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S. Brändstedt · C. Busch · M. Hellström · B. Nordin
M. Häggman

Neo-adjuvant GnRH therapy and radical prostatectomy: effects on tumorous and benign tissue volumes – a morphometric study

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Abstract The effect on tumour and prostate volumes of a 3-month course of neo-adjuvant hormone therapy was studied using computerised planimetry on serially sectioned specimens obtained by radical prostatectomy. Fifty-four specimens from patients not receiving pre-treatment were compared to 38 specimens from patients given the gonadotropin-releasing hormone (GnRH) analogue triptorelin for 3 months before the operation. Glandular volume and volume of the index tumour was determined. To determine the position of the index tumour within the gland, the centre of mass of the tumour was identified and the distance to the gland margin calculated. This value (M_1) represents the sum of the tumour radius and the various amounts of normal tissue. The amount of surrounding tissue could be approximated by correlating M_1 to the corresponding tumour volume.

Results: The two groups differed significantly in total gland volumes, but not in tumour volumes. M_1 was strongly correlated to the tumour volume in the treatment group ($r = 0.73$), whereas in the control group the correlation was found to be significantly weaker ($r = 0.44$), indicating that there was less tissue surrounding the tumour in the pre-treated group. In a multiple regression analysis of all 92 patients, index tumour volume was found to be associated with total gland volume, DNA ploidy pattern, tumour grade but not whether or not pre-treatment was given. This study found that the volumes of the single largest tumour focus were not significantly affected by hormonal pre-treatment, and that "the prostate condenses around the tumour rather than that the tumour shrinks back into the prostate". However, the precise relationship between tumour epithelial volume and stroma with or without neo-adjuvant hormonal pre-treatment remains to be clarified.

Key words Prostate cancer · Radical prostatectomy · Neo-adjuvant treatment · Morphometry

S. Brändstedt (✉) · M. Hellström
Department of Urology, Karolinska Institute, Danderyd Hospital, S-182 88 Danderyd, Sweden

C. Busch
Department of Pathology, University Hospital, Uppsala University, Uppsala, Sweden

B. Nordin
Centre for Image Analysis, Uppsala University, Uppsala, Sweden

M. Häggman
Department of Urology, University Hospital, Uppsala University, Uppsala, Sweden

Introduction

Despite recent improvements in preoperative staging [2, 13, 18, 22, 23], and earlier detection by prostate-specific antigen (PSA) screening/case-finding [1, 19], a large proportion of radical prostatectomy specimens from patients operated on for localised prostate cancer show tumour tissue in contact with the surgical margin [8, 31]. The PSA recurrence rate 10 years postoperatively approaches 25% even in series of strictly selected cases [30]. Local recurrences may be a major cause for the reappearance of the disease [26]. Preoperative or neo-adjuvant hormonal therapy has been suggested as a means of improving surgical outcome in patients with stage T2 and T3 localised cancer [3, 5, 14, 16, 27, 28]. The rationale behind this treatment is the reduction of total gland and tumour volume. Radical excision might thus be easier to achieve, resulting in a higher local control rate. Several studies using clinical and ultrasonographic staging [7, 21, 25] have indicated that, following pre-treatment, there is a decrease in total prostate as well as in tumour volumes. Some studies have shown a more pronounced reduction in tumour volume compared to prostatic volume [21]. The impact of neo-adjuvant therapy seems well documented.

Whether the long-term local control rates are improved remains to be seen in follow-up studies. The aim of this study was, using morphmetric methods, to describe the effect of GnRH treatment on tumour volume and that of benign tissue.

Material and methods

Patients and surgical procedures

Ninety-two radical prostatectomy specimens were studied. The specimens were obtained from two different groups of patients with localised prostate cancer (Table 1). Thirty-eight patients were operated on during 1990–1993, including stage T1b-3a, grade 1–3 tumours. Staging and grading was performed according to the TNM classification of 1992 [24] and the WHO grading system [17], respectively. These 38 patients had received monthly depot injections of triptorelin 3.75 mg (Decapeptyl Depot, Ferring, Sweden), for 3 months before the operation. Cyproterone acetate 150 mg daily was given for 3 weeks at the start of treatment to prevent tumour flare. All pre-treated patients had serum testosterone levels in the castration range after 3 months of therapy. Fifty-four specimens were examined from patients who underwent surgery during 1987–1990. No pre-treatment was given in this group with stage T1b-T2, G 1–3 tumours [8]. A uniform operating technique with nerve-sparing dissection according to Walsh [29] was used in all 92 cases. Distribution according to clinical and pathological tumour stage in the two series is shown in Table 2.

