Prognostic Utility of the Cell Cycle Progression Score Generated from Biopsy in Men Treated with Prostatectomy

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Purpose: The cell cycle progression score is associated with prostate cancer outcomes in various clinical settings. However, previous studies of men treated with radical prostatectomy evaluated cell cycle progression scores generated from resected tumor tissue. We evaluated the prognostic usefulness of the score derived from biopsy specimens in men treated with radical prostatectomy.

Materials and Methods: We evaluated the cell cycle progression score in cohorts of patients from the Martini Clinic (283), Durham Veterans Affairs Medical Center (176) and Intermountain Healthcare (123). The score was derived from simulated biopsy (Martini Clinic) or diagnostic biopsy (Durham Veterans Affairs Medical Center and Intermountain Healthcare) and evaluated for an association with biochemical recurrence and metastatic disease.

Results: In all 3 cohorts the cell cycle progression score was associated with biochemical recurrence and metastatic disease. The association with biochemical recurrence remained significant after adjusting for other prognostic clinical variables. On combined analysis of all cohorts (total 582 patients) the score was a strong predictor of biochemical recurrence on univariate analysis (HR per score unit 1.60, 95% CI 1.35–1.90, $p = 2.4 \times 10^{-7}$) and multivariate analysis (HR per score unit 1.47, 95% CI 1.23–1.76, $p = 4.7 \times 10^{-5}$). Although there were few events (12), the cell cycle progression score was the strongest predictor of metastatic disease on univariate analysis (HR per score unit 5.35, 95% CI 2.89–9.92, $p = 2.1 \times 10^{-8}$) and after adjusting for clinical variables (HR per score unit 4.19, 95% CI 2.08–8.45, $p = 8.2 \times 10^{-6}$).

Conclusions: The cell cycle progression score derived from a biopsy sample was associated with adverse outcomes after surgery. These results indicate that the score can be used at disease diagnosis to better define patient prognosis and enable more appropriate clinical care.

Key Words: prostate, prostatic neoplasms, prostatectomy, biopsy, prognosis

Abbreviations and Acronyms

BCR = biochemical recurrence
CCP = cell cycle progression
DVA = Durham Veterans Affairs Medical Center
IHC = Intermountain Healthcare
MC = Martini Clinic
PH = proportional hazards
PSA = prostate specific antigen
RP = radical prostatectomy

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MEN with newly diagnosed prostate cancer are often treated with RP.[1] Unfortunately 30% of men who undergo RP eventually experience an increase in serum PSA (BCR),[2–6] a disease transition point that is often followed by additional therapy. Physicians use clinicopathological parameters to identify patients likely to experience BCR after surgery.[7]

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However, these parameters are limited by the quality of information that can be obtained through clinical examination of the patient or from the small amount of tumor present in diagnostic needle biopsies. As a result, preoperative prediction models are only moderately prognostic, creating significant patient and physician anxiety, which may lead to unnecessary therapy in men with cancer of low malignant potential or inadequate treatment in men with more virulent disease.[8,9] Biomarkers that improve these models could decrease prognostic uncertainty and enable more appropriate treatment decisions.

The CCP score, based on measuring CCP gene expression, is strongly associated with prostate cancer outcomes.[10–13] In previous studies of surgical outcome prediction the CCP score was measured in the prostatectomy specimen. We evaluated the ability of the score derived from needle biopsy to predict tumor biology as measured by BCR and metastatic disease after RP.

PATIENTS AND METHODS

Cohorts

Cohort 1 included 316 randomly selected men from a consecutive series of patients treated with RP at MC from 2005 to 2006. The original diagnostic biopsies were unavailable. Therefore, a pathologist prepared a simulated biopsy for select patients by randomly removing a tissue cylinder 0.6 mm in diameter from the region of the postoperative formalin fixed, paraffin embedded block containing the largest tumor foci and re-embedded it lengthwise to create a simulated biopsy block. A total of 283 samples (78%) generated good quality CCP scores. Median clinical followup in patients without recurrence was 61 months (IQR 60, 73). BCR was defined as postoperative PSA greater than 0.2 ng/ml or secondary treatment (radiation or androgen therapy) for increasing PSA regardless of attaining the 0.2 ng/ml cutoff point. Metastatic disease was confirmed by positive bone scan, computerized tomography, magnetic resonance imaging or plain x-ray.

