Effect of Neoadjuvant Androgen Deprivation on Circulating Prostate Cells in the Bone Marrow of Men Undergoing Radical Prostatectomy

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ABSTRACT

Our objective was to determine the effect of neoadjuvant hormonal therapy on the presence of circulating prostate cells in patients undergoing radical prostatectomy for prostate cancer. A total of 60 patients at high risk for extraprostatic disease were analyzed for the presence of circulating prostate cells using reverse transcriptase PCR (RTPCR) amplification of the prostate-specific antigen mRNA. Twenty-nine patients underwent radical prostatectomy for a clinical T2b-c tumor or a stage T1-T2a tumor and a serum prostate-specific antigen level ≥10 ng/ml (radical prostatectomy alone), and 31 similarly staged patients received neoadjuvant hormonal therapy before radical prostatectomy (neoadjuvant). Bone marrow samples were used for RTPCR analysis. Twenty-four percent and 58% of the radical-prostatectomy-alone patients and neoadjuvant patients had organ-confined disease, respectively (P = 0.007). In the radical-prostatectomy-alone group, 77% and 14% of patients with extraprostatic and organ-confined disease were RTPCR positive, respectively (P = 0.03). However, in the neoadjuvant group, 46% and 28% of patients with extraprostatic and organ-confined disease were RTPCR positive, respectively (P = 0.29). For patients that were RTPCR positive, 45% of the neoadjuvant patients had organ-confined disease compared with 6% in the radical-prostatectomy-alone patients (P = 0.018). These data suggest that a subset of the neoadjuvant patients are converted to organ confined disease without eliminating the prostate cells in the bone marrow. Our data suggest that hormonal therapy before radical prostatectomy decreases the occurrence of extraprostatic disease but, to a lesser degree, the incidence of circulating prostate cells. This may partially explain why hormonal therapy before radical prostatectomy has not improved disease-free survival.

INTRODUCTION

Despite the widespread use of digital rectal examinations and serum PSA measurements to detect early stage prostate cancer, 40% of patients undergoing radical prostatectomy for clinically localized disease have extraprostatic extension (1, 2). Of these patients, 30–60% will eventually develop a rising serum PSA level after radical prostatectomy, and long-term follow-up indicates that disease recurrence in this situation most often includes a component of distant metastatic disease (3). Although it is theoretically possible that distant metastasis can occur from locally persistent prostate cancer tissue in the pelvis, the majority of distant failures likely result from undetected (occult) micrometastatic disease that was present at the time of radical prostatectomy. Present staging modalities are not sufficiently sensitive to detect low volumes of metastatic disease at the time of diagnosis.

Preoperative neoadjuvant hormonal ablation therapy has been advocated as a method to improve survival in patients with clinically localized prostate cancer (4–6). Analysis of radical prostatectomy specimens shows that this form of therapy has a profound effect on the primary tumor, but it almost never eliminates the tumor entirely. Nonetheless, patients treated with 3–6 months of preoperative hormonal therapy have a 50% higher chance of having organ-confined disease compared with patients undergoing radical prostatectomy alone. Despite the improvement in pathological staging results, the use of neoadjuvant androgen-ablation therapy has not translated into improved disease-free survival. Pathological downstaging of the primary tumor in and of itself might be expected to decrease the rate of local recurrence. This downstaging would be expected to improve disease-free survival only for those few cancers in which the initial metastasis of prostate cancer cells occurs from locally persistent prostate cancer tissue after the radical prostatectomy. As mentioned above, this probably represents a small proportion of eventual distant failures. If neoadjuvant androgen-ablation therapy is to be expected to improve disease-free survival to a significant degree, it will do so only by effective elimination of all of the undetected distant micrometastatic disease before radical prostatectomy.

Using RTPCR amplification of PSA mRNA sequences, we

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3 The abbreviations used are: PSA, prostate-specific antigen; RTPCR, reverse transcriptase PCR.
and others have demonstrated circulating prostate cancer cells in the peripheral blood and bone marrow of patients with clinically localized and metastatic disease (7–11). The presence of circulating prostate cancer cells in the bone marrow correlates with the pathological stage of the primary tumor and disease-free survival, suggesting that the molecular detection of metastatic disease portends distant failure that will eventually become clinically detectable (9, 12). The effect of hormonal therapy on the ability of the RTPCR test to detect prostate cancer cells is unknown. Often in patients with clinically localized or metastatic prostate cancer, the serum PSA level will decrease to undetectable levels after hormonal therapy is started; however, complete tumor eradication is rare (4–6, 13, 14). In these patients, we have few methods to evaluate the disease response until the PSA levels rise again. If the RTPCR test can detect circulating prostate cells even in patients with an undetectable serum PSA level, we may better evaluate the disease response in patients on hormonal therapy.

