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CLINICAL INVESTIGATION **Prostate**

POSTRADIOTHERAPY PROSTATE BIOPSIES: WHAT DO THEY REALLY MEAN? RESULTS FOR 498 PATIENTS

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Purpose: Postradiotherapy (RT) prostate biopsies are prone to problems in interpretation. False negatives due to sampling error, false positives due to delayed tumor regression, and indeterminate biopsies showing radiation effect in residual tumor of uncertain viability are common occurrences.

Methods and Materials: A cohort of 498 men treated with conventional RT from 06/87–10/96 were followed prospectively with systematic transrectal ultrasound (TRUS)-guided post-RT prostate biopsies, starting 12–18 months after RT. If there was residual tumor but further decline in serum prostate-specific antigen (PSA), biopsies were repeated every 6–12 months. Patients with negative biopsies were rebiopsied at 36 months. Residual tumor was evaluated for RT effect and proliferation markers. The 498 men had 978 biopsies. Median time of the first biopsy ($n = 498$) was 13 months, biopsy #2 ($n = 342$) 28 months, biopsy #3 ($n = 110$) 36 months, biopsy #4 ($n = 28$) 44 months, and biopsy #5 ($n = 4$) 55 months. Median follow-up is 54 months (range 13–131). One hundred seventy-five patients (34%) had prior hormonal therapy for a median of 5 months (range 1–60).

Results: Clinical stage distribution was T1b: 46; T1c: 50; T2a: 115; T2b/c: 170; T3: 108; T4: 11; Tx: 1. Distribution by Gleason score was: 28% Gleason score 2–4; 42%: 5–6; 18%: 7; and 12%: 8–10. Seventy-one men have died, 26 of prostate cancer and 45 of other causes. Actuarial failure-free survival by T stage at 5 years is T1b: 78%; T1c: 76%; T2a: 60%; T2b/c: 55%; T3: 30%; and T4: 0%. Actuarial freedom from local failure at 5 years is T1b: 83%; T1c: 88%; T2a: 72%; T2b/c: 66%; T3: 58%; and T4: 0%. The proportion of indeterminate biopsies decreases with time, being 33% for biopsy 1, 24% for biopsy 2, 18% for biopsy 3, and 7% for biopsy 4. Thirty percent of indeterminate biopsies resolved to NED status, regardless of the degree of RT effect, 18% progressed to local failure, and 34% remained as biopsy failures with indeterminate status within the time frame of this report. Positive staining for proliferation markers was associated with both subsequent local failure and also any type of failure. In multivariate analysis, only PSA nadir ($p = 0.0002$) and biopsy status at 24–36 months ($p = 0.0005$) were independent predictors of outcome.

Conclusions: Post-RT prostate biopsies are not a gold standard of treatment efficacy, but are an independent predictor of outcome. Positive immunohistochemical staining for markers of cellular proliferation is associated with subsequent local failure. Indeterminate biopsies, even when showing marked RT effect, cannot be considered negative. **© 2000 Elsevier Science Inc.**

Prostate cancer, Radiotherapy, Postradiation biopsies, Outcome prediction.

INTRODUCTION

Although radiotherapy has been an established therapeutic modality for clinically localized prostate cancer for over four decades, we are still grappling with the best measure of outcome assessment. Clinical outcome, as assessed through digital rectal examination (DRE) and overall survival, markedly overestimates the efficacy of radiotherapy in eradicating the tumor. Clinical outcome may be an appropriate end-point for patients with a life expectancy that is limited to less than 10 years due to age or comorbid disease. It is not appropriate for younger men seeking definitive management.

Ten years of experience with prostate-specific antigen (PSA) has taught us not to expect undetectable values after radiotherapy. A desirable PSA nadir following radiotherapy is 0.5 ng/mL or less (1–4), and relative stability of the PSA following the nadir is important. Three successive increases likely indicate recurrence, and this definition has been recommended by the American Society of Therapeutic Radiology and Oncology (ASTRO) consensus guideline (5).

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However, the absolute value of the PSA and its pattern over time is a game of probabilities for the individual patient. There is no clear-cut PSA threshold distinguishing treatment success from treatment failure (6).

Postradiotherapy prostate biopsies should provide a definitive measure of the success or failure of local treatment. This is unfortunately not the case. Because of long tumor doubling times and the fact that radiotherapy causes postmitotic cell death (7), histologic resolution may take 2–3 years. Biopsies done before this may be difficult to interpret, showing residual cancer cells with evident radiation damage and uncertain viability. Furthermore, sampling error is clearly a problem, especially posttreatment when all that may remain are scattered tumor remnants, only some of which may be viable.

This is a report of a prospective study, begun in 1990, in which systematic transrectal ultrasound (TRUS)-guided biopsies have been performed on 498 men at intervals following radiotherapy. The aim of the study was to determine the time course for histologic tumor resolution, and to correlate biopsy results with PSA and clinical outcome.

METHODS AND MATERIALS

Age range was 49 to 87 years (median: 69). At referral to the Ottawa Regional Cancer Center, patients were evaluated with a full history and physical examination. Clinical tumor stage was determined by DRE by both the referring urologist and the radiation oncologist. Staging investigations included a complete blood count, renal and hepatic function tests, serum alkaline and acid phosphatase, PSA (since 11/89, Abbott IMX assay), chest radiograph, technetium-99m bone scan, and pelvic computed tomography (CT) scan. Staging pelvic lymphadenectomy was performed in 26 patients. Staging is according to the International Union Against Cancer TNM classification of 1992 (8). The stage distribution is 9% T1b ($n = 46$), 10% T1c ($n = 50$), 23% T2a ($n = 115$), 34% T2b/c ($n = 170$), 21% T3 ($n = 106$), and 2% T4 ($n = 11$). Distribution by grade is 20% Gleason 2–4, 31% Gleason 5–6, 13% Gleason 7, and 9% Gleason 8–10. In 27% the Gleason score was not available. When graded as well, moderately, or poorly differentiated, 7% of tumors could not have a grade assigned, 35% were well-differentiated, 49% moderately, and 10% poorly differentiated.

One-third of patients ($n = 175$) received hormonal therapy prior to referral for definitive radiotherapy (median duration 5 months, range 1–60 months). The indications for, and modalities of, hormonal therapy were not standardized and were determined by the referring urologist. Hormonal intervention included both monotherapy with steroidal or nonsteroidal anti-androgens or, less commonly, total androgen blockade with a combination of a luteinizing hormone–releasing hormone (LHRH)-agonist and anti-androgen. The duration of hormonal therapy was often unrelated to optimal cytoreduction. Hormonal therapy was discontinued prior to radiotherapy and not reinstituted except for documented failure.

All patients were treated between 6/87 and 10/96 with external beam radiotherapy using 18-MV photons from a linear accelerator, with a 4-field box technique in accordance with planning CT scan as previously described (9). The treatment volume was limited to the prostate and seminal vesicles for small, well-differentiated tumors and those that were pathologically node-negative. Otherwise the first echelon nodes, to the level of the bottom of the sacroiliac joints, were included in the first phase of the treatment to a dose of 45–46 Gy. Seminal vesicles generally received 50 Gy unless clinically involved, and the prostate received a minimum dose of 65 Gy (median 66 Gy) over 6½ weeks. Conformal radiotherapy was introduced in 1995, but without dose escalation.