Table 1 Clinical tumour stage and grade in hormone- and non-hormone-treated patiens on admission to the study. Figures represent numbers of patients [hormone-treated ($n = 38$)/non-hormone-treated patients ($n = 54$)]. See text for abbreviations

	G1	G2	G3	Total
T1b	1/2	0/3	–	1/5
T2	16/17	3/28	4/4	23/49
T3	4/0	8/0	2/0	14/0
Total	21/19	11/31	6/4	38/54

Table 2 Distribution of patients according to clinical (T-stage) and pathological tumour stage (pT-stage) in prostatectomy specimens

	T1b	T2	T3	Total
Non-hormone-treated patients ($n = 54$)				
pT2	0	8		8
pT3 –	0	15		15
pT3 +	5	26		31
Hormone-treated patients ($n = 38$)				
pT2	0	11	7	18*
pT3 –	0	3	4	7
pT3 +	1	9	3	13

* Significant difference ($P < 0.01$) from finding in the non-hormone-treated group

Morphological procedures

All specimens were fixed in 10% formalin, embedded in paraffin and, serially, transversely sectioned at 5-mm intervals. The whole mount specimens were sectioned at 5 µm thickness and stained with van Gieson's stain. Tumour areas were mapped on the whole mount slides. Total gland and index tumour volumes were calculated from the prostatectomy specimens by a planimetric technique using a computerised workstation (Epsilon Image, Imtec, Uppsala, Sweden). Images of the sections were read into the computer by manual delineation and the volume contribution from each section was estimated of the tumour. Tumour area was calculated and multiplied by the actual thickness of the section. The volume contributions from each of the sections were added. Tissue shrinkage during histopathological preparation was compensated for by a factor of 1.22 [20].

Mathematical assessment

The histological slide, displaying the largest area of invasive cancer in each case, was digitised into the image analysis programme Imp (Diascan AB, Uppsala, Sweden). Three numerical values describing the morphometric relations were obtained in each case:

1. The cross-sectional surface area of the index tumour (tumarea).
2. The cross-sectional surface area of the gland (glandarea).
3. The position and the pixel value (centrepix) of the centre of mass of the index tumour.

As a measurement for the position of the tumour in relation to the gland, the following formula was determined (Fig. 1)

$$M_1 = \frac{\text{Pixel value of the index tumour centre}}{\sqrt{\text{gland area}}}$$

The data were stored and managed in the statistical software program supplied by BMDP Statistical Software, Los Angeles, USA.

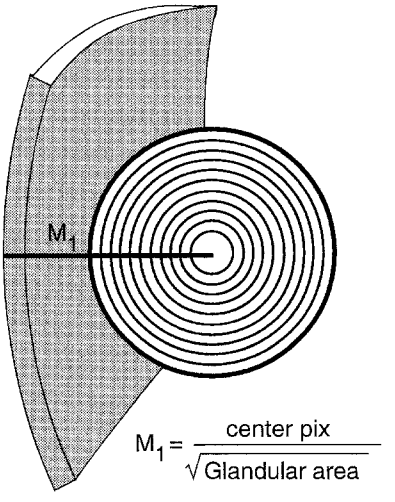

Fig. 1 Definition of M_1

DNA determinations

Flow cytometric DNA-ploidy assessment of multiple samples was performed from each gland. The method is described in detail elsewhere [6, 12, 15]. Briefly, 50-µm-thick tumour sections were taken and analysed by flow cytometry (FCM) with regard to DNA-ploidy pattern. The Go and G2 + M peaks in the FCM histogram were used to define the diploid and non-diploid cell populations, as previously described [9]. A DNA-ploidy pattern was considered non-diploid if the G2 + M peak contained more than 10% of all analysed nuclei or if the ratio between the position of the G2 + M peak and the G0 peak was outside the range of 1.90–2.10.

Statistics

Student's t-test was used to compare group mean values. A multivariate analysis was undertaken using the multiple logistic regression method.

