

Morphologic Effects of Neoadjuvant Hormone Therapy on Prostate Cancer

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ABSTRACT

We have reported that neoadjuvant hormone therapy (NHT) combining the antiandrogen flutamide and a luteinizing hormone—releasing hormone (LHRH) agonist for 3 months leads to decreased tumor volume, capsular penetration, and margin invasion in patients with localized prostate cancer. More recently, we compared patients randomized to 3 and 6 months of NHT. The tumor volume was further significantly decreased after 3 additional months of NHT. The histologic features and biologic characteristics of cancer tissue were also strongly influenced by NHT, and cancer cells had features suggestive of low activity in prostates exposed to hormonal manipulation. Despite an apparently higher Gleason score and loss of androgen receptors, suggesting a selection of more aggressive cell clones, nucleolar diameter and staining with the cell proliferation marker Ki-67 were significantly reduced after 3 months of NHT compared with tumors from patients treated by surgery alone and were further reduced after 6 months of NHT. Although longer follow-up is needed to assess whether those changes will translate into improved patient outcomes, our data clearly demonstrate that NHT in patients with prostate cancer leads to a marked downsizing of the tumor bulk and a marked decrease of cancer cell activity.

INTRODUCTION

RADICAL PROSTATECTOMY is a common procedure for localized prostate cancer, and the prognosis for those patients is largely dependent on tumor volume, organ confinement, margin involvement,^{1,2} and Gleason score.² The addition of neoadjuvant hormone therapy (NHT) to the treatment of patients with localized prostate cancer has been associated with decreased prostate specific antigen (PSA) concentrations and a lower incidence of positive surgical margins,³⁻¹⁰ suggesting that improved local control may be expected. A recent meta-analysis of published randomized controlled trials using nonsteroidal antiandrogens in advanced prostate cancer further supports a beneficial effect of maximum androgen blockade on disease progression and survival.¹¹ Because of the significance of a high Gleason score for patient survival,² it is hoped that NHT would affect, not only the tumor size, but also the biologic activity of cancer cells in order to prevent the development of occult distant metastases. Our experience indicates that not only does NHT induce a downsizing of prostate cancer, but the altered

cancer cells are biologically less active than those not exposed to hormonal manipulation.

MATERIAL AND METHODS

Population

In 1996, we compared the histologic features of prostate cancers from 47 patients treated by NHT followed by radical prostatectomy and 49 patients treated by surgery alone.³ More recently, one of us (THvdK) compared the morphologic changes induced by 3 months (18 patients) and 6 months (22 patients) of NHT (unpublished data).

The randomization process used with those patients has been reported elsewhere.^{3,12} All patients had a thorough clinical evaluation including prostate biopsies, staging and metastatic work-up. All patients had clinical Stage T₂ or T₃ disease. After giving informed consent, the patients were randomly assigned to either surgery with or surgery without 3 months of NHT in one

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study^{3,12} or to either 3 or 6 months of NHT prior to surgery in a second study. The two groups did not differ in age or initial stage of disease.

Of the 136 prostate cancers selected from both randomized studies, the clinical characteristics, including age and initial serum PSA concentrations, were similar in the different arms. In our study comparing surgery alone and surgery with 3 months of NHT,³ patient age averaged 62.1 years. The PSA at diagnosis averaged 19 ng/mL in patients treated by surgery alone and 27 ng/mL in those submitted to 3 months of NHT. In the study comparing 3 and 6 months of NHT, the patient age averaged 61.3 years (3 months) and 63 years (6 months), respectively. The mean PSA concentration was somewhat lower than in the first study, averaging 7.5 and 8.5 ng/mL, respectively.

Radical Prostatectomy

All patients included in these randomized studies underwent nerve-sparing radical retropubic prostatectomy. In both studies, all prostatectomies were performed by the same urologist (YF). The specimen margins were inked, and the tissue was sectioned according to a well-defined protocol.³

Histologic Technique

Hematoxylin and eosin-stained sections of radical prostatectomy specimens from all 136 randomized patients have been reviewed histologically. In both studies, sections were reviewed without knowledge of the treatment arm. The tumor grade, extent, and margin involvement, as well as the presence and histologic appearance of prostatic intraepithelial neoplasia (PIN), were assessed, and the tumor volume and nucleolar diameter were measured by image analysis.