Follow-up ranges from 13–131 months (median: 54 months). Patients who remained free of recurrence were seen quarterly in the first year, every 4 months in the second year, twice yearly in years 3 to 5, and annually thereafter. Follow-up included a functional inquiry, DRE, and a serum PSA drawn prior to DRE. The PSA nadir is defined as the lowest PSA reading achieved after completion of radiotherapy, in the absence of hormonal manipulation. The time to nadir is taken from the last day of radiotherapy. Following neoadjuvant hormonal therapy, the serum PSA often rises immediately after radiotherapy due to testosterone recovery, followed by a decline over the subsequent months from the effect of radiotherapy on the prostate. Under these circumstances, the nadir is taken after the secondary decline.

Since 7/90 postradiotherapy TRUS-guided prostate biopsies have been obtained systematically in all patients. Since the intent of this policy was to determine the time course for histologic resolution of prostate cancer after radiotherapy, the first biopsy was performed at approximately 12 months after completion of radiotherapy. Prostates that showed residual tumor at this time were rebiopsied every 6–12 months, provided the PSA continued to fall and there was no evidence of clinical failure. A positive biopsy in conjunction with a rising PSA or clinical progression was considered as local failure and no further biopsies were obtained. Patients with an initially negative posttreatment biopsy were rebiopsied routinely at 36 months, or at any time for a rising PSA. Four hundred ninety-eight men have had 978 biopsies. Median time of the first biopsy ($n = 498$) was 13 months, biopsy #2 ($n = 342$) 28 months, biopsy #3 ($n = 110$) 36 months, biopsy #4 ($n = 28$) 44 months, and biopsy #5 ($n = 4$) 55 months. Generally, six core samples were taken at each biopsy session (range: 4–7 depending on prostate size). Two passes were usually taken through the site of the original tumor, as indicated on a staging diagram provided to the diagnostic radiologist.

All biopsies were reviewed by one pathologist (S.R.) and stained for PSA and high-molecular-weight keratin to distinguish radiation atypia in benign glands from residual malignancy (10). Residual tumor was evaluated for RT effect and graded according to previously published criteria (11). Immunohistochemical stains for proliferative cell nu-

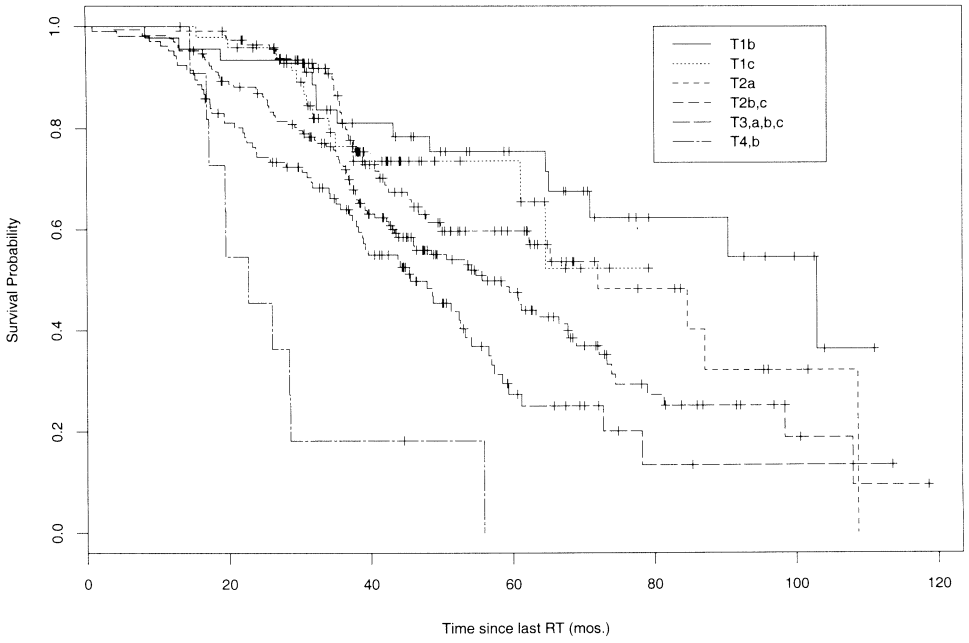

Fig. 1. Kaplan-Meier predicted freedom from local, distant, or biochemical relapse by T stage.

clear antigen (PCNA) (11) and more recently Mib-1, were used on all biopsies showing any evidence of residual tumor, as indicators of proliferative capacity. A biopsy was considered positive for PCNA or Mib-1 if 5 or more nuclei per 100 tumor cells counted showed evidence of positive staining. Negative biopsies with no evidence of residual tumor on hematoxylin–eosin (H & E) were not stained for markers of cellular proliferation. Biopsies were considered either negative (no evidence of residual tumor), positive (no or minimal RT effect), or indeterminate (marked RT effect). Although immunohistochemical staining for proliferative markers was frequently positive in positive biopsies and usually negative in indeterminate biopsies, this was not a criterion in classifying biopsies as positive or indeterminate.

Failures are classified as local, distant, biochemical, or "biopsy-only." Biochemical failure is defined according to the ASTRO consensus criteria (5) as 3 consecutive rises in serum PSA after the nadir. In the absence of residual tumor on biopsy or evidence of distant metastases, such patients were classified as having biochemical failure. Local and distant failures followed the same PSA criteria but with either biopsy evidence of residual tumour or radiologic evidence of systemic disease, respectively. Patients classified as having "biopsy-only" failure had a positive or indeterminate biopsy but maintained a nonrising PSA.

Data were updated in August 1998 and analyzed using Statistica from StatSoft for descriptive statistics. In addition, SAS (12) was used for multivariate analysis and Splus (13) was used for the Kaplan-Meier plots. There are three endpoints of interest in this study: overall survival, failure-free survival, and local failure-free survival. All time-dependent outcomes are calculated from the time of completion of radiotherapy, and patient data are censored where the outcome of interest had not yet occurred at last follow-up.

Because the proportional hazards assumption was tested and found not to be violated, Kaplan-Meier (14) survival curves are used both to estimate overall survival, and for univariate analysis with multilevel categorical variables. The log-rank test (15) is used to compare the survival profiles between groups. Cox proportional hazards (16) modeling is employed in the event that there is more than one covariate to be considered simultaneously as a risk factor for survival outcome.

RESULTS

Seventy-one men (14%) are dead, 26 from prostate cancer and 45 from other causes. Current status of the entire population at the time of last follow-up reveals that 37% manifest no evidence of disease (NED) ($n = 186$), and 15% remain in the indeterminate category of biopsy-failure with no clinical or biochemical evidence of progression ($n = 77$). Failures have been established in 47% of the population; 12% biochemical failure ($n = 62$), 22% isolated local failure ($n = 112$), 7% isolated distant failure ($n = 36$), and an additional 5% ($n = 26$) with simultaneous local and distant failure.

