Prediction of Pathologic Stage and Postprostatectomy Disease Recurrence by DNA Ploidy Analysis of Initial Needle Biopsy Specimens of Prostate Cancer

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Background. DNA ploidy determination of carcinomas in radical prostatectomy specimens has shown significant correlation with patient outcome, but the predictive value of ploidy status of cancers obtained by transrectal ultrasound-guided needle biopsies has not been studied extensively.

Methods. Eighty-nine paired needle biopsy specimens (NBX) and radical prostatectomy (RPX) specimens from patients with early clinical stage (A2–B2) prostate cancer were evaluated for DNA content by image analysis of Feulgen stained tissue sections. Findings were compared with Gleason grading on the same specimens by univariate and multivariate analyses for prediction of local tumor invasion, metastasis, disease recurrence, and serum prostate specific antigen concentration during a 0.9–6.0 year clinical follow-up period.

Results. There was excellent correlation of ploidy status between NBX and RPX specimens ($P < 0.0001$); NBX and RPX grades did not correlate. On RPX specimens, aneuploid status correlated with high tumor grade ($P < 0.0005$). Aneuploidy in NBX specimens was associated with a twofold higher rate of extracapsular spread (ECS) ($P = 0.04$). Aneuploid NBX tumors featured a tenfold greater frequency of metastasis than did diploid NBX tumors ($P < 0.005$). Radical prostatectomy grade correlated with ECS ($P < 0.001$) and presence of metastatic disease ($P = 0.04$). On multivariate logistic regression analysis, aneuploidy in both NBX and RPX specimens was the most significant variable and independently predicted the presence of metastasis ($P = 0.006$ for NBX; $P = 0.028$ for RPX). Tumor grade of NBX and RPX specimens did not independently predict metastatic disease or disease recurrence, but RPX grade was associated independently with ECS ($P = 0.005$). Aneuploid NBX tumors recurred after RPX three times more often than did diploid cases, which was significant on univariate ($P < 0.001$) and multivariate ($P = 0.018$) analyses using the Cox proportional hazards model. There was no correlation with NBX or RPX Gleason score and disease recurrence. Preoperative serum PSA concentration did not correlate with tumor grade or ploidy status, but on multivariate analysis, when paired with ploidy status, independently contributed to the propensity for ECS, metastasis, and disease recurrence.

Conclusions. DNA content analysis of early clinical stage prostate carcinoma needle biopsy specimens by image analysis directly correlates with radical prostatectomy specimen ploidy status and is associated independently, with the presence of metastasis, postprostatectomy disease recurrence, and ECS. Needle biopsy tumor grading did not correlate with prostatectomy grade and did not predict disease outcome accurately. *Cancer* 1994; 74:2811–8.

Key words: prostate cancer, DNA ploidy, tumor grade, needle biopsy, radical prostatectomy.

Serum prostate specific antigen screening followed by transrectal ultrasound-guided, spring-loaded, automatic needle biopsy has led to an increasing detection of prostate cancer in the United States. Recent studies indicating the potential benefits of withholding therapy in older men with limited disease and the potential to predict inoperable cancer in men with biologically aggressive tumors[1,2] has prompted the search for new biomarkers that could be applied for prognostic assessment on the initial narrow bore prostate needle biopsy. Although traditionally regarded as the cornerstone in dis-

ease outcome prediction, tumor grading on needle biopsies may suffer from sampling limitations and artifacts created by the biopsy procedure.[3,4]

Tumor DNA ploidy determination has achieved wide acceptance as a predictor of disease progression in prostate cancer.[4–20] However, most prostate carcinoma DNA content studies have been performed on archival specimens analyzed by flow cytometry or image analysis obtained from radical prostatectomy specimens.[4–24] Although DNA ploidy determination on initial prostate needle biopsies (NBX) has been studied on fine needle aspiration cytology specimens,[25–27] the DNA content of narrow bore core tissue biopsies has not been extensively considered,[28–31] and correlation of ploidy status of NBX specimens with classic prognosis parameters in the disease has not been well documented. We present the needle biopsy DNA content findings in 89 patients with prostate adenocarcinoma and correlate the results with the tumor grade and DNA ploidy status of the subsequent radical retropubic prostatectomy specimens; the final postprostatectomy pathologic stages; the preoperative and postoperative serum prostate specific antigen concentrations; and the frequency of disease recurrence.