In the absence of long-term follow-up data, trials of neoadjuvant androgen-Ablation before radical prostatectomy are presently evaluated by the ability of neoadjuvant androgen to improve the pathological stage of the primary tumor. However, this surrogate end point may not be valid because eventual failure is most likely caused by the incomplete elimination of micrometastatic disease rather than ineffective treatment of the primary tumor. We, therefore, sought to compare the ability of neoadjuvant androgen deprivation to reduce the incidence of prostate cancer cells in the bone marrow versus its ability to downstage the primary tumor. In the future, if the RTPCR test proves to be accurate, a negative result may be a more relevant predictor of treatment success than the ability to achieve organ-confined disease in the surgical specimen.

MATERIALS AND METHODS

To determine the effect of neoadjuvant hormonal therapy on circulating prostate cells in patients with clinically localized prostate cancer, we compared the RTPCR results of 29 patients undergoing radical prostatectomy for clinical stage T2a–T2a tumors or a T1c–T2a tumor and a serum PSA level ≥10ng/ml with 31 similarly staged patients who received neoadjuvant hormonal therapy (15). We chose patients with these preoperative parameters because they have a high risk of extraprostatic disease (70%) and may be candidates for neoadjuvant hormonal therapy. The use of neoadjuvant hormonal therapy in this study was not randomized and was dependent on the individual surgeon’s preference and/or patient’s wishes. All of the patients had histologically proven prostate cancer. A serum PSA level was obtained on all of the patients before the institution of hormonal therapy. Bone marrow was obtained from all of the patients before the institution of hormonal therapy in the radical-prostatectomy-alone patients. No patient had received chemotherapy or radiation therapy before enrolling in this study. All of the patients had clinically localized prostate cancer and had a negative bone scan before the institution of hormonal therapy or surgery.

All of the radical prostatectomy specimens were processed in the same fashion. Briefly, the prostate was placed in 10% formalin and submitted entirely for histological evaluation in standard cassettes. The left side of the prostate was inked in green and the right side in blue. The prostate was step-sectioned at 5-mm intervals, and 8-μm sections were taken from each block and stained with H&E. One of three fellowship-trained genitourinary pathologists reviewed the radical prostatectomy specimens. Tumors were staged in the following manner: (a) organ-confined disease (tumor that is completely confined to the prostate); (b) extraprostatic extension (tumor that penetrates through the prostate capsule but does not touch the ink margin or invade the seminal vesicles); (c) positive surgical margins (presence of a tumor cell at the inked margin); (d) seminal vesicle invasion (any invasion of the seminal vesicle by tumor cells); (e) positive lymph nodes (prostate cancer cells identified in lymph nodes); and (f) distant metastasis (presence of distant metastasis by bone scan).

Prostate Cancer Cell Line. The LNCAP prostate cancer cell (obtained from American Type Culture Collection, Manassas, VA), which expresses PSA, was used as a positive control (7). These cells were grown in RPMI medium at 37°C and supplemented with 10% FCS.

Patients’ Specimens. The human investigations performed in this study have been approved by the Internal Review Board at the University of Kentucky, the Lexington Veterans Affairs Medical Center, and Wayne State University. All of the patients signed an informed consent form. Bone marrow aspirates were collected from patients undergoing radical prostatectomy at the time of surgery and before prostate manipulation. At least 4 weeks had passed after the prostate needle biopsy.

Bone Marrow Sample Preparation. Ten ml of heparinized bone marrow were taken from each iliac crest and pooled. This was layered over a Ficoll-Hypaque gradient (Pharmacia, Piscataway, New Jersey) and centrifuged at 2,000 rpm for 20 min. The cell layer at the interface was collected and washed once in PBS. This cell layer contains mononuclear and epithelial cells (16). Histopathological evaluation of the bone marrow samples was not performed.

