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Robustness of ProsVue™ linear slope for prognostic identification of patients at reduced risk for prostate cancer recurrence: Simulation studies on effects of analytical imprecision and sampling time variation

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ABSTRACT

Objective: The ProsVue assay measures serum total prostate-specific antigen (PSA) over three time points post-radical prostatectomy and calculates rate of change expressed as linear slope. Slopes ≤2.0 pg/ml/month are associated with reduced risk for prostate cancer recurrence. However, an indicator based on measurement at multiple time points, calculation of slope, and relation of slope to a binary cutoff may be subject to effects of analytical imprecision and sampling time variation. We performed simulation studies to determine the presence and magnitude of such effects.

Design and methods: Using data from a two-site precision study and a multicenter clinical trial of 304 men, we performed simulation studies to assess whether analytical imprecision and sampling time variation can drive misclassifications or classification switching of patients with stable disease or recurrence.

Results: Analytical imprecision related to expected PSA values in a stable disease population results in ≤1.2% misclassifications. For populations with recurrent disease, an analysis taking into account correlation between sampling time points demonstrates that classification switching across the 2.0 pg/ml/month cutoff occurs at a rate ≤11%. In the narrow region of overlap between populations, classification switching maximizes at 12.3%. Lastly, sampling time variation across a wide range of scenarios results in 99.7% retention of proper classification for stable disease patients with linear slopes up to the 75th percentile of the distribution.

Conclusions: These results demonstrate the robustness of the ProsVue assay and the linear slope indicator. Further, these simulation studies provide a potential framework for evaluation of future assays that rely on the rate of change principle.

1. Introduction

Prostate cancer (PCa) is the most common male malignancy in the United States (157 cases/100,000 men) and the second leading cause of cancer death (24 deaths/100,000 men) [1]. Treatment of localized cancer generally relies on radical prostatectomy (RP). Following surgery, risk stratification for recurrence traditionally incorporates surgical findings including surgical margin involvement, lymph node involvement, extracapsular extension, seminal vesicle invasion, and pathological factors such as Gleason score and disease staging of the resected tumor [2,3]. In fact, various nomograms including these risk factors and other clinico-demographic variables have been validated and used clinically [4,5]. However, these risk factors and nomograms are generally directed to identification of increased risk for recurrence rather than probability for disease-free survival [2,3,5].

Most recently, the ProsVue Assay has been cleared for commercial use by the US Food and Drug Administration (FDA) as "a prognostic marker in conjunction with clinical evaluation as an aid in identifying those patients at reduced risk for recurrence of prostate cancer for the eight year period following prostatectomy." The assay measures serum total prostate specific antigen (PSA) in post-RP samples and calculates rate of change of PSA over the sampling time period, expressing the outcome as linear slope (pg/ml PSA change per month post-RP). The assay is novel in at least a few respects. First, as the intended use implies, the assay is optimized to identify patients at reduced risk for recurrence, i.e. to identify patients with high likelihood of stable disease. In order to demonstrate efficacy for this indication, the assay employs the immuno-polymerase chain reaction (immuno-PCR) [6] to achieve sensitivity an order of magnitude lower than existing PSA assays [7]. The improved sensitivity allows quantification of PSA at the medically significant [8,9] levels exhibited in stable disease (<5 pg/ml), but which have been historically below the measurement range of former high-sensitivity assays [9,10].

Secondly, the assay is the first to receive clearance based on linear slope of tumor marker concentration versus time post-surgery. Specifically, PSA is measured in three samples taken between 1.5 and

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20 months post-RP, a relevant period of time post-RP in which a patient would wish to know of his long-term risk. The slope (change of PSA with time in the sampling period) is then calculated using simple least squares regression and compared to a threshold of 2.0 pg/ml/month, with values at or below the threshold associated with reduced risk for PCa recurrence.

FDA clearance was received based on a prospectively designed trial employing archived samples from 304 men followed up to 17.6 years post-RP for stable disease (n = 240) or clinical recurrence (n = 64) defined as death due to PCa, positive biopsy or positive imaging for metastases [11]. ProsVue slopes > 2.0 pg/ml/month showed a univariable hazard ratio (HR) for clinical recurrence of 18.3 (95% CI: 10.6–31.8, $P<0.0001$) representing a 94.5% reduction in recurrence risk for patients with slopes ≤2.0 pg/ml/month. Median disease-free survival was 4.8 and >10 years in recurrence and stable disease, respectively ($P<0.0001$). In a multivariable model including pre-RP PSA, pathological stage, and Gleason score, ProsVue HR was 9.8 (5.4–17.8, $P<0.0001$), representing an 89.8% reduction in risk for patients with slopes ≤2.0 pg/ml/month. Sensitivity and specificity for correct classification of recurrence risk were 71.2% (59.2-82.4%) and 94.6% (90.9-97.1%), respectively, and positive and negative predictive value at the study prevalence of 21.1% recurrence were 78.0% (65.3-87.7%) and 92.7% (88.6-95.6%) respectively. (Moul J, et al. NADiA ProsVue PSA slope predicts reduced risk for prostate cancer recurrence following radical prostatectomy: a multicenter study. Submitted to *Urology*).