Patients and Methods

Patients

From an overall group of 105 patients with early clinical stage prostate carcinoma, 89 (85%) patients who registered with the urologic oncology service of the Albany Medical Center Hospital underwent transrectal ultrasound-guided, narrow bore prostate biopsy (NBX) and subsequent radical retropubic prostatectomy (RPX) from 1986 through 1992 were included in this study. Biopsy needles were 18 gauge, 1.8 mm in diameter, spring-loaded devices obtained from the Bard (Bard Surgical Co., Murray Hill, NJ) and Meditek (Meditek Co., Watertown, MA) companies. All patients had clinical Stage A2–B2 disease before RPX. The serum PSA was determined by the Hybritech (Hybritech Co., San Diego, CA) method. The medical records and microscopic slides were reviewed in all patients. Patient eligibility was determined by the presence of sufficient tumoral tissue in the NBX to perform DNA content analysis on a minimum of 100 cells. All RPX specimens were serially sectioned and totally embedded for microscopic study. The clinical follow-up period from the time of biopsy diagnosis ranged from 0.9 to 6.4 years, with a mean of 2.6 years. No patients received hormonal or radiotherapy before the diagnosis of postprostatectomy disease recurrence.

Tumor grade was determined according to the Gleason system[32] on tissue from the original needle bi-

opsy and subsequent radical prostatectomy specimens in all patients. Grading was performed independently by three pathologists and the average score used for each patient. Disease recurrence was defined as an elevation of serum PSA to 0.4 nanograms per milliliter (ng/ml) at any time beginning 1 month after the RPX. All recurrent serum PSA elevations were confirmed by a repeat specimen 1 month after the initial elevated measurement. The incidence of a positive resection margin for tumor was recorded in all patients. Metastasis (Stage D) was defined as lymph node involvement at RPX or biopsy-proven recurrent disease in bone or parenchymal organs outside the pelvis identified during the follow-up period. Extracapsular spread (ECS) was defined as local tumor invasion of seminal vesicle, transcapsular penetration, positive resection margin, or the presence of metastasis (Stages C or D).

Quantitative DNA Analysis

Five micrometers of formaldehyde solution fixed, paraffin embedded tissue sections of the NBX and representative blocks from the RPX specimens were stained by the Feulgen method and analyzed for DNA content using the Roche RPW Image Analyzer (Roche Image Analysis Systems, Elon College, NC). No patients had received medical or radiation treatment before DNA analysis on the biopsy or prostatectomy specimens. The instrument was calibrated using similarly stained rat hepatocytes, and histograms for 100 benign prostate acinar epithelial cells adjacent to the adenocarcinomas were obtained. The DNA indices of the benign internal control cells were adjusted to 1.0. The relative DNA content of the adjacent adenocarcinoma was measured on a minimum of 100 cells, and the tumor DNA index was determined by comparison with the control diploid cells. DNA ploidy was measured on the cells in the highest tumor grade areas in the NBX and RPX specimens. Highest tumor grade areas were used for histogram generation in patients with multiple positive needle biopsies and in patients with multiple tumoral foci on the RPX specimen.