Results

Volume assessments

Total gland volumes differed significantly between pre-treated patients and controls (mean = 23.8 ml, SD = 10.2 ml, vs mean = 35.8 ml, SD = 11.81 ml, $P < 0.001$). Index tumour volumes, however, did not differ between the two groups (mean = 3.3 ml, SD = 3.7 ml vs mean = 5.3 ml, SD = 5.5 ml, NS) (Fig. 2a, b). Total tumour volumes were found to be only marginally larger (mean = 3.5 ± 3.8 ml vs 6.1 ± 5.6 ml)

Mathematical assessment of tumour to gland margin relationship

Eighty-eight cases were available for this analysis (36 + 52). The factor M_1, quantifying the relative distance from the index tumour centre to the edge of the gland, was not significantly different between the groups. M_1 was correlated to the index tumour volume. In the treatment group, the correlation was stronger ($r = 0.73$) than in the control group ($r = 0.44$) (Fig. 3). The slope coefficients of the regression lines differed significantly (0.056 vs 0.021, $P < 0.01$) (Fig. 3).

DNA assessments

The occurrence of samples showing a non-diploid DNA pattern seemed to be related to tumour volume ($P < 0.01$), although all cell lines in the largest single tumour (17 ml) were diploid. Almost half of the tumours displayed a non-diploid ploidy pattern, whether or not the patients had received hormone treatment. Six patients with a non-diploid pattern showed such a pattern in all samples studied. The remaining 17 subjects showed ploidy heterogeneity, i.e. tumours containing a mixture of areas with diploid and non-diploid DNA configurations. The ratio between the number of samples (which had been collected from the largest tumour focus) with a non-diploid and a diploid DNA configuration averaged 1:1. The lack of statistical significance between the two series of patients was confirmed with Kolmogorov-Smirnov's two-sample test.

Multivariate analysis

The multiple regression analysis of all cases showed that the index tumour volume is determined by total gland volume ($P < 0.05$), non-diploid DNA ($P < 0.01$), pre-treatment grade (G2; $P < 0.05$, G3; $P < 0.05$), but not by neoadjuvant hormones (NS) (Table 3).

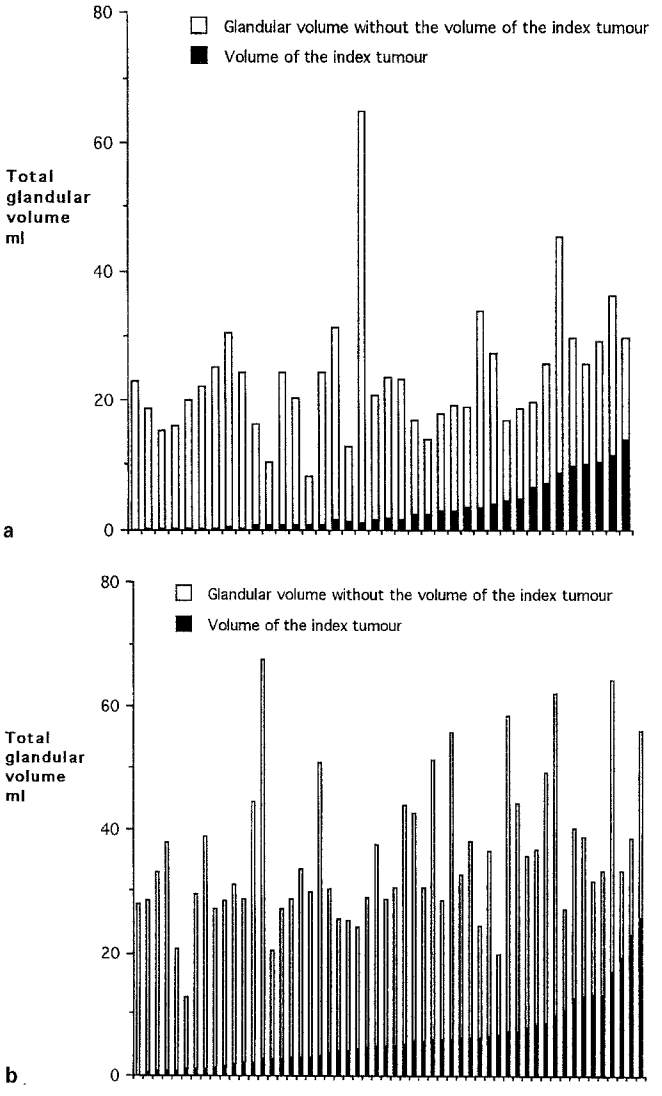

Fig. 2a, b Glandular and index tumour volumes estimated by the planimetric technique in hormone-treated ($n = 38$) and non-hormone-treated patients ($n = 54$), respectively. Glandular volumes differed significantly between the two series ($P < 0.001$). The apparent difference in tumour volumes was not significant