Despite the efficacy findings, certain questions arise in the use of linear slope. First, does analytical imprecision present a potential risk for misclassification by driving errors in the calculated slope that result in classification switching? Since excursions of precision can occur as point sources in single sampling points or in cumulative effect from the three sampling points, the question is worthy of consideration. Similarly, does variation in the time at which samples are taken drive errors resulting in classification switching? Both questions seek to evaluate the robustness of the ProsVue Assay and are properly presented for clinical chemists and physicians evaluating use of the assay in clinical practice. Furthermore, since future diagnostic assays may employ the rate of change principle, it is important to develop statistical methods to evaluate effects of variation. We performed multiple simulation studies to address the questions specific to ProsVue and also provide a potential framework for evaluation of future assays.

2. Materials and Methods

2.1. Source data

Two prior studies provided data used in the simulations. First, an analytical precision study was performed at two independent laboratories (Iris Molecular Diagnostics, Carlsbad, CA and Molecular Diagnostics Services, San Diego, CA) [5]. The study design followed the Clinical Standards Laboratory Institute (CLSI) EP5-A2 guideline, incorporating variance from three operators, two unique reagent lots, two real-time PCR instruments (Applied Biosystems® 7500 Fast Dx, Life Technologies, Inc.), and 25 days of testing with two assays per day for a total of 50 runs. The results thus represented repeatability, between-run, between-day, between-site, between-operator, between-lot, and between-instrument components of variation. In each assay, three male serum pools (Samples 1, 2, and 3) containing increasing concentrations of PSA were analyzed in duplicate determinations. Mean PSA value in pg/ml, Total standard deviation (SD), and Total percent coefficient of variation (CV) were determined for each sample. In addition, pooled SDs from Samples 1 and 2 and Samples 1, 2, and 3 were calculated as the square root of the average of the variances for each sample combination. For simulation studies, mean PSA values and associated SDs provided key input parameters.

The prospectively designed trial employing archived samples from 304 post-RP patients also provided data for use in simulations. The trial followed a case-cohort design [12], was conducted in accordance with the Declaration of Helsinki, and approved by local investigational review boards. Briefly, three blood samples taken between 1.5 and 20 months post-RP from each patient were acquired and tested in the ProsVue assay. ProsVue PSA values (pg/ml) determined from each sample were regressed versus months post-RP via simple least squares analysis and the linear slope calculated in pg/ml/month for each patient. Slopes were dichotomized such that patients with slopes ≤2.0 pg/ml/month were considered "at reduced risk for prostate cancer recurrence" versus "not at reduced risk for prostate cancer recurrence" for patients with slopes > 2.0 pg/ml/month. (The cutoff of 2.0 pg/ml/month was identified as optimal, producing the fewest classification errors, via comparison of group-based values and uncertainty between groups [13]; receiver-operating characteristic curves; and univariable and multivariable logistic and Cox regression analyses in previous pilot studies of ProsVue [14].) Binary ProsVue slope classification was compared to a binary clinical outcome of recurrence or stable disease for each patient. Clinical recurrence (n = 64 patients) was based on death due to PCa, positive imaging, or positive biopsy for local or distant metastases whereas stable disease (n = 240) was indicated by lack of such findings for at least 8 years post-RP. Time to clinical recurrence or censor was incorporated into Kaplan-Meier survival analyses and uni- and multivariate Cox proportional hazards regressions. The results demonstrated that ProsVue was a significant predictor of risk for PCa recurrence independent of traditional variables including Gleason Score, disease stage, and pre-RP PSA measurements. This trial formed the basis for the regulatory clearance of ProsVue. For simulation studies, ProsVue PSA values and slopes in the recurrent and stable disease populations and inter-individual slope SDs in the recurrent population were utilized.

2.2. Simulation 1 - Effects of analytical imprecision in patients with stable disease

Simulations were performed using the SD of Sample 1 from the precision study as well as the pooled SDs from the combination of Samples 1 and 2 and the combination of Samples 1, 2, and 3; the range of mean values exhibited by the stable disease population from the first through third sampling points from the clinical trial; putative patient post-RP sampling points of 3, 5, and 7 months; 3, 6, and 9 months; and 3, 9, and 18 months; and the mean slope of the stable disease population from the clinical trial as a "drift parameter." For each combination of SD, mean value, and time point scenario, 20,000 random data replications were generated assuming independence of values between simulated patient visits. The assumption of independence was appropriate since values in the stable disease population largely reflect intra-individual biological variation and analytical variation rather than the occurrence of an ongoing biological process.

For each combination of input parameters, the simulation provided six random variates for each patient, the first three variates representing the values that would be used to generate an "initial slope" and the second set of three variates representing a "final slope" for each patient. Comparison of the initial to final slopes yielded the critical outcome of the simulation, the change in slope based on initial analysis and final analysis, which was summarized as the frequency of classification switching ("at reduced risk for PCa recurrence" to "not at reduced risk for PCa recurrence" and vice versa).

2.3. Simulations 2 and 3 - Effects of analytical imprecision in patients with PCa recurrence

ProsVue PSA values from visits 1–3 from the clinical trial in the recurrent population displayed significant departures from normality (Shapiro-Wilk $p<0.05$). Attempts to transform the values failed