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A Novel System for Estimating Residual Disease and Pathologic Response to Neoadjuvant Treatment of Prostate Cancer

Claire Murphy, MD¹, Lawrence True, MD¹, Funda Vakar-Lopez, MD¹, Jing Xia, PhD², Roman Gulati², Bruce Montgomery, MD³, and Maria Tretiakova, MD, PhD¹

¹Department of Pathology, University of Washington, Seattle, Washington

²Fred Hutchinson Cancer Research Center, Seattle, Washington

³Department of Medical Oncology, University of Washington, Seattle, Washington

Abstract

Background—Pathologic variables that characterize response of prostate carcinoma to current neoadjuvant therapy have not been characterized in detail. This study reports (1) the histological features of prostate cancer treated with abiraterone and enzalutamide and inter-pathologist variance in identifying these features, and (2) the effect of the novel androgen deprivation agents on residual cancer volume.

Methods—We reviewed sections of prostatectomies from 37 patients treated with neoadjuvant agents and 22 untreated patients, tabulated the frequency of 9 features of cancer (intact cancer glands, isolated cancer cells, poorly formed glands, cribriform architecture, clear spaces, intraductal carcinoma, solid sheets of cancer cells, prominent nucleoli and previously described ABC grouping) and 2 features of benign glands (prominent basal cells and coalescent corpora amylacea). We used several methods, including a novel metric (visual grid system), to estimate residual tumor volume.

Results—The most highly reproducible features were ABC grouping ($\kappa=0.56-0.7$), presence of intraductal carcinoma ($\kappa=0.34-0.72$), cribriform architecture ($\kappa=0.42-0.68$), solid sheets of tumor cells ($\kappa=0.44-0.56$), and coalescent corpora amylacea ($\kappa=0.4-0.54$). Among poorly reproducible features were prominent nucleoli ($\kappa=0.03-0.11$), clear spaces ($\kappa=0.05-0.07$), and poorly formed cancer glands ($\kappa=0.02-0.1$). Determination of tumor mass was excellent regardless of the method used - maximum tumor size ($\kappa=0.9-0.94$), tumor area ($\kappa=0.94-0.96$) and grid-based tumor cellularity ($\kappa=0.9$).

Conclusions—We propose using a set of parameters including maximum tumor size, tumor area/volume, cellularity, volume, and ABC grouping for evaluating radical prostatectomies post neoadjuvant therapy.

Keywords

Prostate cancer; cellularity; interobserver variability; neoadjuvant; residual disease; reproducibility

Introduction

Preoperative systemic (neoadjuvant) therapy is increasingly used in clinical trials for locally advanced prostate cancer. The primary goals are to reduce the size of the tumor, number of tumor cells, increase the likelihood of negative surgical margins, attempt to reach pathological remission, and improve survival.¹² Studies have described the histopathological changes that occur after neoadjuvant therapy, and largely have advocated that Gleason scoring is no longer accurate in assessing severity of disease or prognosis, and therefore assigning a Gleason score is not recommended by the International Society of Urological Pathology.³⁴⁵⁶ This leads to the problem of communicating the pathological findings in treated prostate cancer to the clinician in a way that can help assess response of the neoplasm. Dichotomization of response as pathologic complete response or residual disease is overly simplistic for these objectives, particularly because residual disease after neoadjuvant treatment includes a broad range of actual responses from near-pathologic complete response to partial and minimal responses to frank resistance.⁷

Clinical and pathologic variables that are consistently associated with a better response to neoadjuvant therapy are well characterized for breast cancer, but not for prostate cancer.⁸⁹¹⁰ There are several widely accepted methods to measure residual cancer burden in mastectomy specimens as a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden.¹¹ However, most of these methods cannot be directly applied to prostate due to cancer multifocality, morphologic heterogeneity, intimate association of neoplastic and non-neoplastic components, and high stromal cell density. Therefore, our goal is to critically assess multiple quantitative and semi-quantitative variables that characterize particular dimensions of residual cancer burden in neoadjuvant treated prostatectomy specimens and to evaluate their reproducibility in terms of inter- and intra- observer variability. In our institution, multiple clinical trials that are designed to assess the use of neoadjuvant treatment are ongoing, but long-term patient outcomes are not yet available. Yet, even before any correlation with the long term outcomes is evaluated, verification that candidate pathological variables are reproducible is a critical precondition for clinical use.