PCR-directed Amplification of mRNA Sequences. The oligonucleotide primers, probes, and amplification conditions have previously been published (7). Briefly, the reverse PCR kit from Perkin-Elmer (Norwalk, CT) was used to perform the RTPCR experiments. cDNA was made from 2 μg of total mRNA using random hexamers. One-half of the cDNA sample was amplified with the PSA primers and the other half was amplified with β-actin primers as an internal positive control. One-half of the PSA RTPCR was electrophoresed on a 1.5% agarose gel and transferred to a nylon membrane. The membrane was then hybridized with a PSA oligonucleotide probe as described previously (7). One-half of the β-actin RTPCR product was electrophoresed on 1.5% Agarose gel and stained with Ethidium bromide. Each RTPCR experiment evaluated mRNA from a tube containing no mRNA (negative controls), a 10−5 and 10−6 dilution of the LNCAP cells (positive controls), and the patient samples. The LNCAP dilutions were constructed by mixing LNCAP cells with peripheral blood mononuclear cells from healthy volunteers. Samples that were considered positive for circulating prostate cells had an amplified PSA product, provided that the negative controls were negative. Specimens were considered to be true negative for prostate cancer cells if there was no amplified product for the PSA product but the
For the neoadjuvant group, 23 were treated with Leuprolide only. The average number of months of neoadjuvant hormonal therapy was 3 (range, 2–8 months). Fifteen and 17 patients with T2b-T tumors were in the radical-prostatectomy-alone group and 31 had received Leuprolide with or without flutamide before radical prostatectomy (neoadjuvant androgen ablation was similarly successful in the neoadjuvant androgen-ablation patients as it was in the radical-prostatectomy-alone patients). Specifically, in the radical-prostatectomy-alone group, the patients with extraprostatic disease had a significantly higher incidence of prostate cancer cells in their bone marrow compared with patients with organ-confined tumors [17 (77%) of 22 versus 1 (14%) of 7, respectively; \( P = 0.03 \)]. In contrast, in the neoadjuvant group, the incidence of prostate cancer cells in the bone marrow was not different among the pathological stages [6 (46%) of 13 in the extraprostatic-disease group versus 5 (28%) of 18 in the organ-confined group; \( P = 0.29 \)].

Finally, we compared the pathological stages among only those patients whose bone marrow tested positive for circulating prostate cells. The rate of organ-confined disease among patients whose bone marrow was positive for prostate cancer cells was significantly higher in the neoadjuvant androgen-ablation patients compared with the radical-prostatectomy-alone patients [5 (45%) of 11 versus 1 (6%) of 18; \( P = 0.018 \)]. This suggests that a subset of neoadjuvant androgen-ablation patients are converted to organ-confined disease without eliminating the presence of prostate cancer cells in the bone marrow.

Table 1 shows the demographics of patients undergoing radical prostatectomy. Of the 60 patients who underwent radical prostatectomy, 29 had no previous hormonal therapy (radical-prostatectomy-alone group) and 31 had received Leuprolide with or without flutamide before radical prostatectomy (neoadjuvant group). For the neoadjuvant group, 23 were treated with a combination of Leuprolide and Flutamide and 8 received Leuprolide only. The average number of months of neoadjuvant hormonal therapy was 3 (range, 2–8 months). Fifteen and 17 patients with T2b-T tumors were in the radical-prostatectomy-alone group and neoadjuvant group, respectively. There was no significant difference between the average PSA level or the average Gleason score between the two groups.

As seen in Table 2, neoadjuvant androgen ablation was associated with an increase in the organ-confined disease rate [18 (58%) of 31 versus 7 (24%) of 29; \( P = 0.007 \)] and a decrease in positive margins [3 (10%) of 31 versus 10 (35%) of 29; \( P = 0.017 \)] compared with radical prostatectomy alone. The incidence of extraprostatic extension only, seminal vesicle invasion, or positive lymph nodes was similar between the two groups. Neoadjuvant androgen ablation was similarly successful in reducing the overall incidence of prostate cancer cells in the bone marrow (Table 3). Eighteen (62%) of the 29 radical-prostatectomy-alone patients and 11 (35%) of the 31 neoadjuvant-group patients had circulating prostate cells in their bone marrow (\( P = 0.04 \)).

We then examined the incidence of prostate cancer cells in the bone marrow when stratified by treatment and pathological stage. Overall, 6 (24%) of the 25 patients with organ-confined tumors were RTPCR-positive compared with 23 (66%) of the 35 patients with extraprostatic disease (\( P = 0.001 \)). However, the bone marrow RTPCR result was not associated with the pathological stage in the neoadjuvant androgen-ablation patients as it was in the radical-prostatectomy-alone patients. Specifically, in the radical-prostatectomy-alone group, the patients with extraprostatic disease had a significantly higher incidence of prostate cancer cells in their bone marrow compared with patients with organ-confined tumors [17 (77%) of 22 versus 1 (14%) of 7, respectively; \( P = 0.03 \)]. In contrast, in the neoadjuvant group, the incidence of prostate cancer cells in the bone marrow was not different among the pathological stages [6 (46%) of 13 in the extraprostatic-disease group versus 5 (28%) of 18 in the organ-confined group; \( P = 0.29 \)].

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The nadir PSA level was not associated with the RTPCR status. Of the 17 patients who had a PSA \( \leq 0.1 \) ng/ml on hormonal therapy, 6 were RTPCR-positive. Adequate follow-up data are unavailable on these patients to determine the prognostic significance of the RTPCR status.

### RESULTS

Table 1 shows the demographics of patients undergoing radical prostatectomy. Of the 60 patients who underwent radical prostatectomy, 29 had no previous hormonal therapy (radical-prostatectomy-alone group) and 31 had received Leuprolide with or without flutamide before radical prostatectomy (neoadjuvant group). For the neoadjuvant group, 23 were treated with a combination of Leuprolide and Flutamide and 8 received Leuprolide only. The average number of months of neoadjuvant hormonal therapy was 3 (range, 2–8 months). Fifteen and 17 patients with T2b-T tumors were in the radical-prostatectomy-alone group and neoadjuvant group, respectively. There was no significant difference between the average PSA level or the average Gleason score between the two groups.