The coefficients of variation for the G0/G1 peaks of the internal diploid cells in all tissue histograms ranged from 9% to 23% (mean, 14%). All tumor cell histograms were reviewed without knowledge of the specimen source, and to accommodate the relatively wide coefficients of variation of the normal control cells and tumor cell tissue section histograms, a DNA index of 0.77–1.23 was considered to be diploid. Tumor cell populations with G0/G1 peaks in the tetraploid range were included in the aneuploid group. Thus, the aneuploid tumors were defined as hyperdiploid tumors with DNA index greater than 1.23. Tumors with G0/G1 peaks in the diploid range with G2M components in the tetra-

Table 1. Correlation of DNA Ploidy Status With Prognostic Variables and Outcome for Needle Biopsy and Prostatectomy Specimens

Ploidy status	Number	High grade	Low grade	Serum PSA (ng/ml)	Tumor confined to prostate at RPX	Cases with ECS stage C and/or D	Cases with positive resection margin	Cases with metastases stage D	Cases with disease recurrence	Cases without disease recurrence
Needle biopsy NBX										
Diploid	75	10 (13)	65 (87)	10.5	51 (68)	24 (32)	13 (17)	2 (3)	10 (13)	65 (87)
Aneuploid	14	5 (36)	9 (64)	10.2	5 (36)	9 (64)	7 (50)	4 (29)	7 (50)	7 (50)
Prostatectomy RPX										
Diploid	74	4 (19)	60 (81)	10.4	49 (66)	25 (34)	14 (19)	2 (3)	11 (15)	63 (85)
Aneuploid	15	10 (67)	5 (35)	10.7	7 (47)	8 (53)	6 (40)	4 (27)	7 (40)	9 (60)

Unless otherwise indicated, values are no. (%). PSA: prostate specific antigen; RPX: radical prostatectomy; ECS: extracapsular spread; NBX: needle biopsy.

ploid range of less than 5% of the total cell population were considered to be diploid.

Statistical Calculations

Univariate analysis comparing DNA ploidy status on NBX and RPX specimens with tumor grading and in the prediction of ECS (Stages C or D) at prostatectomy and local or distant metastases (Stage D) was performed using the one-tail Fisher exact method. The criterion for significance was a P value of less than 0.05. Univariate and multivariate analysis of the association of ploidy and grade status with disease recurrence after RPX was examined with the Cox proportional hazards model predicted at 5 years. Multivariate analysis of the prediction of ECS and metastasis was performed using the logistic regression method. Comparison of ploidy status with grade and with the preoperative serum PSA concentration was run by the correlation coefficient method.

Results

Tumor Grade

Of the 89 preprostatectomy needle biopsy (NBX) specimens, 15 (17%) were classified as high grade, with Gleason scores of 7 or greater, and 74 (83%) were low grade, with Gleason scores of 6 or lower (Table 1). Of the radical prostatectomy (RPX) specimens, 24 (27%) were high grade and 65 (73%) were low grade. Of the 89 total specimens, 60 (67%) were concordant for grade and 29 (33%) were discordant. The 29 that were discordant included 19 that were low grade at NBX and high grade at RPX and 10 that were high grade at NBX and low grade at RPX. There was no statistically significant association between NBX and RPX grade (P = 0.37). Linear correlation analysis using the actual Gleason scores, rather than the high and low grade categories,

also showed no significant association between NBX and RPX grade status (r = 0.24).

Tumor Grade versus Ploidy Status

In the 89 NBX specimens, statistical analysis of the association of high grade with aneuploid status did not achieve significance (P < 0.06). Of the 74 low grade NBX specimens, 65 (88%) were diploid and 9 (12%) were aneuploid. Of the nine low grade aneuploid NBX specimens, six (67%) were upgraded to high grade status on the RPX specimen. In the 15 high grade specimens on NBX, 5 (33%) were aneuploid and 10 (67%) were diploid (Fig. 1). Using the RPX specimens, a significant association of ploidy status with tumor grade was found, with 60 of 65 (90%) RPX final low grade specimens having a diploid DNA content and 10 of 24 (42%) final high grade specimens featuring aneuploid histograms (P = 0.00005). Of 15 aneuploid tumors at RPX, 10 (67%) were high grade and 5 (33%) were low grade lesions (Table 1).

