Presurgical Biomarker Performance in the Detection of Gleason Upgrading in Prostate Cancer
Kristina Wittig1, Johnathan L. Yamzon1, David D. Smith2, Daniel R. Jeske2,3, and Steven S. Smith1,4

Abstract

Background: Gleason Score (GS) upgrading is generally considered a trigger for exit to definitive treatment during active surveillance (AS). Predicting the potential for GS upgrading would be of value in assessing AS eligibility.
Methods: We assessed the performance of biomarkers in presurgical specimens of expressed prostatic secretion (EPS) in this setting.
Results: Although EPS volume, total recovered RNA, and RNA expression biomarkers (TMPRSS2: ERG, PCA3, PSA) have been successful in both biopsy outcome prediction, and in the prediction of upstaging in active surveillance eligible patients, they were unable to predict upgrading in patients eligible for active surveillance under National Comprehensive Cancer Network guidelines.
Conclusions: These biomarkers do not improve the prediction of upgrading over indications from standard clinical parameters.
Impact: Additional biomarkers will be needed in this area.

Introduction

Current practice during active surveillance (AS) is to monitor low-risk patients by repeating biopsies periodically. Patients with an initial biopsy Gleason Score (GS) of 6 are offered definitive treatment if a repeat biopsy during surveillance yields a GS \( \geq 7 \). This suggests that the cancer may have progressed during the interval from the previous biopsy. Even so, a sizable proportion of GS 7 patients may have been misclassified GS 6 due to sampling error on low-volume tumors (1, 2). For this reason, a noninvasive test that could detect this relatively subtle change in Gleason sum would be of value.

Results with post-DRE urine (3, 4), have consistently mirrored those with expressed prostatic secretion (EPS; refs. 5, 6), suggesting that either specimen can be used in the detection and classification of prostate cancer. In previous work, improvements in AUC values of \( \geq 0.1 \) (3–5) have been obtained for the prediction that cancer will be found at biopsy using these specimens. In this study, we sought to assess the performance of biomarkers in predicting Gleason upgrading in cohorts of men who underwent radical prostatectomy (RP). Our sample sizes were chosen for a statistical power of greater than 90% for an AUC improvement over baseline of \( \geq 0.1 \).

Materials and Methods

EPS specimens were obtained by milking the urethra after an attentive DRE before the patients underwent a Robot-Assisted Radical Prostatectomy (RARP). Of 646 men who consented under an Institutional Review Board–approved protocol for the collection and evaluation of biomarkers in EPS, 642 had complete clinical data, 224 met the 2012 National Comprehensive Cancer Network (NCCN) criteria, and 206 met the 2014 NCCN criteria for active surveillance based on their preoperative characteristics.

EPS collection and processing were as described previously (5, 6). Standard clinicopathologic data (age, Gleason sum, ethnicity, presurgical PSA, T stage, DRE, and race), direct measurements of EPS volume, total recovered RNA, and quantitative reverse transcription PCR values for TMPRSS2:ERG, PCA3, and PSA RNAs were obtained.

ROC analysis of biomarker performance was carried out in an attempt to predict upgrading. Statistical power for AUC differences attributable to biomarkers was estimated with PASS software. Minimal models for each cohort emerged from stepwise logistic regression analysis on six clinical and ten laboratory variables (Table 1).

Results

For the 2012 NCCN AS cohort EPS volume, total RNA and RNA expression biomarkers did not significantly increase the AUC value beyond the 0.6969 AUC achieved by clinical variables for ethnicity and serum PSA in the prediction of upgrading post-surgery (Fig. 1A). For the 2014 NCCN AS cohort, these same biomarkers failed to significantly enhance the AUC value beyond the 0.7222 value achieved with age, ethnicity, and serum PSA (Fig. 1B). For the 2012 cohort with complete clinical and laboratory data and sample sizes (125 not upgraded and 99 upgraded), the power to detect an increase in the AUC of 0.05 and 0.10 was

Reference:
1. Division of Urology, Department of Surgery, City of Hope, Duarte, California. 2Division of Biostatistics, Department of Information Sciences, City of Hope, Duarte, California. 3Department of Statistics, University of California – Riverside, Riverside, California. 4Beckman Research Institute of the City of Hope, Duarte, California.

Corresponding Author: Steven S. Smith, City of Hope, Fox North, Rm 1100A, 1500 E. Duarte Rd., Duarte, CA 91010. Phone: 626-301-8316; Fax: 626-256-8774; E-mail: sssmith@coh.org

doi: 10.1158/1055-9965.EPI-16-0488
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approximately 40% and 90%, respectively. For the 2014 cohort with 206 patients with complete clinical and laboratory data, the sample sizes (112 not upgraded and 94 upgraded), the power to detect an increase in the AUC of 0.05 and 0.10 is also approximately 40% and 90%, respectively.

