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DNA Ploidy, Gleason Score, Pathological Stage and Serum PSA Levels as Predictors of Disease-Free Survival in C-D_1 Prostatic Cancer Patients Submitted to Radical Retropubic Prostatectomy

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Gleason score
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Abstract
We report our experience with 85 prostatic cancer patients aged 51–79 years, who underwent radical retropubic prostatectomy from 1989 to December 1994 (mean follow-up 35 months). In order to get a more relevant analysis we chose to describe in detail only pathological C-D_1 cases and to subdivide the patients, according to the Gleason sum, into G_2–G_5 and G_6–G_{10} groups. Means of pre- and postsurgery PSA levels were ranked by DNA ploidy and presence or absence of recurrence: aneuploid patients showed lower levels of PSA production that may be due to cell dedifferentiation. However, in patients who developed recurrence, postsurgery PSA levels were higher ($p < 0.005$). The influence of DNA ploidy on disease-free survival was evaluated: the cumulative survival proportion was better in diploid (0.3581) than in aneuploid patients (0.2996). Using the Cox proportional hazard model with age, Gleason sum, DNA ploidy and presurgery PSA levels as covariates, we demonstrated that, in our series, only the presurgery PSA level was an important and significant predictor of recurrences ($p < 0.005$). Considering global recurrences with age, Gleason sum and presurgery PSA levels kept fixed, DNA aneuploidy conferred a relative risk 2.3 times higher than diploidy. When, in the same analysis, we introduced postsurgery PSA levels, only DNA ploidy and the latter variable kept statistical significance with a relative risk of 2.5. Considering only local and distant recurrences (with exclusion of those identified by elevated PSA levels) the relative risk was 3.9 and 3.8, respectively. These data support the critical role of nuclear DNA analysis as predictor of outcome after surgery even in this discussed subset of patients (C-D_1).

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Introduction

Radical prostatectomy has been recognized as a proper form of therapy for diseases that are clinically localized to the primary organ [1]. Examination of serial sections of the prostate has increased the knowledge on the extension of local spread of prostate cancer. Considerable disagreement exists, however, regarding the role of the surgical technique to prevent positive surgical margins. In addition, the persistence of cancer is associated with an increased risk of subsequent metastases. This study addresses the outcome of surgery in the most controversial subset of patients where pathological examination revealed local extension and lymph node invasion by neoplastic cells (pathological C-D_1 cases).

Of 85 radical prostatectomies performed in our department, no operative mortality was reported. After surgery, the histological evaluation showed that in 28 patients the prostate tumor was confined to the gland and that pelvic lymph nodes were negative, whereas in 57 cases evidence of extension outside the prostate or lymph node involvement was found.

Considering all 85 cases treated, the overall survival after a follow-up of 11–70 months was 95.8%, whereas the disease-specific survival and the progression-free survival were 98.6 and 58.9%, respectively.

The percentage of recurrence was 41.1% of which 20% were local failure, 20% distant spread, 10% local and distant and 50% were demonstrated by an isolated elevation of serum PSA. The high percentage of prostate cancers pathologically extending outside the prostate confirms the significant error of clinical staging and justifies the need to determine the impact of therapy in these cases.

In an attempt to identify histopathological criteria that would predict the time of progression in the 57 patients whose disease was pathologically staged C-D_1, we performed a statistical analysis using the Cox method.

The age of the patient, pathological stage, Gleason score, DNA tumor ploidy as well as preoperative and postoperative serum PSA levels have been considered as independent variables in relation to survival and progression from prostate cancer.

Materials and Methods

From February 1989 to December 1994, 85 patients with histologically proven prostatic adenocarcinoma underwent bilateral lymphadenectomy and radical prostatectomy. Clinical staging was determined on the bases of physical, radiological (transrectal ultrasound, CT and isotopic bone scan) and serological examination. The evaluation of serum PSA has also been performed preoperatively and during the follow-up in all cases. The mean age was 66.0 years (range 51–79). All patients were studied at regular intervals with a follow-up ranging from 11 to 70 months. In this study, we considered only 57 patients who pathologically demonstrated an extension of disease outside the anatomical limits of the prostate or/and a pelvic lymph node involvement (C-D_1) after radical prostatectomy. In these C-D_1 prostate cancers, therapy impact was assessed by determining the first evidence of treatment failure. Treatment failure could be characterized by either a local recurrence (biopsy proven) or a positive bone scan, positive nodes and death of metastatic disease or isolated elevation of PSA values. In the last group, we considered a progressive elevation of the postoperative PSA, from 0.4 ng/ml, as indicator for recurrence. Projected survival curves to local and systemic recurrence were constructed. The progression-predictive value of different parameters has been analyzed. Parameters studied were age, pathological stage, Gleason score, DNA tumor ploidy and preoperative and postoperative serum PSA. All radical prostatectomy specimens were examined, and all surgical margins were checked. Multiple sections from the prostate and all lymph nodes removed were examined by frozen section and later by routine paraffin sections; the Gleason score was recorded in all cases. DNA tumor ploidy has been analyzed by the flow-cytometric method, as described in a previous study [2].