Freedom from local, biochemical, or distant relapse by T stage is shown in Fig. 1. Men dying of other causes without prior evidence of recurrence are withdrawn at the time of death as being relapse-free. At 5 years, relapse-free survival is 78% for T1b, 77% for T1c, 60% for T2a, 55% for T2b/c, 30% for T3, and 0% for T4. Freedom from local relapse is shown in Fig. 2. Those patients failing distantly or biochemically were confirmed to have negative posttreatment biopsies at the time of failure. These patients are withdrawn

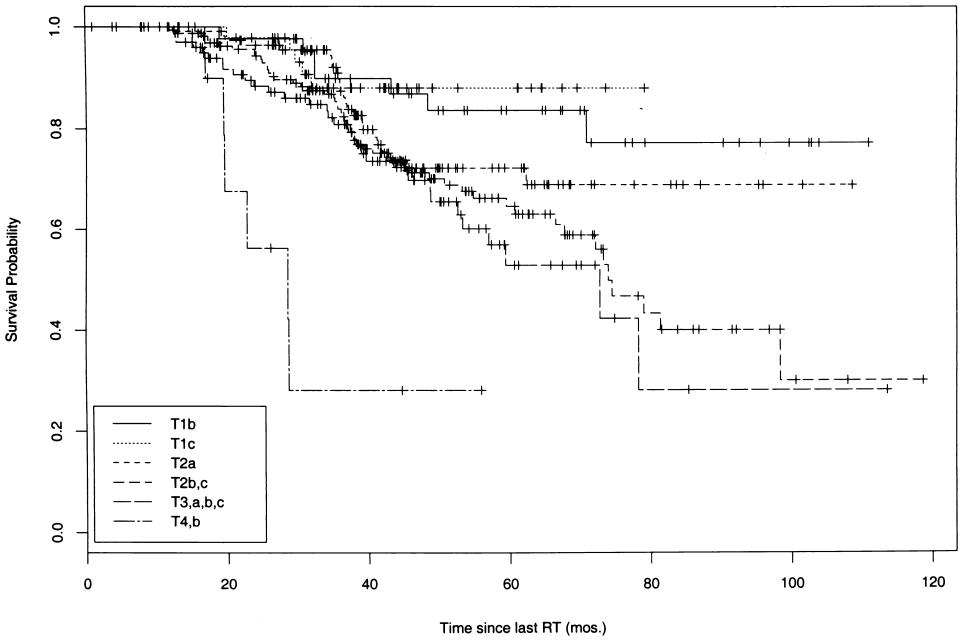

Fig. 2. Kaplan-Meier predicted freedom from local relapse by T stage (positive biopsy and/or abnormal DRE plus rising PSA).

from the analysis of local outcome at the time of hormonal intervention for failure. At 5 years, freedom from clinical or histologic evidence of local recurrence is seen in 83% for T1b, 88% for T1c, 72% for T2a, 66% for T2b/c, 58% for T3, and 0% for T4. In univariate analysis, T stage is predictive of both freedom from any failure ($p = 0.0001$) and freedom from local failure ($p = 0.0001$).

One-third of patients (175) received prior neoadjuvant hormonal therapy at the discretion of the referring urologist for a median duration of 5 months. There was no difference in disease-free survival of these patients compared to those without prior hormonal therapy ($p = 0.53$, Fig. 3). Even when patients were grouped as early stage (T1b/c, T2a) or more advanced (T2b/c,T3), there was no advantage for those receiving prior hormonal therapy in either freedom from local failure (Fig. 4) or freedom from any failure (Fig. 5).

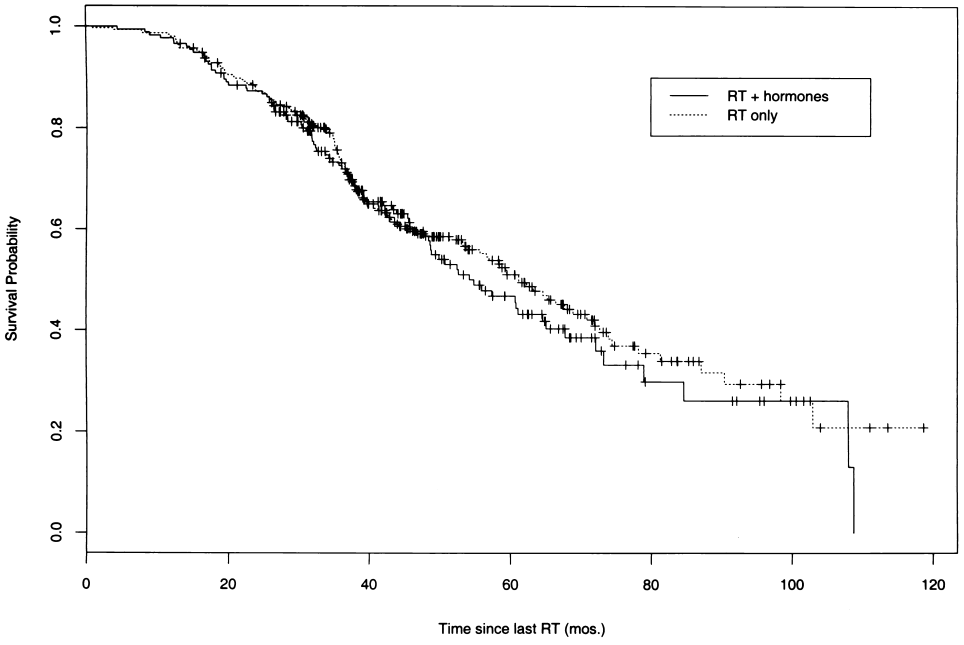

Fig. 3. Kaplan-Meier predicted freedom from any relapse for radiotherapy alone vs. radiotherapy plus prior hormonal therapy.