Tumor Grade versus Prognosis Parameters

When the specimens separated into high grade and low grade lesions on the needle biopsies or when individual Gleason scores were used, the association of grade with ECS at RPX (Stage C or D), incidence of metastases (Stage D alone) at RPX or during the follow-up period, and postprostatectomy disease recurrence all were nonsignificant (Figs. 2–4). However, the final RPX tumor grade, in contrast to the NBX specimen findings, significantly predicted on univariate analysis the presence of ECS at RPX, with high grade lesions having a threefold greater likelihood of ECS status than did low grade lesions (P < 0.0001). RPX tumor grade also significantly predicted metastatic disease (P = 0.04) and disease recurrence at 5 years (P = 0.012).
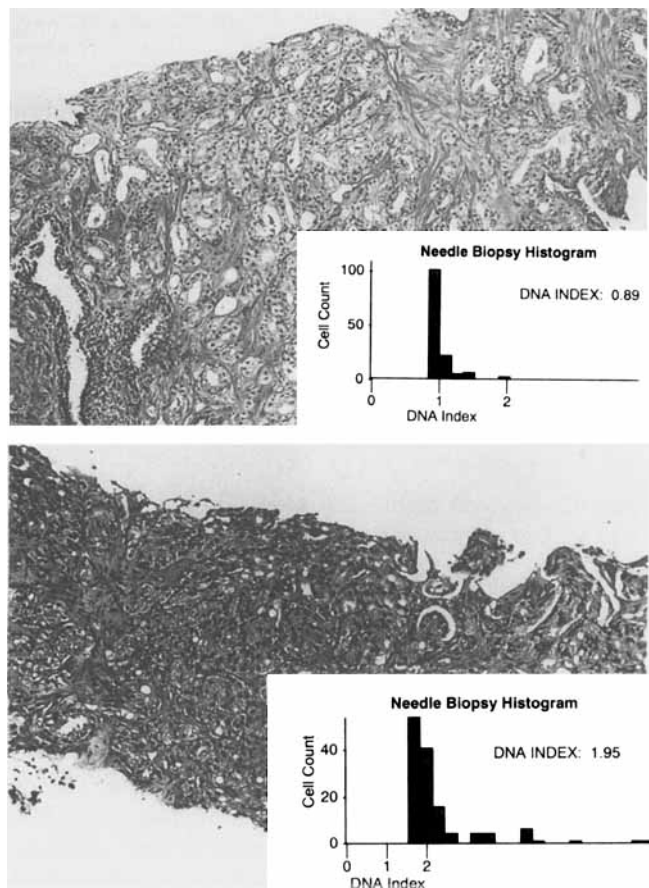
Figure 1. (Top) Low power photomicrograph of spring-loaded, automatic needle biopsy of low grade prostate adenocarcinoma. (Inset) Diploid DNA histogram with DNA index of 0.85 (H & E, original magnification ×40). (Bottom) Low power photomicrograph of spring-loaded, automatic needle biopsy of high grade prostate adenocarcinoma. (Inset) Near tetraploid aneuploid DNA histogram with DNA index of 1.95 (H & E, original magnification ×40).

Resection Margin Status

Of the 89 patients, 20 (22%) had a microscopically proven positive margin of resection at RPX (Table 1). These patients included 13 with high grade and 7 with aneuploid NBX tumors. There was a significant correlation between positive resection margin status and disease recurrence during the follow-up period ($P < 0.001$).

DNA Ploidy Status

There was a highly significant correlation between the NBX specimen DNA histograms and those obtained from the corresponding subsequent RPX specimens ($P < 0.001$). Of the 89 patients, 14 (16%) had aneuploid tumors at NBX and 15 (17%) had aneuploid tumors at RPX (Table 1). There were three (3%) discordant tumors, which included two NBX diploid tumors that

were aneuploid at RPX and one that was aneuploid at NBX and diploid at RPX. Linear correlation analysis revealed significant correlation between NBX and RPX ploidy status ($R = 0.70$; $P < 0.01$).