Statistical Evaluation

The Cox proportional hazard model has been used to analyze recurrence rates. In tables 1–4, coefficients and statistical significances (t value) obtained with the Cox model are summarized. The model, based on life tables, considers the events (recurrences) in their temporal sequence and generates survival probabilities (exemption from the event) for a fixed set of covariates (risk factors).

Results

After radical prostatectomy, of the 57 patients with pathological C-D_1 prostate cancer, 51 (89.4%) were C and 6 (10.6%) were D_1. The evaluation of the Gleason score demonstrated that in 10 (17.5%) cases the Gleason score was ≤ 5, while in 47 (82.5%) it was >5. Moreover, 37 (64.9%) prostate cancers were shown to be diploid and 20 (35.1%) aneuploid.

Figure 1 shows the Gleason score distribution in the different classes of ploidy. Nuclear DNA ploidy appears evenly distributed among the Gleason groups. Diploid cases are twice as common as aneuploid ones. Due to the limited number of cases, the Gleason sum was classified in two groups: well differentiated (G_2–G_5) and moderately to poorly differentiated (G_6–G_{10}). Of the 57 patients, 16 (28%) had positive surgical margins, while the disease was confined to the specimen in the remaining 41 (72%).

After surgery patients were followed for 11–70 months (median follow-up 35 months). All patients underwent adjuvant hormone therapy with a luteinizing-hormone-releasing hormone analogue (goserelin acetate, 3.75 mg

Table 1. Analysis of local and distant recurrences and those evidenced by elevated PSA (C_1-D_1 n = 57; recurrences n = 26)

	Coefficient	t value
Age	0.0205	0.4509
Gleason score	0.0551	0.0936
DNA ploidy	0.8251	1.8495
Presurgery PSA	0.0250	2.0999*

Relative risk (ploidy): 2.28; * $p < 0.05$. Statistical analysis using the Cox proportional hazard model conducted regarding local and distant recurrences and those evidenced by elevated PSA shows statistical significance only for presurgery PSA levels as predictors of recurrence.

Table 2. Analysis of local recurrences and distant metastases (C_1-D_1 n = 57; recurrences n = 12)

	Coefficient	t value
Age	0.1503	2.0591*
Gleason score	−0.8346	−0.8722
DNA ploidy	1.3500	2.0093*
Presurgery PSA	0.0036	0.1263

Relative risk (ploidy): 3.88; * $p < 0.05$. When the same analysis is conducted only regarding local recurrences and distant metastases, statistical significance is attained for age and DNA ploidy.

Table 3. Analysis of postsurgery PSA levels (C_1-D_1 n = 57; recurrences n = 26)

	Coefficient	t value
Age	0.0457	0.9569
Gleason score	−0.0958	−0.1610
DNA ploidy	0.9428	2.0145*
Presurgery PSA	0.0218	1.7969
Postsurgery PSA	0.0295	2.2503*

Relative risk (ploidy): 2.52; * $p < 0.05$. Postsurgery PSA levels, when introduced into the Cox model, reach statistical significance as predictors of recurrence, along with DNA ploidy.

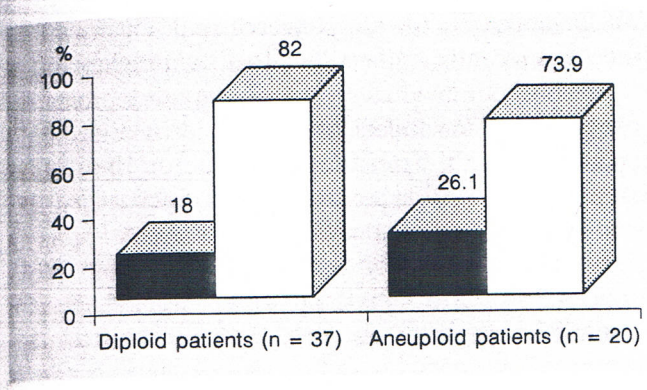
Table 4. Analysis of local recurrences and distant metastases when postsurgery PSA is included (C_1-D_1 n = 57; recurrences n = 12)