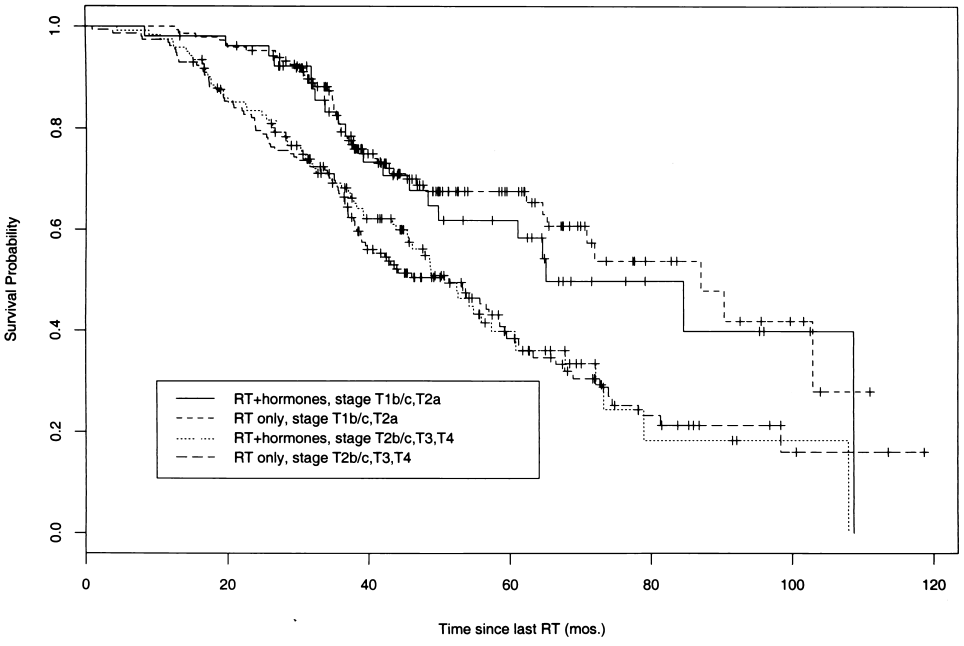

Fig. 4. Kaplan-Meier predicted freedom from any relapse for radiotherapy alone vs. radiotherapy plus prior hormonal therapy according to T stage (early: T1b/c, T2a vs. advanced: T2b/c, T3, T4).

Biopsy data

Delayed tumor regression (false positive). Of patients showing residual tumor on their first posttreatment biopsy, 30% ($n = 81$) showed delayed tumor regression with eventual conversion to negative biopsies and NED status at a mean time of 30 months. The time to nadir PSA for this group was 26.4 months.

Indeterminate biopsies. The proportion of indeterminate biopsies decreases with time, being 33% for the first biopsy (median interval since RT: 13 months), 24% for the second biopsy (median interval since RT: 28 months), 18% for the third biopsy (median interval since RT: 36 months), and 7% for the fourth biopsy (median interval since RT: 44 months). Thirty percent of indeterminate biopsies eventually cleared at a mean time of 31.6 months with the patient achieving NED status. Eighteen percent progressed to local failure at

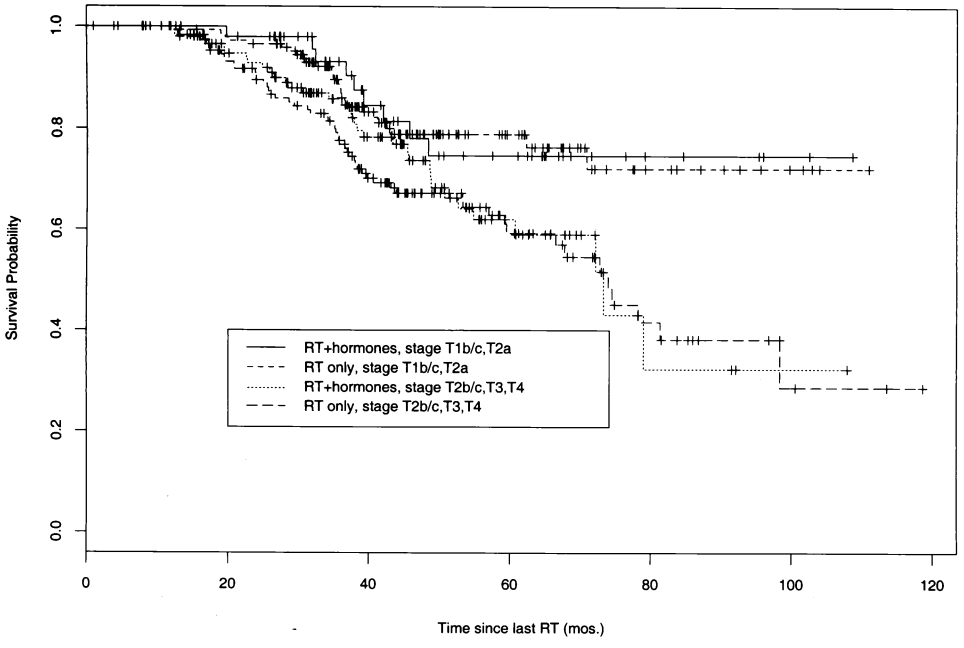

Fig. 5. Kaplan-Meier predicted freedom from local relapse for radiotherapy alone vs. radiotherapy plus prior hormonal therapy according to T stage (early: T1b/c, T2a vs. advanced: T2b/c, T3, T4).

Table 1. Summary of biopsy results by time interval postradiotherapy*

Months since RT (+/− 3)	# Patients with positive bx	# Patients with indeterminate	# Patients with negative bx	Total # biopsied in interval
12	67	116	149	332
18	36	75	96	207
24	37	29	39	105
30	27	17	24	68
36	53	18	66	137
42	18	4	26	48
48	6	2	18	26
54	5	1	5	11
60	4	0	9	13
66	4	0	3	7
72	8	1	1	10
≥ 78	5	0	2	7

* Note that biopsies done after 3 years were usually performed to investigate a rising PSA.

a mean time of 38 months. Thirty-four percent remained as biopsy failures with no other evidence of disease within the time frame of this report. In the remainder, intervention for distant failure curtailed further observation of the local outcome. RT effect was scored as mild (1–2/6), moderate (3–4/6), or marked (5–6/6) according to the previously published scoring system (11). The degree of RT effect was not predictive of outcome in indeterminate biopsies. The number and results of biopsies performed in each time interval after radiotherapy are shown in Table 1.

Proliferation markers. Positive staining for proliferation markers in biopsies 1 or 2 was associated with both subsequent local failure and any failure (local, biochemical, or distant) (chi-square). In our pathology department, there was excellent concordance between PCNA and Mib-1 staining (37), so during the course of the study Mib-1 replaced PCNA as the immunohistochemical stain of choice. In biopsy 1 the association between PCNA/Mib-1 positivity and subsequent local failure attained a significance level of $p = 0.004$, and in biopsy 2, $p = 0.0009$. Positive staining for proliferation markers was also associated with the risk of any failure, with $p = 0.01$ for positive staining in biopsy 1 and $p = 0.004$ for positive PCNA/Mib-1 staining in biopsy 2 (Table 2).

False negatives. In patients who achieved a negative postradiotherapy biopsy ($n = 308$), 19% ($n = 60$) were subsequently found to have residual local disease either on a systematic 36-month rebiopsy, or on biopsies specifically ordered to investigate a rising PSA. This reversion from negative back to positive was detected at a mean time of 43 months after radiotherapy.

Predictive value of biopsies. To evaluate the relative importance of biopsy status in predicting ultimate clinical outcome, only those biopsies performed between 24–36 months were considered. Those biopsies performed before 24 months of follow-up were eliminated because of the phenomenon of delayed tumor regression seen in 30% of patients with early positive posttreatment biopsies. Beyond 36 months biopsies were not continued systematically, and the majority performed late in the course of follow-up were triggered by a rising PSA. These should be considered diagnostic of failure rather than predictive and their inclusion would bias the analysis in favor of the utility of biopsy. In univariate analysis, biopsy status between 24–36 months was highly predictive of ultimate outcome ($p = 0.0001$).