Cohort 2 included men treated with RP at DVA from 1994 to 2005 who received treatment within 2 years of diagnosis. All eligible patients were included in study unless the diagnostic biopsy was not performed at DVA and, thus, it was unavailable for analysis. CCP scores were generated from the diagnostic biopsy. A total of 186 samples were included, of which 176 (95%) generated good quality CCP scores. Median clinical followup was 88 months (IQR 69, 119). BCR was defined as postoperative PSA greater than 0.2 ng/ml, 0.2 ng/ml for 2 consecutive determinations at least 3 months apart or secondary treatment (radiation or androgen therapy) for increasing PSA regardless of attaining the 0.2 ng/ml cutoff. Metastatic disease was confirmed by positive bone scan, computerized tomography, magnetic resonance imaging or plain x-ray.

Cohort 3 was treated with RP at IHC between 1997 and 2004. CCP scores were generated from the diagnostic biopsy. A total of 151 patients were selected for study, of whom 123 (81%) had good quality molecular data available. We included all 36 available recurrent cases in which BCR developed at any time after treatment up to November 2011. We also included 87 men randomly sampled from the eligible population (case-to-control ratio approximately 1:2) who were free of BCR as of September 2011. BCR was defined as serum PSA greater than 0.2 ng/ml after a recorded nadir PSA of zero. Metastatic disease was confirmed by whole body bone scan.

All patients were diagnosed with prostate adenocarcinoma without evidence of lymph node or bone metastasis. Patients with preoperative PSA greater than 100 ng/ml, other evidence of systemic disease or insufficient remaining tumor to generate a CCP score were excluded from analysis. Patients who received neoadjuvant hormones or radiation preoperatively were also excluded because of the potential to alter the CCP score. Institutional review board approval was obtained at all study sites.

Sample Preparation and CCP Score

Formalin fixed, paraffin embedded tumor blocks containing a simulated (MC) or diagnostic (DVA and IHC) biopsy were analyzed at Myriad Genetics. A board certified pathologist (ZS) identified the cancer area, measured its length in mm and circled it. For each patient with at least an approximately 1 mm tumor on hematoxylin and eosin staining we cut 10, 10 μm sections for RNA isolation.

Select carcinoma regions were macrodissected according to pathologist (ZS) instructions. Carcinoma was deparaffinized and RNA was extracted using miRNeasy (Qiagen®) as described by the manufacturer. Gene expression was measured using TaqMan® Low Density Arrays as previously described.[12] All samples were run in triplicate.

The CCP score was calculated from the expression data of 31 CCP genes normalized by the expression of 15 housekeeper genes as previously described.[12] CCP scores were rejected if more than 9 CCP genes were missing or the SD of CCP scores in the triplicate value was greater than 0.5.

Data Management and Statistics

Patient samples were de-identified before CCP score determination. CCP scores were sent to the collaborator, who unblinded and returned clinical data. Analysis was collaborative and guided by a prespecified statistical analysis plan.

The final data set for combined analysis consisted of 582 eligible patients with complete CCP scores, and data on PSA, biopsy Gleason scores, adjuvant therapy and elapsed time from surgery to last followup, BCR or metastatic disease. Clinical stage and percent of positive cores were also available but incomplete. CCP score was modeled as a continuous predictor. PSA was transformed by the natural logarithm of 1 + PSA in ng/ml to achieve a more symmetrical distribution and suppress variability near the detection level. Gleason scores were obtained from the original pathology reports except those from DVA, where they were centrally re-reviewed for this study. Gleason scores were categorized as a 3-level factor (less than 7, 7 and greater than 7) but converted to integers (1, 2 and 3) to calculate the Pearson correlation coefficient.

Survival analysis was performed with Cox PH methods using date of surgery as the starting time and time to BCR