Ploidy Status as a Prognosis Parameter

Aneuploid tumor status on NBX significantly correlated with ECS (Fig. 2), metastasis (Fig. 3), and postoperative disease recurrence predicted at 5 years (Fig. 4). On NBX, univariate analysis revealed that aneuploid tumors were twice as likely to achieve ECS at prostatectomy ($P < 0.03$); ten times more likely to metastasize ($P = 0.005$), four times more likely to recur during the observed period (50% versus 13%), and twice as likely to recur during the 5-year predicted (99% versus 56%) postoperative follow-up period ($P = 0.001$). Aneuploid status of the RPX specimen also significantly predicted the presence of metastasis ($P = 0.007$) and postoperative disease recurrence ($P = 0.009$) but did not significantly associate with ECS.

Serum Prostatic Specific Antigen Level

There was no statistically significant relationship between the serum PSA concentration before prostatectomy and the tumor grade or DNA ploidy status of either the NBX or RPX specimens. The mean preoperative
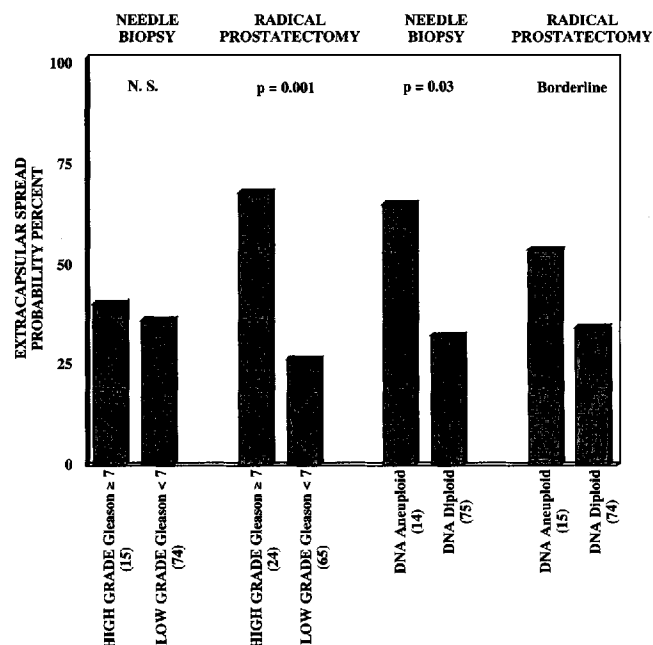

Figure 2. Comparison of tumor grade versus ploidy status on NBX and RPX specimens in the prediction of extracapsular spread (Stages C or D) in prostate cancer. Statistical comparison by univariate analysis using the one-tail Fisher exact method. The number of patients is shown in parentheses.
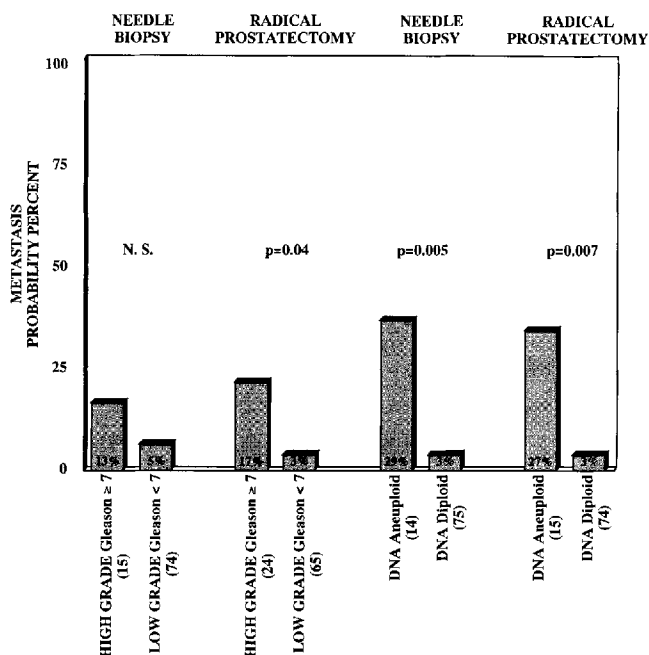
Figure 3. Comparison of tumor grade versus ploidy status for NBX and RPX specimens in the prediction of metastasis (Stage D) in prostate cancer. Statistical comparison by univariate analysis using the one-tail Fisher exact method. The number of patients is shown in parentheses.