Discussion

Following neo-adjuvant hormonal ablation therapy, a decrease in prostate gland volume as well as a marked regression of tumour size has been demonstrated previously [3, 5, 7, 11, 14, 16, 25, 27]. Most of these studies are based on volume determination by transrectal ultrasound (TRUS), where tumours are detected as hypoechogenic lesions. Hypoechogenicity, however, is a relative concept and cannot be used reliably as

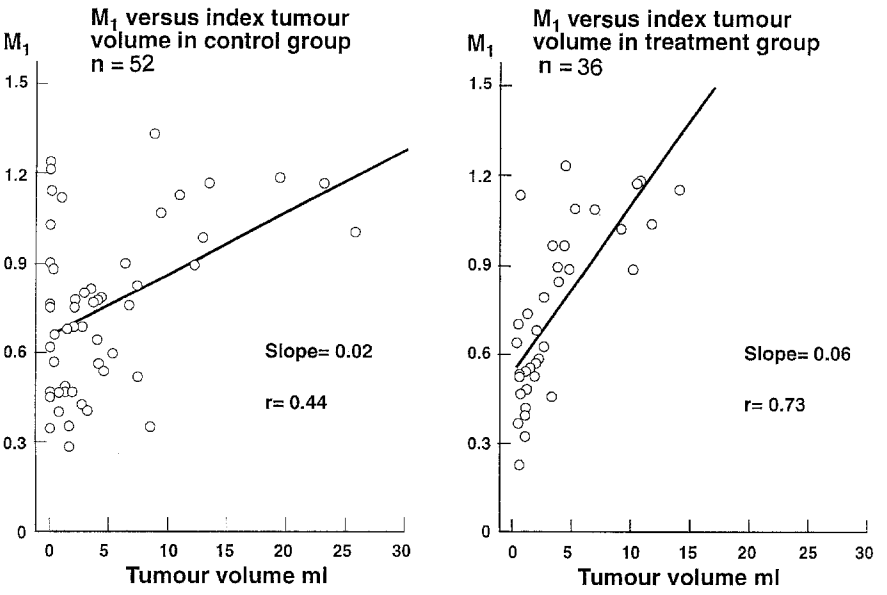

Fig. 3. Scatter diagram of individual M_1 in relation to index tumour volume in hormone-treated and non-hormone-treated patients. In the treatment group the correlation was stronger ($r = 0.73$) than in the control group ($r = 0.44$)

Table 3 Multiple regression analysis using index tumour volume as the dependent variable

Independent variable	Coefficient	*P* value
Total gland volume	0.10	<0.05
Non-diploid DNA	3.09	<0.01
Tumour grade 2	2.18	<0.05
Tumour grade 3	4.99	<0.05
Neoadjuvant hormones	−0.52	NS

a parameter to determine tumour size [13, 18]. Significant histopathological changes in non-malignant and malignant prostate tissue after hormonal pre-treatment have been described [11]. A decrease in the rate of positive surgical margins, following neo-adjuvant treatment, was found in an earlier non-randomised study [7]. Several other studies have also demonstrated a lower rate of positive surgical margins following neo-adjuvant therapy [3, 5, 14, 25]. The decreased incidence of positive surgical margins has been attributed to the observed reduction of tumour volume [21].