PSA DATA

Pretreatment PSA. Failure-free survival according to pretreatment PSA is shown in Fig. 6. The PSA groupings are ≤ 5 ng/mL, 5.1–10 ng/mL, 10.1–20 ng/mL, and > 20 ng/mL. In univariate analysis, pretreatment PSA is predictive of NED survival ($p = 0.0001$). At 5 years, 90% of patients with a pretreatment PSA < 5.0 ng/mL are free of any relapse, 62% for PSA 5.1–10 ng/mL and 26% for PSA 10.1–20.0 ng/mL. Only 18% of patients presenting with a PSA > 20 ng/mL are free of failure by 5 years.

Nadir PSA. Nadir PSA is strongly predictive of outcome in univariate analysis ($p = 0.0001$). Failure-free survival by nadir PSA is shown in Fig. 7. Results were analyzed in four groups: PSA nadir ≤ 0.5 ng/mL, 0.6–1.0 ng/mL, 1.1–2.0 ng/mL, and > 2.0 ng/mL. At 5 years the NED survival is

Table 2. Association of positive proliferative staining with subsequent failure*

		PCNA +	Mib-1 +	PCNA & Mib-1 neg	Chi^2
Biopsy 1	NED	6	1	47	
	LF	26	10	66	0.004
	All F	34	13	104	0.01
Biopsy 2	NED	0	1	15	
	LF	31	18	46	0.009
	All F	32	18	64	0.004

* Note that only those biopsies showing residual tumor are stained for proliferative markers. The status of the patient is that of last follow-up, not the status at the time of the biopsy. The chi-square statistic is used to compare the total number staining positive for either proliferative marker in NED patients to either those with local failure or those with any type of failure.

LF = local failure; All F = all failures.

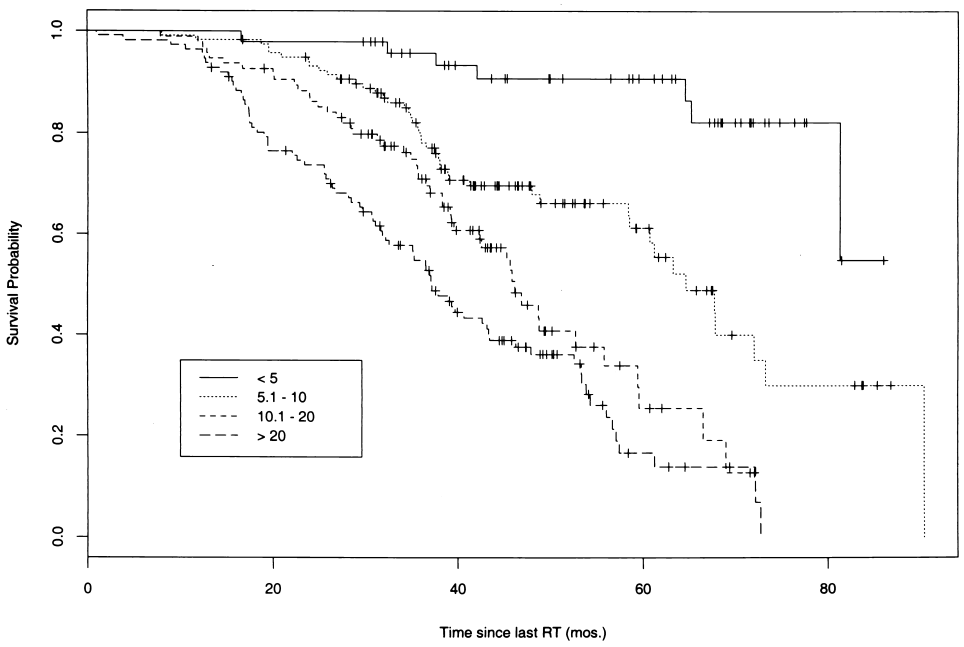

Fig. 6. Kaplan-Meier predicted freedom from any relapse by pretreatment PSA group (group 1: PSA ≤ 5.0 ng/mL; group 2: PSA 5.1–10.0 ng/mL; group 3: PSA 10.1–20.0 ng/mL; group 4: PSA > 20.0 ng/mL).

75% for patients with nadirs ≤ 0.5 ng/mL, 50% for nadirs 0.6–1.0 ng/mL, and 32% for nadirs 1.1–2.0 ng/mL. However, only those patients achieving a nadir ≤ 0.5 ng/mL show a stable plateau in the NED survival curve, with 60% NED at 8 years. For PSA nadirs of 0.6–1.0 ng/mL, although at 5 years the Kaplan-Meier predicted freedom from failure is 50%, by 8 years this has fallen to 15%, with no evidence of a plateau even at this level.

Mean and median PSA nadirs and time to nadirs are summarized in Table 3. The median nadir for NED patients ($n = 186$) is 0.4 ng/mL achieved at a mean time of 24 months postradiotherapy. For those patients in the "biopsy-failure" group ($n = 77$) it is 0.5 ng/mL at 24 months ($p =$ nonsignificant [NS]). For all other types of failure, the PSA nadir is significantly higher and achieved earlier ($p < 0.0001$). For biochemical failures, the median nadir is 0.9

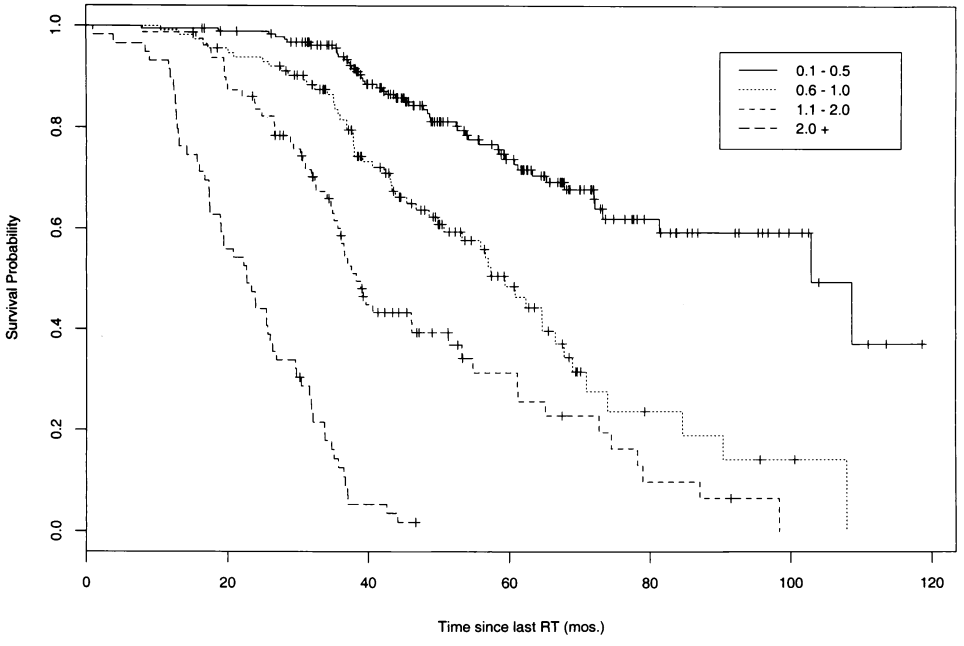

Fig. 7. Kaplan-Meier predicted freedom from any relapse by nadir PSA group (group 1: PSA nadir < 0.5 ng/mL; group 2: 0.6–1.0 ng/mL; group 3: 1.1–2.0 ng/mL; group 4: > 2.0 ng/mL).