PSA concentration of the 89 patients with early stage prostate cancer was 10.4 ng/ml. In 88 of the 89 (99%) patients, the PSA concentration fell below 0.1 ng/ml after prostatectomy. The 17 patients included in the group with recurrent disease had a mean initial postoperative recurrent elevation of the PSA of 2.3 ng/ml. These 17 patients included 16 in whom the PSA concentration rose after prostatectomy to greater than 0.4 ng/ml and one patient in whom the PSA concentration failed to return to below 0.1 ng/ml after RPX. On univariate analysis, the peak preoperative PSA concentration did not correlate with the risk of postoperative disease recurrence ($P = 0.117$). Although a stronger association of preoperative PSA concentration was obtained for prediction of ECS ($P = 0.052$) and metastatic disease ($P = 0.062$), the results did not reach the significance cutoff limits.

Multivariate Analysis

Multivariate analysis revealed that aneuploid status on NBX was the most significant predictive variable and achieved independent statistical significance in the association with metastasis by logistic regression analysis ($P = 0.0006$) and postoperative disease recurrence by Cox proportional hazards testing ($P < 0.001$). DNA content analysis of the NBX specimens also independently predicted ECS (direct transcapsular invasion or

metastasis) ($P = 0.025$). Tumor grade on the NBX specimens did not independently associate with any of the tumor outcome variables. However, RPX tumor grade independently correlated with ECS ($P = 0.028$). The preoperative serum PSA concentration, not a significant predictor of outcome on univariate analysis, when paired with the NBX DNA ploidy status also independently predicted ECS ($P = 0.038$), metastatic disease ($P = 0.031$), and disease recurrence ($P = 0.028$). RPX grade, but not NBX grade or NBX or RPX ploidy status, independently associated with limited direct invasion beyond the capsule, seminal vesicle involvement, or positive surgical margins (Stage C only) without concurrent or subsequent metastasis ($P = 0.02$).

Discussion

The use of radical retropubic prostatectomy as the major primary therapy for patients with newly diagnosed prostate adenocarcinoma has been called into question.[1,2] Many older men with biologically indolent forms of the disease may be treated by far more conservative methods, and younger men with aggressive tumors may be have inoperable disease at the time of diagnosis. Thus, a significant clinical, ethical, and epidemiologic issue has arisen regarding which patients should undergo radical surgery. The current study evaluated the DNA content histogram of the initial transrectal ultrasound-guided, prostate cancer, needle biopsy
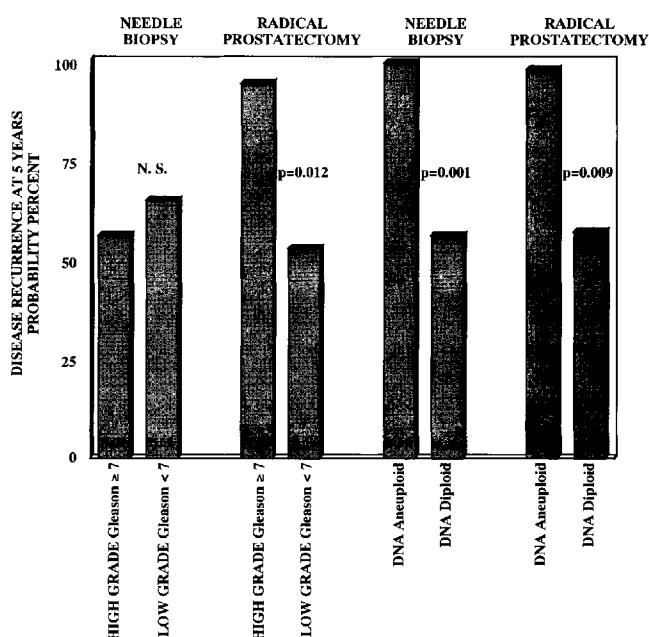