The specimens from the two groups of patients in this study differed significantly in the rate of positive surgical margins [7]. Therefore it seemed relevant to use these specimens for further analysis using a morphometric approach. Our working hypothesis was that a meticulous study of our archival material should reveal differences in tumour volumes if they existed. We found significant differences in gland volumes but not in index tumour volumes. The apparent difference in tumour volumes (Fig. 2a, b) was not significant. It should be emphasised that the planimetric calculations of volumes do not reflect the composition of cells within cancerous or benign tissue, i.e. the epithelial vs stromal tissue relationship. Evidence of relative increased amounts of stromal tissue after hormone ablation have been reported previously [4, 11]. Further analysis using epithelial-cell-specific cytokeratin staining and colour-based image analysis has made it possible to quantify the amount of epithelial cells vs stroma. These studies show an increased relative amount of stroma within the tumour area following hormonal pre-treatment [10].

To further analyse the spatial relationship between the tumour and the margin in the individual specimen, computerised planimetry and image analysis was used. Several numerical values describing the morphometric relations of the index tumour within the gland were obtained, such as the shortest and longest distance from the index tumour to the edge of the gland. We found, however, due to the variability in size and shape of the tumour, that single-dimensional physical parameters were less useful.

From a technological standpoint it is important to find features that add to the discrimination and can be reliably measured. In this study the combination of the volume of the index tumour and the distance of the centre of mass of the tumour to the margin of the gland were found to be expedient. The numerical value M_1 represents the amount of tissue between the tumour centre of gravity and the margin of the gland. Thus the M_1 value is the sum of the tumour radius and the various amounts of surrounding normal tissue. The greater the M_1 of the tumour tissue, the stronger the correlation of M_1 to tumour volume.

M_1 was strongly correlated to the tumour volume in the treatment group ($r = 0.734$), whereas in the control group the correlation was significantly weaker. This reflects a difference in the amount of tissue surrounding the tumour. The significantly increased slope coefficient as shown in Fig. 3 further emphasises this finding,

indicating that there was less benign tissue surrounding the tumour tissue in the treatment group.

This study demonstrates that the main volume difference depended on a decrease in benign tissue, whereas the gland volume occupied by the cancer remained relatively unchanged. The multiple regression analysis of all 92 patients demonstrated that factors other than the neo-adjuvant hormonal treatment were determinants of index tumour volume, such as abnormal DNA-ploidy pattern and higher tumour grade. In a further study [10] we have shown, however, that the stroma to tumour-epithelium ratio is higher after pretreatment.