Consequently, this study collated different pathological findings in prostate cancers treated in a neoadjuvant fashion from the literature and evaluated how reproducibly pathologists can score them.⁴¹²¹³¹⁴¹⁵¹⁶¹⁷ The tumor features we studied included maximum tumor size, tumor area by point-counting, tumor cellularity (both visual assessment and automated scoring), histopathologic grouping of tumor response and 11 therapy-induced histologic and cellular features. By examining variability of scores between pathologists and by the same pathologists over time this study can help identify variables that are most reproducible and demonstrate potential for classification of pathologic responses to neoadjuvant therapies in clinical trials.

Materials and Methods

Case selection

Having institutional review board approval through the University of Washington, we studied 37 neoadjuvantly treated patients from ongoing prostate cancer clinical trials covering a broad spectrum of neoadjuvant therapies, including enzalutamide, lupron, dutasteride, ketoconazole, estrogen, bicalutamide, and/or abiraterone. From each patient the entire radical prostatectomy slide set was reviewed by a senior pathologist to select a single representative slide with a sufficiently large tumor focus for further assessment. Selected H&E slides were arranged alongside a representative slide from 5 non-treated prostatectomies, which were re-used in each examination. All patients were selected from ongoing clinical trial cohorts who were matched for patient age, Gleason score (4+3 or 4+4), tumor volume, and stage. The slides were labeled and reviewed by four pathologists in the same order once at time zero (round 1), and again after a 6-month washout period (round 2). In the provided data sheet, the various tumor characteristics were recorded independently by the participating pathologists. Additional 17 non-treated prostatectomies were examined for tumor cellularity only, to ensure the presence of high cellularity tumors.

Quantitative (continuous) tumor variables

The size of the dominant tumor focus was measured as the maximum linear dimensions of microscopic residual tumor bed in centimeters, similar to the methodology of Chen et al (2003).¹⁸ Tumor bed area was estimated by point-counting where the residual tumor bed was overlaid by a grid, and the number of positive points (carcinoma within a grid square) represented an estimate of the tumor size in reference to the entire specimen.^{19,20,21}

The assessment of cellularity was based on the schema developed for breast cancer at MD Anderson Cancer Center to measure residual cancer burden after neoadjuvant therapy and resection, where percent cellularity was estimated by determining the fraction of the tumor bed containing carcinoma.¹⁰ A sheet containing examples of percent cellularity was provided with the scoring packet, taken from the “Graphical Illustrations of Percentage Cancer Cellularity” provided on the MD Anderson Residual Cancer Burden Calculator website (supplemental materials, www.mdanderson.org/breastcancer_RCB). Cellularity was then analyzed using automated computer calculation on the Aperio image system and compared to the pathologist's visual estimate.

Immunostaining and Automated Image Analysis

Four micron sections were deparaffinized, washed and immunostained using several different antibodies including P504, high- and low-molecular weight cytokeratins, PSA, AR, and apoptosis markers bcl2 and Caspase 3 (data not shown). The immunostaining was performed in a CAP certified diagnostic immunohistochemistry laboratory according to a standardized protocol as previously described.²² Of all tested antibodies, PSA showed the best discriminatory pattern between brightly stained tumor glands and individual cells against negative or weakly stained benign structures. For this staining we used rabbit polyclonal antibody against prostate-specific antigen (PSA, dilution 1:8,000, DAKO, Carpinteria, CA, USA) on an automated immunostainer (Bond III (TM), Leica Biosystems,

Germany). The PSA-stained slides were scanned on Aperio ScanScope AT (Aperio Technologies, Vista, CA, USA) at 200× magnification. High resolution digital image files were created and saved in the web-based Spectrum Plus digital slide manager. The tumor areas, strongly highlighted by PSA immunostaining, were then subjected to automated analysis using the Positive Pixel Count algorithm. Non-specific labeling of non-cancerous elements, non-cellular secretions and benign glands were excluded by annotation tool and setting up color thresholds prior to applying computer algorithm for quantitating residual tumor cellularity. The percent cellularity was determined as the ratio of positively stained tumor area in brown pixels to total stained tissue area (Figure 1).