Table 3. Median and Mean PSA nadirs and time to nadirs*

Status	Number	Median nadir (ng/mL)	Mean nadir (ng/ML)	Time to nadir (mos)	Current PSA (ng/mL)
NED	186	0.4	0.5	24	0.5
Biopsy F	77	0.5	0.6	24	0.9
Biochem F	62	0.9	1.3	19	N/A
Local F	112	1.1	1.9	17	N/A
Distant F	62	2.2	7.8	10	N/A
24–36 mo bx +	64	1.15	1.6		
24–36 mo bx −	54	0.65	1.7		
24–36 mo bx Indeterminate	38	0.65	1.1		

* Nadir and time to nadir for NED and "biopsy failure only" groups differs significantly from all other failure categories ($p < 0.0001$).
Abbreviations: NED = no evidence of disease; Biopsy F = biopsy evidence of tumor; Biochem F = rising PSA/negative biopsy; Local F = positive biopsy + rising PSA and/or abnormal DRE; Distant F = distant failure.

ng/mL at 16 months. For those patients with local failure, it is 1.1 ng/mL at 17 months, and for those with distant failure it is 2.2 ng/mL at 10 months.

Mean and median PSA nadirs were examined according to 24–36 month biopsy status (Table 3). The median nadir for those patients with a positive 24–36 month biopsy is similar to that of the local failure category (1.15 ng/mL). The median nadir for patients with a negative 24–36 month biopsy is higher than that for NED patients and the mean is much higher, indicating the contamination of this group with patients failing distantly and biochemically. Prostate biopsies add important information when assessing treatment outcome, permitting one to distinguish between local and systemic failure.

Current PSA. Although there is no difference in PSA nadir or time to nadir between the group of patients who are NED and those who are in the "biopsy-failure" category, the median current (at last follow-up) PSA is higher in the biopsy failure group ($p < 0.001$). The NED patients are maintaining a stable PSA with the median current PSA being 0.5 ng/mL (median nadir was 0.4 ng/mL). For the "biopsy-failure" group the median current PSA is 0.9 ng/mL (compared to a median nadir of 0.5 ng/mL).

In univariate analysis, T stage, Gleason score, pretreatment PSA, nadir PSA, and 24–36 month biopsy result were all significant predictors of outcome (Table 4). Age and prior hormonal therapy were not significant. Multivariate analysis was carried out to examine the relative importance of these various potential prognostic indicators. PSA was treated as both a continuous and a grouped variable. The only two independent predictors of outcome were PSA nadir ($p = 0.0002$) and 24–36 month biopsy result ($p = 0.00004$) (Table 4).

Gleason score ($p = 0.1$) was of borderline significance, while T stage (0.4) and pretreatment PSA (0.3) were no longer predictive.

Table 4. Summary of multivariate analysis of prognostic factors showing univariate significance

Variable	Univariate (K–M)*	Multivariate (Cox)†
T stage	0.0001	0.4
Gleason score	0.0001	0.14
Pretreatment PSA	0.0001	0.3
Nadir PSA	0.0001	0.0002
24–36 month biopsy	0.0001	0.00005

* Univariate analysis by Kaplan-Meier method.
† Multivariate analysis by Cox proportional hazard method.

DISCUSSION

Serum PSA is an excellent biochemical marker of disease status following radiotherapy for prostate cancer. However, it does not distinguish between local and distant recurrence. With standard dose radiotherapy, the majority of treatment failures have a local component. In the current series only 7% of patients had isolated distant failure, and another 12% purely biochemical failure with negative prostate biopsies, while 27% had local failure, either as an isolated event or combined with concurrent distant failure. With standard radiotherapy, positive rebiopsy rates range from 29–93% (17–23). The results presented in this series illustrate the suboptimal tumor eradication seen with standard dose radiotherapy. Significant improvements in biochemical disease-free survival have been observed in dose escalation studies with 5-year no biochemical evidence of disease (bNED) rates improving even for high-risk patients from 10–20% for standard radiotherapy to 32–55% (24–26) for those treated with higher doses. The margin for further improvements through optimization of local treatment can be inferred from the difference between the rate of PSA failures after radical prostatectomy as compared to radiotherapy for patients stratified by known pretreatment prognostic factors (27). When radiotherapy is optimized to eliminate local failures, the PSA failure rate should be no higher than that seen after a radical prostatectomy with negative margins. Only the component of distant failure remains and this should be equivalent for both surgery and radiotherapy.

The definition for biochemical disease-free status for this

protocol is according to the 1997 ASTRO consensus guideline (5). A desirable PSA reading after prostate radiotherapy is clearly lower than the laboratory limit of normal, but there is not an obvious absolute threshold separating cure from failure. PSA stability is the most important indicator of disease-free status. Stability is seldom seen with readings above 2–3 ng/mL (6). This prospective study commenced before the ASTRO consensus guideline was available. The definition of biochemical disease-free status that was initially chosen reflected the state of knowledge of the times. Our initial PSA criteria for failure were a PSA >2.0 ng/mL and > 1 ng/mL above the nadir. When the analysis by the ASTRO consensus guideline is compared to that done by the initial failure criteria, 12 NED patients are reclassified as biochemical failures, and 10 patients move from the biopsy failure category to local failure (Table 5). Those patients meeting the NED criteria by either definition demonstrate remarkable PSA stability, with a median nadir PSA of 0.4 ng/mL and a median current PSA of 0.5 ng/mL.

Table 5. Comparison of failure status by ASTRO criteria and initial study criteria

Status	ASTRO criteria*	Percentage	Initial criteria†	Percentage
NED	186	37.3%	199	39.9%
Biopsy failure	77	15.4%	88	17.6%
Biochemical failure	62	12.4%	49	9.8%
Local failure	112	22.4%	101	20.2%
Distant failure	36	7.2%	36	7.2%
Local + distant	26	5.2%	26	5.2%

* ASTRO criteria for biochemical failure for 3 consecutive PSA increases after the nadir.

† Initial study criteria were a PSA > 2.0 ng/mL and > 1 ng/mL above the nadir.

Postradiotherapy prostate biopsies should provide an accurate assessment of the local efficacy of radiotherapy. By elucidating the pattern of failure, we can better distinguish patients who will benefit from more aggressive local therapy from those who are destined to fail distantly despite successful management of the primary tumor. The experience reported in this series involving sequential biopsies in 498 patients helps to define the utility and limitations of postradiotherapy biopsies.