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References

1. Cooner WH, Mosley BR, Rutherford CL, Beard JH, Pond HS, Terry WJ, Igel TC, Kidd DD (1990) Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. J Urol 143:1146
2. Enlund A, Pedersen KV, Boeryd B, Varenhorst E (1990) Transrectal ultrasonography compared to histological assessment for local staging of prostatic carcinoma. Acta Radiol 31:597
3. Fair WR, Aprikian A, Sogani P, Reuter V, Whitmore WF Jr (1993) The role of neoadjuvant hormonal manipulation in localized prostatic cancer. Cancer 71(Suppl):1031
4. Ferguson JD, Franks LM (1953) The response of prostatic carcinoma to oestrogen treatment. Br J Surg 40:422–428
5. Flamm J, Fischer M, Höltl W, Pfluger H, Tomschi W (1991) Complete androgen deprivation prior to radical prostatectomy in patients with stage T3 cancer of the prostate. Eur Urol 19:192
6. Häggman M, de la Torre M, Brändstedt S, Norlen BJ, Norberg M, Busch C (1994) Pre- and postoperative DNA ploidy patterns correlated to pT-stage, histological grade and tumour volume in total prostatectomy specimens. Scand J Urol Nephrol 28:59
7. Häggman M, Hellström M, Aus G, Pedersen K, Wijkström H, Stege R, Elvin A, Tisell Å, de la Torre M, Busch C, Brändstedt S (1993) Neoadjuvant GnRH-agonist treatment (triptorelin and cyproterone acetate for flare protection) and total prostatectomy. Eur Urol 24:456
8. Häggman M, Norberg M, de la Torre M, Fritjofsson Å, Busch C (1993) Characterization of localized prostatic cancer: distribution, grading and pT-staging in radical prostatectomy specimens. Scand J Urol Nephrol 27:7
9. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA (1983) Method for analysis of cellular DNA content of paraffin-embedded material using flow cytometry. J Histochem Cytochem 31:1333
10. Hellström M, Ranefall P, Wester K, Brändstedt S, Busch C: Effect of androgen deprivation on epithelial and mesenchymal tissue components in localized prostate cancer. Br J Urol. Accepted
11. Hellström M, Häggman M, Brändstedt S, de la Torre M, Pedersen K, Jarsfeldt I, Wijkström H, Busch C (1993) Histopathological changes in androgen deprived localized prostatic cancer. A study in total prostatectomy specimens. Eur Urol 24:461
12. Hellström M, Wester K, Häggman M, Brändstedt S, Busch C (1996) DNA ploidy patterns in androgen-deprived localized prostate cancer. Eur Urol 29:420–424
13. Lee F, Gray JM, McLeary RD, Meadows TR, Kumosaka GH, Borlaza GS, Staub WH, Lee F Jr, Solomon MH, MacHugh TA, Wolf RM (1985) Transrectal ultrasound in the diagnosis of prostate cancer: location, echogenicity, histopathology and staging. Prostate 7:117
14. Macfarlane MT, Abi-Aad A, Stein A, Danella J, Belldegrun A, DeKernion JB (1993) Neoadjuvant hormonal deprivation in patients with locally advanced prostate cancer. J Urol 150:132
15. Malmström P-U, Vasko J, Wester K, Norlén BJ, Busch C (1989) Flow cytometric analysis of DNA content of deparaffinized nuclei in urinary bladder carcinomas. APMIS 97:811–819
16. Monfette G, Dupont A, Labrie F (1989) Temporary combination therapy with Flutamide and Tryptex as adjuvant to radical prostatectomy for the treatment of early stage prostate cancer. In: Labrie F, Lee F, Dupont A (eds) Early stage prostate cancer: diagnosis and choice of therapy. Excerpta Medica, Amsterdam, pp 41–51
17. Mostofi FK (1975) Grading of prostatic carcinoma. Cancer Chemother Rep 59:111
18. Norberg M, Häggman M, Andersson T, Busch C, Magnusson A (1993) Evaluation of localized prostate cancer – a comparative study of transrectal ultrasonography versus histopathology. Eur Radiol 3:166
19. Oesterling JE (1991) Prostate-specific antigen: a critical assessment of the most useful tumour markers for adenocarcinoma of the prostate. J Urol 145:907
20. Partin AW, Epstein JI, Cho KR, Gittelsohn AM, Walsh PC (1989) Morphometric measurement of tumour volume and percent of gland involvement as predictors of pathological stage in clinical stage B prostate cancer. J Urol 141:341
21. Pinault S, Tetu B, Gagnon J, Monfette G, Dupont A, Labrie F (1992) Transrectal ultrasound evaluation of local prostate cancer in patients treated with LHRH agonist and in combination with Flutamide. Urology 39:254
22. Ritchie JP (1994) Management of patients with positive surgical margins following radical prostatectomy. Urol Clin N Am 21:717
23. Salo JO, Rannikko S (1994) The role of radical surgery in the treatment of prostatic cancer. Ann Med 26:147
24. Schröder F, Hermanek P, Denis L, Fair WR, Gospoddarowicz MK, Pavone-Macaluso M (1992) The TNM classification of prostate cancer. Prostate Suppl 4:129
25. Schulman CC, Sassine AM (1993) Neoadjuvant hormonal deprivation before radical prostatectomy. Clin Invest Med 16:523
26. Takayama TK, Lange PH (1994) Radiation therapy for local recurrence of prostate cancer after radical prostatectomy. Urol Clin North Am 21:687
27. Tunn UW, Goldschmidt AJW (1990) Effects of temporary antiandrogenic treatment prior to radical prostatectomy. In: Murphy G, Khoury S, Chatelain C, Denis L (eds) Recent advances in urological cancers – diagnosis and treatment. SCI, Jersey, p 102
28. Vallet BS (1944) Radical perineal prostatectomy subsequent to bilateral orchiectomy. Del Med J 16:18
29. Walsh PC, Lepor H, Eggleston JC (1983) Radical prostatectomy with preservation of sexual function: anatomical and pathological considerations. Prostate 4:473
30. Walsh PC, Partin AW, Epstein JI (1994) Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol 152:1831
31. Zietman AL, Shipley WU, Willett CG (1993) Residual disease after radical surgery or radiation therapy for prostate cancer. Clinical significance and therapeutic implications. Cancer 71 (Suppl 3):959