	Coefficient	t value
Age	0.1512	2.1650*
Gleason score	−0.8054	−0.8528
DNA ploidy	1.3373	1.9868*
Presurgery PSA	−0.0003	−0.0111
Postsurgery PSA	0.0256	0.7931

Relative risk (ploidy): 3.81; * $p < 0.05$. Considering only local recurrences and distant metastases, statistical significance is evidenced only for age and DNA ploidy.

monthly) and cyproterone acetate (300 mg weekly). A total of 26 (45.6%) patients experienced progression. Of the 16 cases with positive surgical margins, 8 (50%) had progression, while of the 41 with negative surgical margins 18 (43.9%) presented progression.

In cases with positive margins who experienced progression, treatment failure was characterized by local recurrence alone in 52%, distant metastases alone in 8.3%, local recurrence and distant metastases in 12.5% and elevated PSA in 27.2%. On the contrary, in cases with negative margins who experienced progression, treatment failure was characterized by local recurrence alone in 10.1%, distant metastases alone in 24.2%, local recurrence and distant metastases in 13.9% and elevated PSA in 51.8%. In the 57 C-D_1 prostate cancers, the overall survival was 94.8%; the disease-specific survival and the progression-free survival were 98.2 and 53.4%, respectively. Figures 2 and 3 show the percentage of recurrence in the different classes of Gleason score and ploidy. In order to evaluate the influence of age, Gleason score, DNA ploidy and PSA levels on recurrence rates of the disease, the Cox proportional hazard model was used. Using as covariates age, Gleason score, ploidy and preoperative PSA levels, only PSA was shown to be important and significant for recurrences ($p < 0.05$), while all the other covariates did not attain statistical significance. Considering the previous covariates for only local and distant relapses as events, PSA values (preoperative) lose significance, while ploidy and age become important predictors of the event ($p < 0.05$). In this case the relative risk conferred by aneuploid DNA content is 3.9. When we repeated the analysis and considered also postprostatectomy PSA values (at day 28) only ploidy and presurgical PSA levels were pre-



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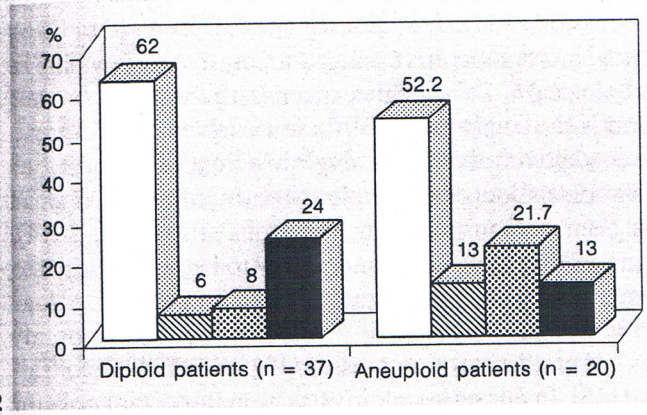

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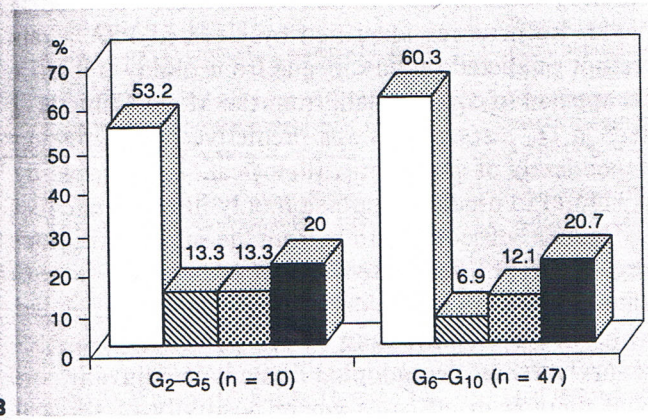

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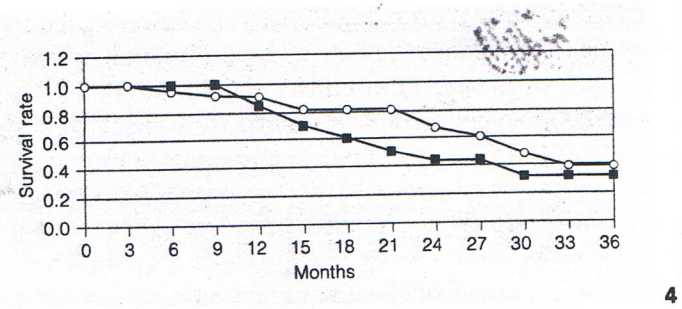