Immunohistochemistry Techniques

Recently, one of us (THvdK) measured cell proliferation with the use of the monoclonal antibody MIB-1 (Immunotech, France), which recognizes the Ki-67 antigen on paraffinized sections.¹³ The areas with the highest staining count were located at low power. The percentage of proliferating cells was obtained by calculating, on 400 to 500 cells, the ratio of stained cancer cells to total cancer cells at 400 \times magnification.

Image Analysis

The tumor volume and nucleolar diameter of the specimens from both randomized studies were measured by image analysis as defined earlier.³ The cancer surface was obtained by tracing, with the mouse, along the tumor edges marked with a pen. The cancer volume was obtained by measuring the cancer surface on each section. The sum of each individual area was multiplied by the thickness (5 mm) of each slice and by a correction factor to compensate for tissue shrinkage attributable to Formalin fixation.³ The mean nucleolar diameter was obtained by measuring, by image analysis, the diameter of 50 manually selected nucleoli on a digitized image obtained with the 60 \times objective.

RESULTS

Downsizing of Prostate Cancer

Table 1 summarizes the surgical pathologic stage of prostate cancers included in the study by Vaillancourt and colleagues,³ and generated from our recent study comparing 3 months and 6 months of NHT. It appears that, whereas stage pT₂ tumors account for 49% of cancers in patients treated by surgery alone, they represent close to 80% of cases after 3 months of NHT, and this proportion is maintained with 3 additional months of treatment. This result shows that more tumors are organ confined after NHT, which suggests a downsizing of the cancer tissue by NHT. Furthermore, whereas positive surgical margins were significantly decreased, by 25% ($P < 0.001$), after 3 months of NHT compared with surgery alone,³ a low rate of positive surgical margins was maintained after 3 additional months, but the difference did not reach significance ($P = 0.21$). Our tumor volume measurements confirm those data. After 3 months of NHT, tumor volume was reduced by 44% ($P < 0.007$) compared with prostates not exposed to NHT.³ The median tumor volume averaged 2.7 cc in surgically treated patients and 1.5 cc in those receiving 3 months of prior NHT. Tumor volume was further decreased by 60% with 3 additional months of NHT. In this study, the tumor volume was measured closer to the cancer glands and averaged 0.5 cc with 3 months of NHT and 0.2 cc with 6 months ($P = 0.002$).

Morphologic Changes in Prostatic Tissue and Cancer Cells

After NHT, there was marked atrophy of normal prostatic tissue with prominence and hyperplasia of the basal-cell layer (Fig. 1). The cancer cells were mostly isolated or formed small clusters or small glands, features reminiscent of Gleason patterns 3 to 5 (Fig. 2). The nucleolar size was markedly affected by NHT. In our prior study,³ whereas only 53% of prostate cancers had an average nucleolar diameter $< 1.5 \mu\text{m}$ in the surgically treated arm, the proportion reached 89% in those patients submitted to 3 months of NHT ($P < 0.001$), and this small nucleolar size was maintained with 3 additional months of NHT

TABLE 1. COMPARISON OF pT STAGES IN PATIENTS TREATED BY SURGERY ALONE OR SUBMITTED TO 3 OR 6 MONTHS OF NHT BEFORE SURGERY

pT Stage	Surgery*	Neoadjuvant Hormone Therapy		
		3 Months*	3 Months [†]	6 Months [‡]
0	0 [‡]	0 (0)	0 (0)	2 (9)
2	24 (49)	39 (83)	14 (78)	17 (77)
3	25 (51)	8 (17)	4 (22)	3 (14)
Total	49 (100)	47 (100)	18 (100)	22 (100)
P value		< 0.001		NS

*Randomized study comparing surgery alone and 3 months of NHT before surgery.³

[†]Randomized study comparing 3 months and 6 months of NHT before surgery (THvdK, unpublished).

[‡]Number of cases (% of total).



FIG. 1. Atrophic prostatic tissue after 6 months of NHT with prominence and hyperplasia of basal-cell layer. (H&E; original magnification 250 \times)

(88%). Finally, the average rate of cancer cell proliferation decreased significantly, from 4.8% in surgically treated cancers to 2.6% after 3 months of NHT and 1.8% with 3 additional months of treatment ($P < 0.01$). These data support the concept that cancer cells are biologically less active after NHT.

It is possible, however, that the biologic activity of cancer cells may be reactivated in certain cases after the cessation of NHT. In fact, of the 40 patients entered in the study comparing 3 and 6 months of NHT, five stopped their NHT 8 to 38 days prior to surgery, and all five belonged to the 6-month arm. Whereas only 4 (11%) of the 35 patients who stopped NHT at the time of surgery had Ki-67 staining greater than 6% and 6 (17%) had a nucleolar diameter $>1.5 \mu\text{m}$, of those five whose surgery was delayed after the cessation of NHT, two had Ki-67 $>6\%$ and three had nucleolar size $>1.5 \mu\text{m}$.

