 0.5 - 1</b>	9,924 (34.81)	142 (9.13)	136 (11.37)	10,202
<b>1 - 1.5</b>	5,914 (20.75)	245 (15.75)	226 (18.9)	6,385
<b>1.5 - 2</b>	3,398 (11.92)	275 (17.67)	228 (19.06)	3,901
<b>2 - 3</b>	3,208 (11.25)	473 (30.4)	324 (27.09)	4,005
<b>3 - 4</b>	1,418 (4.97)	402 (25.84)	256 (21.4)	2,076
<b>Total</b>	28,505	1,556	1,196	31,257
<b>Missing PSA</b>				29