Discussion

For NCCN AS cohorts, the AUC values achieved with standard clinical markers remained unchanged by the inclusion of biomarkers that have been successful in the prediction of biopsy outcome and upstaging. Although an increase in AUC value of 0.05 due to these biomarkers cannot be excluded, it is unlikely that such an increase would warrant the clinical use of these biomarkers in counseling patients in this setting in spite of their success in other areas of treatment. Moreover, the general concordance between results obtained with EPS specimens and those obtained with urine samples obtained after an attentive DRE argues that the results obtained with EPS will mirror those obtained with post-DRE urine. We conclude that in this cohort

Table 1. Logistic regression model P values for prognostic factors for Gleason upgrading

<table>
<thead>
<tr>
<th>Variable</th>
<th>NCCN Cohorts</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>2012 (N = 224; yes = 99, no = 125)</td>
<td>UV</td>
<td>MV</td>
<td>UV</td>
<td>MV</td>
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<td>Pretreatment Gleason Score</td>
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<tr>
<td>Ethnicity</td>
<td>0.0598</td>
<td>0.0460</td>
<td>0.0702</td>
<td>0.0395</td>
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<td>Race</td>
<td>0.2404</td>
<td></td>
<td>0.4513</td>
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</tr>
<tr>
<td>Age</td>
<td>0.1590</td>
<td></td>
<td>0.0080</td>
<td>0.0066</td>
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<td>DRE</td>
<td>0.1026</td>
<td></td>
<td>0.1462</td>
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<tr>
<td>T Stage</td>
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<td></td>
<td>0.0934</td>
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<td>Prebiopsy serum PSA</td>
<td>0.0001</td>
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<td>&lt;0.0001</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
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<td>PCA3 RNA</td>
<td>0.6811</td>
<td></td>
<td>0.7230</td>
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<td>PCA3 RNA/Input RNA</td>
<td>0.6914</td>
<td></td>
<td>0.7215</td>
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<tr>
<td>PCA3 RNA/PSA mRNA</td>
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<td>0.1906</td>
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<td>TMPRSS2:ERG</td>
<td>0.5614</td>
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<td>0.5773</td>
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<tr>
<td>TMPRSS2:ERG/Input RNA</td>
<td>0.5888</td>
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<td>0.5956</td>
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<tr>
<td>TMPRSS2:ERG/PSA mRNA</td>
<td>0.5188</td>
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<td>0.5385</td>
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<td>PSA mRNA</td>
<td>0.2326</td>
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<td>0.2563</td>
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<tr>
<td>PSA mRNA/Input RNA</td>
<td>0.2356</td>
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<td>0.2320</td>
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<td>Expressed volume prostatic fluid (μL)</td>
<td>0.7702</td>
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<td>0.5221</td>
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<tr>
<td>Total RNA in specimen (ng)</td>
<td>0.5982</td>
<td></td>
<td>0.5917</td>
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NOTE: Univariate (UV) P values are from single variable models. Multivariate (MV) P values are from the final model selected from a stepwise selection procedure.

Figure 1.
ROC for multivariate analysis of biomarkers in the prediction of upgrading. A, NCCN 2012 cohort of 225 patients. 2012 NCCN guidelines permit low- and very low-risk patients with a Gleason sum (GS) ≤ 6, a clinical diagnosis of T1–T2a, and a serum PSA value <10 ng/mL to enter active surveillance. Patients who have less than 10 years life expectancy are permitted to enter AS if they exhibit only one of the following risk factors: GS 7, or T2b–T2c, or PSA 10–20. Baseline GS, serum PSA, and ethnicity gave an AUC value of 0.6969 with or without EPS volume, total recovered RNA, and quantitative reverse transcription PCR values for RNA expression levels from TMPRSS2:ERG, PCA3, and PSA. Patient demographics: ethnicity: Hispanic, 6.3%; non-Hispanic, 92%; unknown, 1.8%. Race; African American, 3.6%; Asian American, 6.3%; Caucasian American, 87.5%; American Indian, 0.4%; Other, 2.2%. B, NCCN 2014 cohort of 206 patients. NCCN guidelines issued in 2014 permit only low- and very low-risk patients with a GS ≤ 6, a clinical diagnosis of T1–T2a, and a serum PSA value <10 ng/mL to enter AS. Baseline GS, serum PSA, age, and ethnicity gave an AUC value of 0.7222 with or without EPS volume, total recovered RNA, and quantitative reverse transcription PCR values for RNA expression levels from TMPRSS2:ERG, PCA3 and PSA. Patient demographics: Ethnicity, Hispanic, 6.3%; non-Hispanic, 91.7%; Unknown, 1.9%. Race; African American, 3.9%; Asian American, 5.8%; Caucasian American, 87.8%; American Indian, 0.5%; Other, 1.9%.
of men, models for predicting upgrading are dominated by the standard clinicopathologic parameters with no significant contribution from current biomarkers of RNA expression or prostate function tested in EPS.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: K. Wittig, S.S. Smith
Development of methodology: K. Wittig, S.S. Smith
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Wittig
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Wittig, J. Yamzon, D.D. Smith, D.R. Jeske, S.S. Smith
Writing, review, and/or revision of the manuscript: K. Wittig, J. Yamzon, D.D. Smith, D.R. Jeske, S.S. Smith
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Wittig, D.D. Smith, S.S. Smith
Study supervision: K. Wittig, S.S. Smith

Grant Support
This work was supported by NIH NCI grants CA 102521 and CA 136055 (to S.S. Smith).

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Received June 13, 2016; accepted July 6, 2016; published OnlineFirst August 19, 2016.

References
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Cancer Epidemiol Biomarkers Prev Published OnlineFirst August 19, 2016.

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