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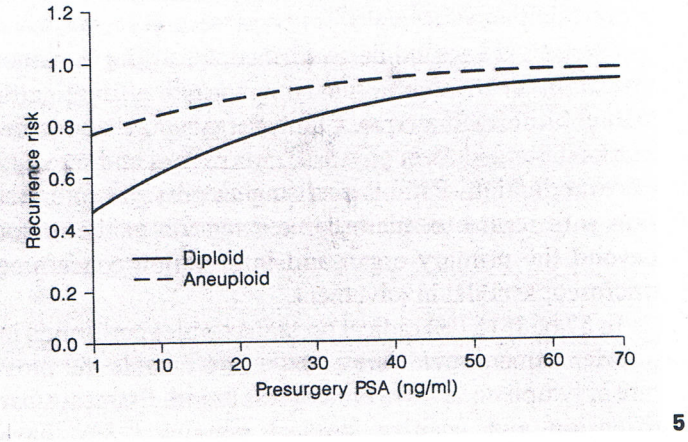

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Fig. 1. Percentage of recurrence in Gleason classes. ■ = G_2–G_5; □ = G_6–G_{10}.

Fig. 2. Percentage of recurrence in ploidy classes. □ = No recurrence; ▨ = local; ⊠ = local and/or distant; ■ = PSA elevated.

Fig. 3. Gleason sum in DNA ploidy classes. □ = No recurrence; ▨ = local; ⊠ = local and/or distant; ■ = PSA elevated.

Fig. 4. Life table survival curves in diploid (○) and aneuploid (●) patients.

Fig. 5. Solution of the Cox model. Recurrence risk related to presurgery PSA levels in diploid and aneuploid patients, with age and Gleason score kept fixed.

dictive and significant for recurrence ($p < 0.05$) with a relative risk for aneuploidy of 2.5. Postprostatectomy PSA values were used as 'internal statistical control' to analyze the accuracy of the test. Considering only local and distant recurrences, ploidy and age reach statistical significance and the relative risk is 3.8.

Projected survival curves to local and systemic recurrence in diploid and aneuploid patients are depicted in figure 4. Analyzing figure 4, a significant difference regarding survival between the diploid and aneuploid groups is evident. If we consider all recurrences (local, distant, PSA increase), the cumulative ratio of survival is 0.3581 for the diploid group and 0.2996 for the aneuploid one. Otherwise, if we consider only local and/or distant recurrences (excluding those based only on PSA increase), the cumulative ratio of survival is 0.7556 for the diploid and 0.4063 for the aneuploid group. In figure 5 we have depicted a graphical representation of the Cox model solution. The aneuploid DNA content seems to offer a 50% higher risk of recurrence compared to the diploid

DNA content. With rising PSA levels, however, the risk seems to be balanced in both groups. The analysis is conducted with age, Gleason score and pTNM exactly matched.

Conclusions

Many clinicopathological studies have shown the importance of tumor grade, volume, surgical margins and DNA tumor ploidy as independent variables in relation to progression and survival from prostate cancer. We insist that these data should be confirmed according to pathological rather than clinical staging because of the significant clinical staging error. Clinical staging uses the information obtained from physical, radiological and serological examination. Pathological staging provides information with regard to microscopic extension of the tumor beyond the primary organ and information concerning microscopic nodal involvement.

In 57 of 85 (67%) radical prostatectomies performed in our department, evidence of extension outside the prostate or lymph node involvement was found. Extracapsular extension and positive surgical margins (28%) have proved to be common features of radical prostatectomy. Therefore we attempted to identify the long-term effectiveness of radical prostatectomy combined with adjuvant therapy in pathological stage C-D_1 prostate cancer. End points such as disease-free survival and progression-free survival showed percentages of 98.2 and 53.4%, respectively. These high values of survival may depend on the relatively short follow-up (11–70 months, median 35 months).