The primary goal of this protocol was to determine the time course over which biopsies converted to negative following radiotherapy. Of patients showing residual tumor in their first posttreatment biopsy, usually performed at 12–18 months after completion of radiotherapy, 30% showed eventual clearance of tumor at a mean time of 30 months after radiotherapy. The time to nadir PSA in this group was 26.4 months. This would seem to indicate that biopsy status will not give us earlier feedback on treatment outcome than one would obtain by following PSA. Despite the significant proportion of early biopsies that do eventually revert to negative, initial biopsy status is indeed predictive of ultimate failure, whether that be local or systemic ($p < 0.0001$). Nonetheless, to minimize false positives in the face of a declining PSA, biopsies should not be obtained before 30–36 months after radiotherapy, or before PSA nadir has been reached.

The interpretation of postradiotherapy biopsies can be problematic in that a large proportion will show residual tumor with radiation effect of varying intensity. Despite obvious radiation damage and doubtful viability, these biopsies cannot be called negative. However, neither are they clearly positive. This indeterminate category decreases over time, being one-third of first biopsies but decreasing to 7% for biopsies done beyond 36 months. Residual irradiated tumor eventually declares itself if given enough time. In this series 30% resolved with no trace of detectable tumor on subsequent biopsies (these patients also being biochemically NED) at a mean time of 31.6 months and another third remained indeterminate within the time frame of follow-up. However, 18% progressed to local failure at a mean time of 38 months. For this reason, we feel that the indeterminate category should remain distinct and be subjected to closer long-term follow-up.

The degree of radiation effect can be reproducibly graded based on cytoplasmic and nuclear changes as described by Crook et al. (11). In this earlier publication, no tumors exhibiting severe radiation change (Grade 5–6 on a scale of 6) recurred and it seemed likely that these cells had been rendered biologically inactive. Some investigators have elected to consider these biopsies negative (28). However, at the time of the previous analysis, this group had a shorter follow-up of 18–89 months (mean: 43 months). With longer follow-up, the degree of radiation effect no longer appears to be predictive of outcome. Because it is unlikely that cells showing such a severe degree of radiation effect could subsequently recover and progress, a more plausible explanation involves heterogeneous radiation effect and the limitations of sampling error. As demonstrated in the 1997 report (11), 30% of prostates harboring residual tumor showed different degrees of radiation effect within the same biopsy (Fig. 8). Goldstein *et al.* (29) also observed this heterogeneous RT effect in 54% of post-RT biopsy specimens but had performed the biopsies only 18 months after radiotherapy. If the predominant tumor is radiosensitive, any residual tumor revealed in early biopsies may show severe radiation effect. As this component of the tumor regresses over time, it is less likely to be sampled on subsequent biopsies. Neighboring foci of better-preserved tumor continue to grow and become evident with later biopsies. Prostate cancer is known to be multifocal and different tumor clones may exhibit different inherent radiosensitivity. Variations in oxygen tension in the local microenvironment may also influence radiosensitivity (30). Another possible explanation of late recurrences after previous negative biopsies is the development of a new primary tumor, either from preexisting prostatic intraepithelial neoplasia (PIN) or *de novo*.

Sampling error is certainly a limitation of biopsy evaluation of the prostate, either pretherapy or post-therapy (11).

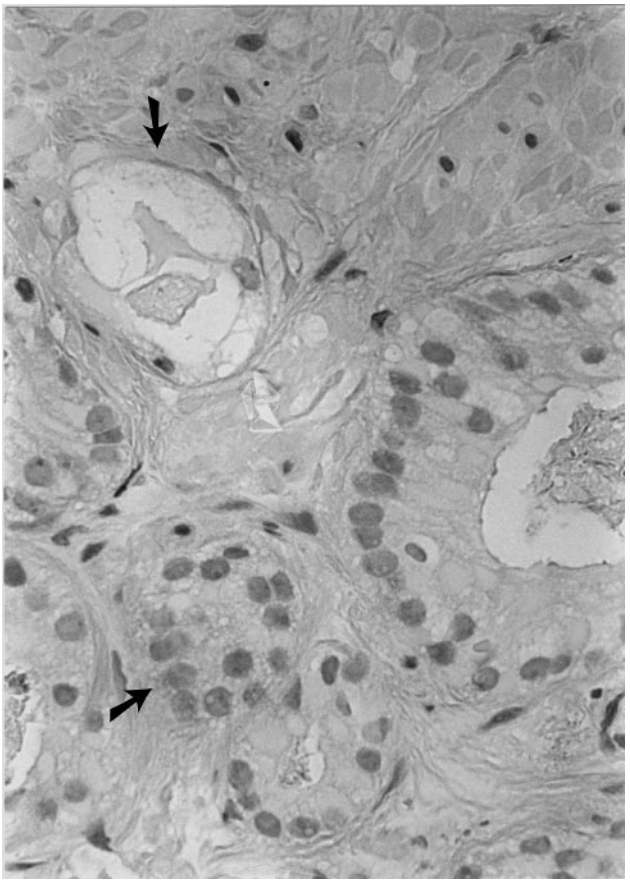

Fig. 8. H & E stain of biopsy showing areas of tumor with severe radiation effect (upper left) next to well-preserved tumor (lower left) (400× magnification).

Increased yield of a repeat series of sextant biopsies has been well documented in screening series. Fleshner *et al.* (31) have reported 30% positive rebiopsies in a series of 130 men suspected of having prostate cancer but whose initial TRUS-guided biopsy was negative. Fifteen to 30% of repeat sextant biopsies either just before (32–34) or immediately after (35) radical prostatectomy have false-negative results. After radiotherapy, TRUS rarely directs biopsy to residual hypoechoic nodules (36). Minimal scattered microscopic tumor remnants can easily be missed. Svetec *et al.* (36) repeated sextant biopsies prior to radical prostatectomy in 90 patients, 68% of whom had received neoadjuvant hormone therapy, a situation analogous to the postradiotherapy situation. Despite pathologically confirmed tumor in the radical prostatectomy specimen, 45% had negative sextant biopsies. In the present series, the diagnostic radiologist was directed toward the area of original tumor by including a staging diagram with the TRUS requisition. Although the usual and median number of cores taken at each biopsy session was 6, reduction in the number of cores (usually for reasons of patient discomfort) was associated with a higher false-negative rate. The median number of cores taken at a false-negative biopsy was 4.

We have found immunohistochemical staining for markers of proliferation (Fig. 9) to be highly predictive of outcome for the 24–36 month biopsies. PCNA is maximum during S phase of the cell cycle but is not specific to proliferating cells as it is also detected during DNA repair. It is formalin-sensitive and to be reliable, a strict protocol concerning the processing of biopsy specimens must be followed. PCNA was all that was available to evaluate proliferation immunohistochemically when this biopsy protocol was initiated. Ki 67 antigen is expressed exclusively in proliferating cells and is detected using mouse monoclonal antibody Mib-1. As it is formalin-stable, it is less sensitive to the details and timing of processing of the biopsy and has now replaced PCNA in our laboratory. The correlation between Mib-1 and PCNA in our laboratory was excellent when samples were fixed in formalin for $<$ 48 hours (37). When biopsies were performed $<$ 18 months after radiotherapy, the correlation diminished slightly, presumably because of ongoing DNA repair in those samples stained with PCNA. Ljung *et al.* (38) reported on the use of PCNA and Mib-1 in 37 post-RT prostate biopsies showing residual tumor at a mean time of 6.8 years after radiotherapy. Mib-1 positivity was seen in 67% and PCNA in 94%. In 10 cases that were not concordant, all were PCNA-positive and Ki 67–negative.