Histopathologic grouping (ABC) of tumor response

This grouping classification developed by Dr. Troncoso and co-authors was directly taken from Efstathiou, et al. (2010), who determined three distinct histopathologic groups A, B and C in neoadjuvant treated prostatectomy specimens that were stronger predictors of biochemical relapse than pathologic stage.⁴ Group A consisted of cell clusters, cords, and isolated cells; group B consisted of intact and fused small glands; and group C consisted of any cribriform growth pattern or intraductal tumor spread.⁴ The figure from that study was provided with the scoring packet to the participating pathologists (see supplemental materials).

Qualitative characteristics of therapy-induced changes

The morphological changes that have previously been associated with therapy effect include changes in the stroma, non-neoplastic glands as well as tumor cells. 11 characteristics collected from the literature were chosen, and included prominent basal cells, coalescent corpora amylacea, prominent nucleoli, intact cancer glands, isolated cells, clear spaces, poorly formed glands, cribriform glands, intraductal carcinoma, and solid sheets.²³²⁴⁴¹²¹³¹⁴¹⁵¹⁶¹⁷ The qualitative variables were recorded in a semi-quantitative fashion using both a 3-tier system (0-absent, 1-focal, and 2-diffuse), and a 2-tier system (0-absent, 1-present).

Statistical Analysis

An agreement analysis was performed to evaluate reproducibility. For the continuous tumor characteristics, and the relation between the visual vs. computer-determined cellularity, the intra-class correlation coefficient (ICC) is used. In particular, we used a version—termed ICC(2,k) in Shrout, et. al. (1979).²⁵ that accounts for random effects for specimen (e.g., a particular specimen may be scored systematically higher by all pathologists) and for pathologist (e.g., a particular pathologist may give systematically higher scores to all specimens). The ICC measures absolute agreement on a scale of 0 to 1 where 1 indicates perfect agreement and 0 indicates no agreement. For the qualitative tumor characteristics, Fleiss's kappa coefficient is used.²⁶ The Kappa coefficient is a robust measurement that accounts for agreement occurring by chance; a value of 1 indicates perfect agreement and 0 indicates no agreement. Leave-one-out cross-validation was used to correlate with years of experience and identify outlier pathologists.

Results

Each of the H&E sections of the neoadjuvantly treated patients selected exhibited at least one of the post-treatment histologic characteristics. The maximum two-dimensions of the tumor bed represented ranged from 0.1 to 2.5 cm, the tumor area ranged from 0.01 to 3.5 cm², and the visually estimated tumor cellularity ranged from 1% to 80%. The histology of the treated tumors ranged from a large tumor bed with occasional isolated cells to diffuse tumor glands including the ones with cribriform architecture and intraductal spread. The most common histologic features in the case collection were prominent basal cells in non-neoplastic glands (86%), intact neoplastic glands (83%), and poorly formed glands (80%). The least common findings were clear spaces, presumed areas left by cancer cells (22%) and cell sheets (13%).

Comparison of ICC for continuous and qualitative tumor characteristics in all cancers is shown in Table 1 and Figure 1A. During round 1, interobserver agreement was excellent for tumor maximum size ($\kappa=0.94$), tumor area ($\kappa=0.94$) and tumor cellularity ($\kappa=0.90$), and good for ABC grouping ($\kappa=0.56$). Interobserver agreement for qualitative morphologic features using 3-tier system was modest for presence of coalescent corpora amylacea ($\kappa=0.49$), cribriform glands ($\kappa=0.42$), solid sheets ($\kappa=0.44$) and intraductal carcinoma ($\kappa=0.34$), while for the remaining selected characteristics of therapy-induced changes agreement was poor ($\kappa=0.03-0.29$).

During round 2, agreement improved for ABC grouping ($\kappa=0.7$) and intraductal carcinoma ($\kappa=0.55$), while other variables remained virtually unchanged. Agreement remained highest for the presence of coalescent corpora amylacea ($\kappa=0.40$), cribriform glands ($\kappa=0.45$), and solid sheets ($\kappa=0.45$). Agreement especially remained poor for characteristics such as poorly formed glands ($\kappa=0.02$), clear spaces ($\kappa=0.05$), and prominent nucleoli ($\kappa=0.07$).

Comparison of 3-tier versus 2-tier system showed consistently higher kappa coefficients for the latter except for “intact” cancer glands, “clear” spaces and poorly formed glands (see Table 1, Figure 2B). Of the 11 morphologic parameters, the most reproducible histologic characteristics in both rounds and regardless of the tier system were coalescent corpora amylacea ($\kappa=0.4-0.54$), cribriform glands ($\kappa=0.42-0.68$), intraductal carcinoma ($\kappa=0.33-0.72$) and solid sheets ($\kappa=0.44-0.56$).