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high risk of having circulating prostate cells in their bone marrow could effect the RTPCR status. At our institution, patients with a PSA ≥ 10ng/ml have a 60% chance of having circulating prostate cells in their bone marrow and an 85% risk of having extraprostatic disease in the pathological specimen. Neoadjuvant hormonal therapy did have an effect on the presence of circulating prostate cells in the bone marrow. Overall, patients treated with radical prostatectomy alone had a higher incidence of circulating prostate cells in their bone marrow compared with patients treated with neoadjuvant hormonal therapy before radical prostatectomy (62% versus 35%, respectively; *P* = 0.04).

Similar to our previously published results (8, 12), the RTPCR status was statistically associated with the pathological stage of disease in the untreated patients. However, this difference was abrogated in patients on neoadjuvant hormonal therapy. In the radical-prostatectomy-alone group, 77% and 14% of patients with extraprostatic and organ-confined disease were RTPCR-positive, respectively (*P* = 0.03). In the neoadjuvant group, the incidence of prostate cancer cells in the bone marrow was not different among the pathological stages (46% in the extraprostatic-disease group versus 28% in the organ-confined group, *P* = 0.29). Most striking, is the fact that for patients who were RTPCR-positive, 45% of the neoadjuvant patients had organ-confined disease compared with 6% in the radical-prostatectomy-alone patients (*P* = 0.018). This suggests that neoadjuvant hormonal therapy may have a greater effect on the primary tumor than on the circulating prostate cells in the bone marrow. This may explain why the downstaging from neoadjuvant hormonal therapy has not translated into an improved disease-free survival rate.

Because this study was not randomized, other confounding factors may have been involved in the apparent inability of neoadjuvant hormonal therapy to fully eradicate circulating prostate cells. Additionally, it is unknown whether a longer duration of neoadjuvant hormonal therapy would further decrease the incidence of circulating prostate cells in the bone marrow. We have shown previously (12) that the RTPCR status is an independent prognostic indicator of disease-free survival in patients undergoing radical prostatectomy who are hormone naive. Our follow-up is too short and the study size inadequate to determine whether the RTPCR status will be predictive of disease-free survival in patients treated with neoadjuvant hormonal therapy.

Our data are similar to a recent study by Su et al. (21), using RTPCR of the prostate-specific membrane antigen mRNA in peripheral blood. They evaluated 17 patients treated with neoadjuvant hormone therapy before radical prostatectomy and 21 patients who underwent radical prostatectomy alone. Seventeen percent of the patients treated with neoadjuvant hormone therapy were RTPCR-positive versus 62% of patients undergoing radical prostatectomy alone. They did not have enough patients to determine whether this difference was statistically significant. We did not routinely obtain peripheral blood specimens from our radical-prostatectomy patients or perform prostate-specific membrane antigen RTPCR. It is unclear whether our results would be different if we had used peripheral blood instead of bone marrow, but in our experience, the incidence of circulating prostate cells in the peripheral blood is lower than in the bone marrow, even in men with metastatic disease (8).

It is possible that neoadjuvant hormonal therapy has decreased the production of PSA mRNA by prostate cells in the bone marrow rather than eliminate the cells. However, we can detect PSA mRNA in LNCAP cells grown without androgens (7). In addition, we were able to identify PSA mRNA in 35% of patients with an undetectable serum PSA level at the time of radical prostatectomy. More likely is that the number of circulating prostate cells in the bone marrow has been decreased to a level below the sensitivity of the RTPCR test. Therefore, neoadjuvant hormonal therapy seems to decrease the number of patients that have detectable levels of PSA mRNA in their bone marrow. By identifying PSA mRNA in the bone marrow of patients with prostate cancer despite a PSA ≤ 0.1ng/ml, we have shown that RTPCR is a very sensitive method to detect prostate cancer cells even in this subgroup of patients. This may be a useful tool to evaluate the tumor burden of patients on hormonal therapy who have an undetectable serum PSA level.

In summary, we have shown that preoperative hormonal therapy can increase the incidence of organ-confined disease in patients at high risk for extraprostatic disease before radical prostatectomy. To a lesser degree, neoadjuvant hormonal therapy decreases the incidence of circulating prostate cells in the bone marrow of patients undergoing radical prostatectomy. This may partially explain why neoadjuvant hormonal therapy has not translated into an improved disease-free survival.

### REFERENCES


Effect of neoadjuvant androgen deprivation on circulating prostate cells in the bone marrow of men undergoing radical prostatectomy.


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