Figure 4. Comparison of tumor grade versus ploidy status in NBX and RPX specimens in the calculated prediction of disease recurrence 5 years after prostatectomy. Statistical comparison by univariate analysis using the Cox proportional hazards model.

specimen as a prognostic indicator and examined the potential of DNA ploidy status as a factor to be used in conjunction with established parameters in the selection of treatment.

Tumor grading has long served as the hallmark for predicting prognosis in prostate carcinoma. Although many previous studies have shown direct statistically significant correlation between grading/stage and recurrence rate/survival, these studies used large-bore needle biopsy, transurethral resection, or total prostatectomy specimens.[32–35] The transrectal, ultrasound-guided, spring-loaded, automatic biopsy, by far the most common type of initial prostate adenocarcinoma specimen evaluated by surgical pathologists today, produces a narrow bore specimen prone to fragmentation, crush artifact, and tumor sample limitations. Recent studies have confirmed the general moderate to weak correlation between the initial grade of the NBX compared with the final grade for patients who undergo subsequent radical retropubic prostatectomy.[3,4]

In the current study, when specimens were stratified into low grade and high grade or when actual Gleason scores were used, there was no statistically significant correlation between NBX grade status and tumor stage at radical prostatectomy, incidence of pelvic nodal or distant metastases, or frequency of postoperative disease recurrence during the follow-up period. In addition, in contrast to the NBX results and in keeping with previous studies,[32–35] tumor grade on the RPX specimens associated with aggressive tumor growth and independently predicted postoperative disease recurrence. The 26% discordant rate, featuring a trend for upgrading of the NBX grade at the time of prostatectomy, is similar to previously published results.[3,4] Similarly, although there was a significant correlation between tumor grade and DNA content in the current results and in the literature for prostate resection specimens,[36–37] in the current study DNA ploidy status and tumor grade did not significantly correlate for the NBX specimens. This finding reflects the significant number of aneuploid low grade NBX specimens that were upgraded at RPX and underscores the relative inaccuracy of tumor grading on narrow bore needle biopsies that significantly limits this prognosis variable in the prediction of pathologic stage at prostatectomy and subsequent clinical outcome.

A variety of techniques have been used to determine prostate cancer DNA content, including image analysis of whole cell fresh touch preparations, tissue sections, and fine needle aspirations, and flow cytometric analysis of fine needle aspirations, fresh tissue, and archival paraffin embedded tissue disaggregation specimens. Most of these studies used RPX specimens, and data evaluating prospective prediction of disease outcome by DNA analysis of transrectal, ultrasound-

guided, prostate narrow bore needle biopsies generally is lacking in the literature. In a series of studies from the Karolinska Hospital,[25–27] flow cytometric DNA analysis of prostate cancer fine needle aspirations revealed that tumor ploidy status may contribute significant objective data regarding the malignant potential of prostate carcinoma. Of recently performed core biopsy DNA content studies, Scrivner et al.[29] reported an association between flow cytometrically determined DNA aneuploidy and a high bromodeoxyuridine labeling index in NBX carcinoma specimens; van den Ouden et al.[28] reported that DNA ploidy status on disaggregated paraffin embedded NBX specimens studied by flow cytometry produced meaningful results in the prediction of prognosis; and Konchuba et al.,[30] in another retrospective flow cytometric study, reported correlation between ploidy status and tumor grade on NBX specimens. More recently, Leung et al.[31] reported an excellent agreement between NBX tissue section ploidy status on image analysis with the histogram from a nuclear suspension of the matched subsequent RPX specimen.