The pathologists experience ranged from 1 to 37 years and was assessed by geometric average distance agreement (range 0.2-0.31). The agreement results did not correlate with years of experience ($p=0.19$). Also, there was no significant difference for the agreements by the four pathologists, suggesting there was no outlier pathologist for all therapies.

The intra-observer correlation coefficients for the continuous tumor characteristics showed moderate to high agreement by the observers between rounds ($\kappa=0.54-0.97$). The intra-class correlation for the computer analyzed cellularity and visual cellularity was 0.74 (95% CI is 0.536-0.861, $p<0.0001$) indicating there is high correlation between the two types of measurements.

Discussion

This is the first study showing that tumor features that have been previously shown to be associated with therapy effect, such as tumor size and cellularity previously validated in breast cancer, appear to also be highly reproducible measurements in prostate cancer. Interobserver agreement for ABC grouping, cribriform glands, intraductal carcinoma, solid sheets and coalescent corpora amylacea were modest but improved with practice or using 2-tier system, indicating that they may be highly reproducible with adequate training and methodology. Poor interobserver agreement for other qualitative histologic characteristics of prostate cancer may be due to variable interpretation of the different features, thus suggesting a need for consensus guidelines before evaluation or that qualitative tumor characteristics are not reproducible enough and should not be used to describe tumor response to therapy in clinical decision-making.

With regard to the highly reproducible quantitative parameters, the agreement is excellent ($\kappa=0.94$), although still not perfect. This could be explained by abundance of paucicellular cancer areas in these neoadjuvantly treated tumors that can be difficult to distinguish from benign structures and reliably measure. Other contributing factors is frequent presence of intraductal spread in absence of frankly invasive tumor foci, treatment effects within benign glands mimicking malignancy with nucleomegaly and prominence of nucleoli, profound stromal changes, and inflammatory cells mimicking rare tumor cells. Despite these challenges, this study indicates that regardless of how many years in practice or method of measurement, these 2D measurements were highly reproducible. Although it is not very surprising to see such a high reproducibility of direct measurements, this type of agreement serves as an internal control of our methods, and the pathologists' ability to interpret therapy-related changes. There was no observable correlation between certain histological effects and a specific therapy, similarly noted in other studies, with all treatments demonstrating a variety of morphological changes.²⁷²⁸

Many investigators have been evaluating the use of tumor volume and its relation to outcomes, rather than using TNM stage only. Many studies have suggested that tumor volume is a significant factor in patient's disease free survival.²⁹³⁰³¹³²³³³⁴³⁵³⁶ While many clinical trials using neoadjuvant therapy have shown a reduction in pathologic T stage, this did not necessarily correlate with a decrease in clinical or biochemical progression.³⁷ Additionally, in some studies, the significance of tumor volume remains unclear. In this study we did not directly analyze tumor volume mainly for logistical reasons since hundreds of slides would have to be circulated among the pathologists, twice. Instead, we used one representative slide as a surrogate for the entire case and the study pathologists determined the tumor area by point-counting on this slide with high reproducibility.

Tumor cellularity is another important aspect of treatment response. This is a subjective measurement that has intrinsic bias due to the differences in individual perception, and variability in mental subtraction of benign structures. This metric also showed high agreement between pathologists ($\kappa=0.90$), which may be due in part from the use of the cellularity images from "Graphical Illustrations of Percentage Cancer Cellularity" provided on the MD Anderson Residual Cancer Burden Calculator website. At the same time, it may

have been problematic to extrapolate the breast cancer-based images to prostate cancer due to the morphologic differences. Interestingly, there is no “gold standard” for how to measure cellularity. To investigate this we used computer-assisted methodology to selectively highlight and count tumor cells. PSA showed the best discriminatory pattern between brightly stained tumor glands and individual cells against negative or weakly stained benign structures. This allowed percentile measurement of brown pixels vs all stained pixels as a close approximation of percent tumor cellularity. Non-specific labeling of non-cancerous elements, non-cellular secretions and benign glands can artificially increase percent cellularity, therefore it was minimized by the exclusion tool and setting up color thresholds prior to applying the computer algorithm to quantify residual tumor cellularity. The computer-analyzed cellularity versus visually estimated cellularity showed strong agreement ($\kappa=0.74$). Overall, it appears that cellularity can be accurately measured between pathologists. One limitation of the study was the relatively small number (N=17) of untreated patients. However, despite this limitation, we were able to demonstrate high reproducibility of selected quantitative parameters.