Intraepithelial neoplasia was also modified after NHT. Nucleoli were smaller than in prostates not exposed to NHT. After 6 months, the tufting was less pronounced, and the epithelial proliferation was flat in most cases (Fig. 3).

DISCUSSION

We recently demonstrated that, compared with surgery alone, NHT prior to surgery leads to a downsizing of prostatic cancer.^{3,12} By image analysis on histologic sections,³ we found that

the tumor volume was decreased by 44% compared with prostates not exposed to NHT ($P < 0.007$). Other investigators also reported a significant decrease of tumor volume by image analysis. In fact, tumor density was reduced by 25% in one study¹⁴ and by 40% in another,¹⁵ and the area occupied by cancer cells was decreased by 50% in another.¹⁶ In our hands, residual tumor was often minimal, and its detection necessitated a careful search. In 8% of our cases, residual tumor was present only as single cells floating within branching spaces that we designated as a hemangiopericytoma-like pattern.³ Furthermore, in 2 of our 47 patients treated by NHT prior to surgery whose prostates were totally embedded, no residual tumor was found. Additional step-sections and immunostaining for cytokeratins were performed on all posterior sections of the prostate, and, in both cases, a single microscopic focus of cancer was found.¹⁷ The rate of capsular penetration dropped from 51% in surgically treated cases to 17% after NHT ($P < 0.001$). Margin invasion was also significantly decreased after NHT ($P = 0.002$). Comparable findings have been reported by others.⁴⁻¹⁰ The use of immunostaining for cytokeratins, PSA, and prostatic acid phosphatase did not help to identify more cases with margin involvement that might have been missed by simple histologic examination.⁴ More recently, our data have been complemented by one of us (THvdK) who compared patients randomized to 3 and 6 months of NHT. This study showed that

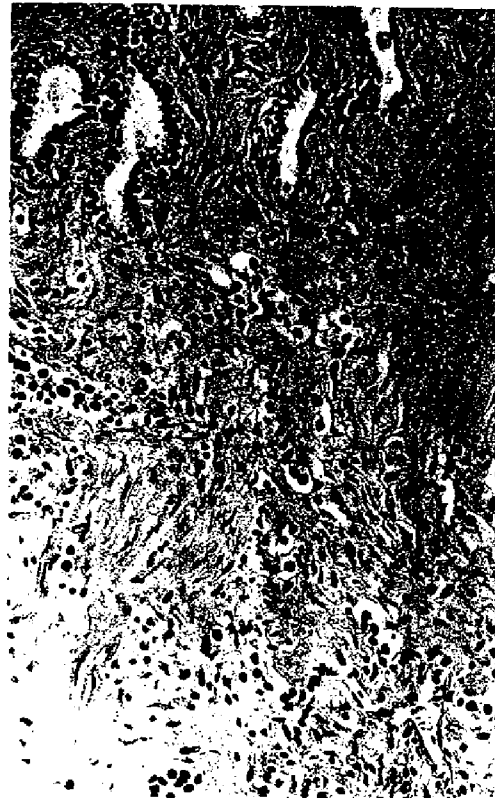


FIG. 2. Cancer cells after 3 months of NHT with features of high Gleason pattern. (H&E; original magnification 250 \times)



FIG. 3. Intraepithelial neoplasia after 3 months of NHT showing flattened pattern and inconspicuous nucleoli. (H&E; original magnification 250 \times)

tumor volume was further decreased by 60% after 6 months of NHT compared with 3-month treatments and that capsular penetration and margin involvement remained low. Smaller tumor size, organ confinement, and lower margin involvement are predictive of better disease control.^{1,2}

The histologic features and biologic characteristics of cancer tissue were also strongly influenced by NHT. Well-formed acinar structures were uncommon under NHT influence, and cancer cells were mostly arranged in diffuse sheets or small glands or as single cells in more than 90% of cases,³ features reminiscent of Gleason patterns 3 to 5. This apparently increased Gleason score has been reported by others.^{15,18,19} In fact, Armas and coworkers¹⁸ reported that 94% of prostate cancers had Gleason scores of 7 to 10 after NHT compared with only 26% in pretreatment biopsies.