Although most reports have indicated a positive correlation between ploidy status and outcome, not all studies of DNA content in prostate cancer have achieved statistically significant prognostic results.[21–24] These noncorrelating studies generally have featured retrospective flow cytometric analysis of disaggregated paraffin embedded, formalin fixed RPX specimens. Falkmer[38] has reviewed methodologic sources of potential errors in noncorrelating studies of DNA analysis in prostate cancer and concluded that enzyme disaggregation techniques and possible intratumoral heterogeneity may be important factors to be considered in evaluating the noncorrelating studies.

On a typical prostate needle biopsy, given the small number of tumor cells generally available for measurement and the need to preserve tissue for permanent record, the image analysis tissue section technique as outlined by Bacus et al.[39] has become the preferred method for DNA content determination for this type of specimen in our laboratory. Although requiring a relatively thin (5-μm) section for nuclear separation that results in partial nuclear visualization, when tumor cell Feulgen staining intensity is compared with that of similarly sectioned benign internal control prostate acinar epithelial cells, results from this method generally have correlated in an excellent fashion with whole cell image analysis companion specimens when studied at prostatectomy.[4,40] Given that the tissue section method results in a wider coefficient of variation of the G0/G1 peak, the current technique has used a relatively higher cutoff point for the DNA index of the diploid range. This approach may, in part, account for the 16% rate of aneuploidy on the NBX specimens measured in the current

study being at the lower end of the frequency of aneuploidy reported for prostate cancer. It also is possible that aneuploid tumors with near-diploid aneuploid cell populations were not identified by this DNA ploidy measurement technique.

Although potential sampling error and intratumoral DNA ploidy heterogeneity have been of concern to several investigators,[41,42] in the current study excellent correlation between needle biopsy ploidy and companion follow-up prostatectomy ploidy status was achieved with 86 of 89 (97%) specimens being concordant. In the study by Leung et al.,[31] 11 of 12 (92%) specimens were concordant.[31] In a recent study by Takai et al.,[43] there was agreement in ploidy status for disaggregated NBX specimens measured by image analysis and corresponding disaggregated RPX specimens studied by flow cytometry in 74% of patients. In a comparison study of RPX specimens, Sisson Hardt et al.[44] reported that the most effective determination of ploidy status in prostate carcinoma was the use of fine needle aspiration analyzed by image analysis, which proved more sensitive than the flow cytometric technique.

The findings in this study of a more accurate prediction of ECS, incidence of metastasis, and disease recurrence by the NBX ploidy status than the RPX ploidy status is of uncertain significance. No specific technical issues can be cited other than the generally greater numbers of tumor cells available for analysis in the RPX specimens. As more patients are added to studies of NBX DNA content and postprostatectomy follow-up periods are lengthened, it will be interesting to see if this finding is maintained.

Although a variety of additional tumor markers have been evaluated for their potential prognostic value in prostate cancer, including dominant oncogene expression and tumor suppressor gene mutation or deletion,[45-47] no specific factor independently indicating the metastatic phenotype of prostate adenocarcinoma has been found. However, in the current preliminary study, despite a relatively short follow-up period, given the tenfold increase in metastases risk imparted by aneuploid tumoral DNA content in the initial NBX specimen, it appears that ploidy status can play a significant role in contributing to the selection of patients for diagnostic procedures, such as laparoscopic pelvic lymph node biopsy to confirm inoperability before scheduling a radical prostatectomy.

Conversely, a small carcinoma focus with limited tumor volume associated with low estimated grade and diploid status of an NBX specimen in an older man may prove sufficient to consider nonsurgical approaches to the disease, including the recently advocated strategy of an initial conservative management and delayed hormonal therapy.[48] In summary, the DNA content analysis of prostate needle biopsies can be readily determined by the tissue section image analysis method, directly correlates with ploidy status at prostatectomy, and in contrast to needle biopsy grading, is associated independently with the presence of ECS, metastasis, and postprostatectomy disease recurrence. We conclude that NBX ploidy determination may be of significant clinical value as a predictor of future disease course in prostate cancer.

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