Some of the qualitative histological characteristics were not reproducible; as the agreement was not acceptable for commonly seen characteristics in treated prostatectomies such as poorly formed glands, clear spaces, and prominent nucleoli. What also seemed problematic was quantifying the qualitative variables in a 3-tier system. For the most part, the pathologists indicated the presence of the characteristics, and quantified them differently (e.g. focal vs diffuse). Agreement was improved when using a 2-tier system reflecting merely absence or presence of certain morphologic features. Interestingly, there was no significant difference between rounds 1 and round 2, for any pathologist, indicating that each pathologist has a consistent internal calibration for qualitative features. In a future study, a consensus conference may be useful to establish an approach to quantifying the qualitative characteristics.

The agreement between histopathologic (ABC) grouping was modest ($\kappa=0.56$), but improved in the second round ($\kappa=0.70$). This indicates a better reflection of morphologic tumor response and cellularity, where instead of quantifying a qualitative characteristic, a tumor group is assigned. Although ABC grouping, has not been entirely validated in the setting of patient outcomes, this study shows that their morphologic classification is reproducible and should continue to be a viable and practical way to grade or classify neoadjuvantly treated prostatic carcinomas. We also found that other reproducible morphologic characteristics of prostate cancer were intraductal carcinoma ($\kappa=0.34-0.72$), cribriform architecture ($\kappa=0.42-0.68$) and solid sheets of tumor cells ($\kappa=0.44-0.56$).

This study was primarily of prospective value for future clinical trials. We propose using a set of parameters such as maximum tumor size, tumor area/volume, cellularity, volume, and ABC grouping that proved to be most reproducible for evaluating radical prostatectomies post neoadjuvant therapy. To validate the prognostic significance of these metrics, large multi-institutional studies are warranted to further correlate them with clinical response, treatment resistance and patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
This figure illustrates the PSA staining and areas circled for the computer-assisted cellularity measurements, and examples of software-estimated cellularity: A - <10%, B - 15%, C - 25%, D - 30%, E - 40%, F - 50%, G - 60%, H - 70%, I - >80%.

A

B

Figure 2. Intra-class correlation coefficients for the tumor characteristics comparing: A - round 1 and round 2, B - interobserver agreement using 2-tier versus 3-tier system.

Table 1
Intra-class correlation for continuous tumor characteristics and Fleiss's Kappa for the qualitative tumor characteristics

Parameter	Round 1	Round 2	Round 1	Round 2
Continuous Tumor Characteristics				
2D size 1st	0.94 ****	0.90 ****		
2D size 2nd	0.94 ****	0.92 ****		
Eyeball %	0.90 ****	0.90 ****		
Est tumor area	0.94 ****	0.96 ****		
Qualitative Tumor Characteristics				
ABC group	0.56 ****	0.70 ****		
Tier-system	3-tier (absent, focal, diffuse)		2-tier (absent, present)	
Basal cells	0.10 *	0.08	0.14 *	0.19 **
Coalescent corpora amylacea	0.49 ****	0.40 ****	0.54 ****	0.45 ****
Prominent nucleoli	0.03	0.07	0.06	0.11
“Intact” cancer glands	0.17 **	0.24 ****	0.00	0.01
Atrophic cancer glands	0.29 ****	0.21 ****	0.34 ****	0.30 ****
Isolated cells	0.19 **	0.30 ****	0.31 ****	0.37 ****
“Clear” spaces	0.07	0.05	0.06	0.05
Poorly formed glands	0.06	0.02	0.04	0.10
Cribriform glands	0.42 ****	0.45 ****	0.68 ****	0.62 ****
Intraductal carcinoma	0.34 ****	0.55 ****	0.53 ****	0.72 ****
Solid sheets	0.44 ****	0.45 ****	0.56 ****	0.53 ****

* denote significance at the 10% levels

** denote significance at the 1% levels

*** denote significance at the 0.1% levels

**** denote significance at the 0.01% levels