This apparent increase in the Gleason score might be interpreted as a feature of greater aggressiveness. Similarly, a marked decrease in androgen receptor (AR) expression was seen by immunohistochemistry after NHT.²⁰ However, additional studies instead showed features of decreased activity in cancer cells after NHT. In fact, nucleolar diameter, as measured by image analysis, was reduced by close to 26% ($P < 0.001$) after 3 months of NHT compared with the value in those patients submitted to surgery alone and was further, although not significantly, reduced ($P = 0.07$) after 6 months of NHT compared with 3 months of therapy. Several publications also emphasize the small nucleolar size of prostate cancer cells exposed to NHT.^{18,19,21} Finally, cell proliferation, as measured by Ki-

67 (MIB-1) immunostaining, was reduced by 46% after 3 months of NHT compared with surgery alone and by 33% after 6 months NHT compared with 3 months ($P < 0.01$). Similar data have been reported by others. Armas and coworkers¹⁸ found that, of prostate cancers exposed to NHT, 67% had $<10\%$ of their cells in proliferation and 75% were diploid, whereas 90% of prostates not exposed to NHT had $>10\%$ proliferating cells, and 80% were nondiploid. Cancer-cell proliferation was also reported to be decreased in prostates exposed to 8 months of NHT.⁴ Furthermore, decreased mitotic activity has been reported in cancer cells exposed to NHT.²¹ Our data and those in the literature reveal that, although cancer cells present some features of increased aggressiveness after NHT, they in fact show morphological features of lower biologic activity than do cells in prostates not exposed to hormone manipulation. The evaluation of the Gleason score using the standard criteria is questionable after NHT because of the marked changes in cancer morphology, as discussed elsewhere in this issue. In fact, other investigators found no significant upgrading after NHT,^{4,6} which raises a possible problem of reproducibility in grade scoring after hormone therapy. This result further supports the concept that the Gleason score is of no utility after NHT because of the lack of clear criteria to grade those tumors and the absence of clinical relevance.

Neoadjuvant hormonal therapy was also found to decrease the prevalence of expression of certain markers. All patients submitted to NHT in our studies experienced a drastic reduction of serum PSA as well as a marked decrease in PSA staining by immunohistochemistry.²² Whereas all cases had strong staining of both normal and cancer cells in prostatic tissue not submitted to NHT, merely 25% had such strong staining after 3 months of NHT. Comparable results were reported by others.¹⁴ Similarly, as mentioned above, a marked decrease of AR expression was seen by immunohistochemistry after NHT²⁰ in both normal cells ($P < 0.001$) and cancer cells ($P = 0.048$). However, certain receptors are upregulated after NHT. For instance, whereas estrogen receptors (ER) are virtually absent from prostatic tissue in patients treated by surgery alone, ER are more frequently expressed after NHT,²³ although this expression is limited to stromal cells around prostatic glands. The significance of this finding is not clear.

Our data also revealed that NHT induced marked changes in benign prostatic tissue. Prostatic atrophy was present in $>70\%$ of cases, whereas basal-cell prominence and hyperplasia and epithelial-cell vacuolization were found $>80\%$ of cases.³ High-grade PIN was present in 67% of cases in prostates treated by surgery alone but only 6% of prostates after NHT ($P < 0.001$). A significant reduction in the frequency^{6,24} or a more focal distribution¹⁸ of high-grade PIN has also been reported. The identification of PIN after NHT may be difficult, however, because of the marked changes in the cell morphology. Indeed, nucleolar prominence, which is a hallmark of high-grade PIN, is much less evident after NHT.^{3,18,21} We also observed that the tufted pattern, which is predominant in untreated prostates and, to a lesser extent, in prostates exposed to 3 months of NHT, is virtually absent in prostates exposed to 6 months of NHT, and the flat pattern is predominant. Although the clinical significance of all these changes is unclear, they reflect a marked reduction of preneoplastic cell activity.

Finally, five patients submitted to 6 months of NHT underwent surgery 8 to 38 days after the cessation of the med-

ical treatment. In those cases, there was a higher prevalence of high proliferation and large nucleoli than in patients whose therapy was stopped at the time of surgery. This finding suggests that, in certain cases, there might be reactivation of residual cancer cells if they cease to be exposed to androgen blockade. Those findings are in keeping with the report that the cessation of short-term NHT is followed by an early rise in PSA.²⁵

Our morphologic studies clearly demonstrate that NHT in patients with prostate cancer leads to a marked downsizing of the tumor bulk and a marked decrease in cancer cell activity. Longer follow-up is now needed to assess whether those changes will translate into an improved outcome for those patients.

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