Currently, there is little consensus among urologists on the most effective form of therapy for these pathological stages [3]. We focus on identifiable factors concerning the biology of prostate cancer that would place patients at increased risk for failure of a definitive treatment. The analysis of surgical margins, Gleason score and DNA ploidy demonstrated that these variables are interdependent. However, it is necessary to discuss the controversy regarding the classification of tetraploid tumors. This controversy is highlighted by 2 major studies from the same institution. Montgomery et al. [4] reported that, of 283 pathological stage B prostatic adenocarcinomas, 68% were diploid, 28% tetraploid and 4% aneuploid. In these studies, patients with tetraploid tumors had a favorable outcome, comparable to those with diploid tumors. In another study reported by Nativ et al. [5], tetraploid tumors were considered to be closer to aneuploid tumors. The European Organization for the Research and Treatment of Cancer has recently studied 98 patients with lymph node metastasis who immediately underwent radical prostatectomy [6]. They concluded that tetraploid tumors should be combined with diploid lesions based on the similar rates of progression. On the contrary, Carmichael et al. [7] reported that, in patients with aneuploid tumor, progression was not significantly higher than that of tetraploid lesions. According to Nativ et al. [5] and Carmichael et al. [7] we did not separate tetraploid from aneuploid cancer either.

We used the Cox analysis to verify the survival and progression-predictive values of age, Gleason score, DNA ploidy and preoperative and postoperative serum PSA in pathological C-D_1 prostate cancer, with the aim to understand if the combination of these variables may improve the predictive value of the single histological factors.

A correlation of DNA ploidy with pathological stage has been demonstrated in numerous studies [5, 8–14]. Some studies have not found ploidy to be proportional to stage [15, 16] and to give information on predicting stage beyond Gleason score [5], while in other studies ploidy was not predictive of progression if grade was also considered [13]. In our series, ploidy has been important only for predicting local and/or distant recurrences ($p < 0.05$; table 2). Recurrences identified by elevated PSA levels were not predicted by any means from ploidy (table 1). This appears to confirm data from the Mayo Clinic [17] where in D_1 cases ploidy was predictive of progression independently of grade. In pathological C stages, ploidy was helpful to predict progression only in low-grade disease [5]. In a subsequent study from the same institution, Morgan et al. [18] submitted 54% of pathological stage C patients to an early hormonal deprivation. They noted that hormone therapy significantly influences disesase-free survival. Since we adopted immediate adjuvant hormonal therapy in all cases where positivity of surgical margins or extracapsular extension was noted, progression rates may have been influenced by the inability of the Gleason score to predict the likelihood of recurrence. Moreover, our data are based on pathological C-D_1 cases where progression has been demonstrated not to be greatly influenced by the Gleason score [19]. Although we are aware of the risks of grouping a heterogeneous subset of patients such as this, where patients with nodal metastases may have a well-confined local disease, most series do not show major differences in survival rates when these groups are taken separately. Therefore it seems reasonable to treat data concerning these patients uniformly. Ring et al. [16] found no correlation of ploidy with pro-

gression in 54 patients using elevated PSA levels as first sign of recurrence. In a multivariate analysis comparing grade, ploidy, capsular penetration and surgical margins, the Gleason sum was the best predictor of progression ($p < 0.0001$). DNA ploidy was able to predict recurrence only in a particular subset of patients with well to moderately differentiated tumors who failed ($p < 0.03$). In this study the state of surgical margins did not significantly affect recurrences. As the authors admit, the follow-up was short and the impact of surgical margins on disease-free survival was difficult to assess. The preoperative serum PSA concentration has been demonstrated to be directly proportional to the pathological stage, but it has been found to be unreliable in predicting the final pathological stage. Kleer et al. [20] were able, in a multivariate logistic regression analysis combining local clinical stage, tumor grade and ploidy, to greatly enhance the predictive power of PSA. We adopted a similar approach to assess the role of preoperative and postoperative serum PSA levels. In our series, high preoperative PSA levels seemed to be correlated with the probability of local or distant recurrence ($p < 0.05$; tables 1, 2), while it had no influence on the rise of PSA levels after surgery (tables 3, 4). This held true even for $C1–C_2$ cases ($n = 51$, t value = 1.9958) which, anyway, showed the same behavior as the $C\text{-}D_1$ group. The decision of submitting a patient with locally advanced prostatic cancer to radical surgery is a difficult one. Moreover, most times it has to be made intraoperatively, when unexpected local extension or lymph node involvement is discovered. The need of new and more powerful means of predicting the outcome of surgery is great. Nuclear DNA content may be one of the parameters to consider when dealing with possible clinical understaging. Our experience based on pathological findings seems to suggest that routine use of ploidy may provide critical information beyond the Gleason sum.

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