Because there are multiple biopsy evaluations for each patient, in assessing the relative importance of the biopsy vis á vis other potential prognostic factors, biopsy results were selected from a limited time interval. Only biopsies performed after 24 months were included to avoid the problem of falsely interpreting a biopsy as positive before histologic regression was complete. Beyond 36 months, biopsies were not systematically continued. The majority performed beyond this period were to investigate a rising PSA and were therefore diagnostic rather than predictive. Including them would overestimate the utility of systematic biopsies in predicting outcome. Biopsy status alone did not determine failure category but required corroboration by either an abnormal DRE or a rising PSA. Those with a positive (or indeterminate biopsy) as the only evidence of disease remained in a biopsy-failure category until there was clinical or biochemical evidence of failure. Thus it is reasonable to use biopsy status as a predictor of ultimate outcome.

Multivariate analysis revealed only PSA nadir and biopsy status at 24–36 months to be independent predictors of outcome. Previous analysis has shown PSA nadir to be the dominant predictor when combined in a model with pretreatment factors such as baseline PSA, Gleason score, and T stage. Although this is common practice, one should not really combine true pretreatment prognostic factors with posttreatment evaluation of response. Both PSA nadir and biopsy status fall into this latter category of evaluating the response to treatment. It is not surprising that biopsy-proven presence of residual disease at 24–36 months postradiotherapy is an independent predictor of ultimate failure. Although biopsies are not required in routine follow-up, they do serve as an indicator of the site of failure and are useful in the evaluation of potential improvements in local

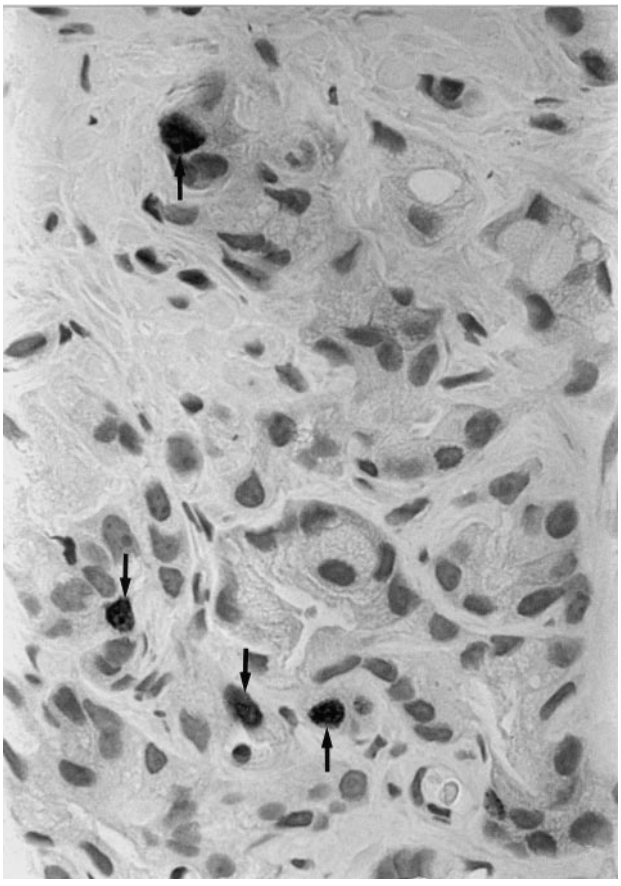

Fig. 9. Residual tumor showing positive Mib 1 nuclear staining (arrows). (400× magnification).

therapy. The fact that biopsy status is predictive of overall outcome reflects the fact that the majority of radiotherapy failures are local or have a local component.

The biopsy failure category ($n = 77$) is indistinguishable from those patients who are NED ($n = 186$) in terms of nadir PSA and time to nadir. This group of biopsy failure patients still has a nonrising PSA and nonsuspect DRE. Many of them (60%) have indeterminate biopsies showing severe radiotherapy effect and may remain free of clinical failure indefinitely. However, the median current PSA for the biopsy-failure category is 0.9 ng/mL as compared to 0.5 ng/mL for NED patients ($p < 0.001$) indicating that a proportion of the biopsy failure group does indeed have active disease and will fail with time.

We failed to demonstrate any difference in outcome with respect to either overall freedom from failure or local control for those patients who had prior hormonal therapy compared to those treated with radiotherapy alone. As this was not a randomized trial and therefore prone to all the pitfalls of selection, no conclusion can be drawn from the data. The Radiation Therapy Oncology Group (RTOG) 86-10 trial randomized patients with locally advanced prostate cancer to 4 months of total androgen blockade plus standard radiotherapy or radiotherapy alone (39, 40). There is a significant improvement in local control, disease-free survival and a trend to improved overall survival for the combined treatment arm. Laverdière (41) has also shown a decrease in positive 24-month biopsy rates comparing 64 Gy of radiotherapy alone to combined treatment with either 3 months neoadjuvant total androgen blockade or 3 months neoadjuvant followed by 6 months adjuvant total androgen blockade. Only properly controlled clinical trials can resolve the issues concerning selection of patients for neoadjuvant hormonal therapy prior to radiotherapy, and the optimal duration of hormonal therapy.

CONCLUSIONS

Serum PSA is a reliable indicator of disease status postradiotherapy but cannot distinguish between local and systemic failure. This distinction is essential in appreciating the frequency with which standard radiotherapy leaves behind viable cancer cells in the prostate, and in spurring the development and implementation of improved techniques and dose delivery. Similarly, postradiotherapy prostate biopsies are useful in evaluating innovations such as dose escalation protocols, or combination treatments involving brachytherapy or hormones. The majority of locally persistent or recurrent tumors will be detected by biopsy before being clinically evident.

Although postradiotherapy prostate biopsies can help to determine the site of failure, they are not indicated in routine follow-up. For the individual patient whose rising PSA will be managed with androgen deprivation, the distinction between local and distant failure may be academic. If radical local salvage is an option, then biopsy proof of locally recurrent or persistent tumor should be mandatory prior to any attempted radical local salvage maneuver.

The timing of the biopsy is critical and seems to be most predictive of outcome in the period 24–36 months after radiotherapy. Grading of the degree of radiotherapy effect helps in the interpretation of the biopsy result, as those with little or no radiotherapy effect are clearly positive. Indeterminate biopsies with marked RT effect may resolve over time or remain biologically inactive, but should be followed as a separate category and not considered negative as a significant percentage will progress with sufficiently long follow-up. Immunohistochemical stains for markers of cellular proliferation are useful in assessing the biologic activity of residual tumor.

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