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PROGNOSTIC VALUE OF DNA PLOIDY AND NUCLEAR MORPHOMETRY IN PROSTATE CANCER TREATED WITH ANDROGEN DEPRIVATION

JOSÉ M. MARTÍNEZ-JABALOYAS, JOSÉ L. RUIZ-CERDÁ, MIGUEL HERNÁNDEZ, ANA JIMÉNEZ, AND FERNANDO JIMÉNEZ-CRUZ

ABSTRACT

Objectives. To assess the prognostic value of flow cytometry and nuclear morphometry in prostate cancer after androgen deprivation treatment.

Methods. A total of 127 patients with a prostate cancer diagnosis who had undergone androgen suppression were retrospectively studied. The DNA content by flow cytometry and nuclear morphometry was studied from biopsy specimens. In the patients with Stage M0, two multivariate analyses by the Cox proportional regression model were performed to determine whether the experimental variables (DNA content and nuclear area) added independent information to the classic prognostic factors (Gleason score and stage). Using the statistical analysis results, risk groups were created.

Results. T and M categories, Gleason score, DNA ploidy, and mean nuclear area proved to have prognostic value in the univariate analysis. For the group of patients free of metastasis (M0), it was possible to create low, intermediate, and high-risk groups using stage and Gleason score with statistically significant differences in survival. Multivariate analysis, combining the classic and experimental variables, selected Gleason score and DNA content as prognostic independent factors. Also, risk groups with statistically significant differences in survival were created. However, the net result of combining both kinds of factors was at least as valuable as the combination of stage and Gleason score in predicting survival.

Conclusions. The determination of DNA ploidy and mean nuclear area do not add enough independent information to improve the predictive value to justify their use in this group of patients treated with hormonal therapy. UROLOGY **59:** 715–720, 2002. © 2002, Elsevier Science Inc.

The usefulness of DNA content determined by flow cytometry and nuclear morphometry to predict the behavior of a variety of neoplasias has been reported.[1,2] In prostate cancer, DNA ploidy adds clinically useful prognosis information after radical prostatectomy, antiandrogen therapy, and radiation therapy.[3,4] With regard to nuclear morphometry, although the first studies reported appeared very promising[5] in predicting prostate cancer behavior, its usefulness has not been well defined owing to contradictory results.[6–9]

The aim of our study was to evaluate the ability of DNA ploidy content and nuclear morphometry to predict mortality in patients with prostate cancer who had undergone androgen deprivation.

MATERIAL AND METHODS

Patient Characteristics

A total of 127 patients with prostate adenocarcinoma diagnosed in our hospital from 1975 to 1988 were selected. All patients had received androgen deprivation, had more than 10 years of follow-up, and had undergone pretreatment staging. Stage information was obtained from digital rectal examination, transrectal and abdominal ultrasonography, chest radiography, abdominopelvic computed tomography, and bone scanning. The TNM classification of 1997[10] was used. No discrimination was made between patients with Stage N0 and N1.

Pathologic Examination

Needle biopsy specimens were used to for the pathologic examination. Histologic sections from tumor tissue specimens of each case were taken from the pathology files and those with higher tumor content and less differentiation were selected. Paraffin-embedded blocks were chosen, and five sections were performed on each block: one of 5 μm, 3 of 50 μm, and finally

From the Departments of Urology and Pathology, Hospital Universitario La Fe, Valencia, Spain

Reprint requests: José M. Martínez-Jabaloyas, M.D., Department of Urology, Hospital Clínico Universitario, Avda de Blasco Ibáñez, 17, Valencia 46010, Spain

Submitted: July 24, 2001, accepted (with revisions): January 2, 2002

0090-4295/02/$22.00
PII S0090-4295(02)01530-3

TABLE I. *Distribution of patients by age, stage, Gleason score, and DNA ploidy: results of univariate analysis of survival*

Variable	Patients (%)	Median Survival (mo)	Relative Risk (Cox Test)	*P* Value (Log-Rank Test)
Age (yr)				
≤65	30 (23.6)	55	1.00	0.37
66–75	63 (49.6)	51	1.35	
>75	34 (26.8)	43	1.57	
T category				
T1–2	28 (22.0)	105	1.00	<0.001
T3	59 (46.5)	53	2.51	
T4	40 (31.5)	23	4.81	
Metastasis				
M0	76 (59.8)	78	1.00	<0.001
M1	51 (41.2)	28	2.57	
Gleason score				
2–6	48 (37.8)	80	1.00	<0.001
7	43 (33.9)	53	1.62	
8–10	36 (28.3)	21	5.00	
DNA ploidy				
Diploid	75 (65.2)	78	1.00	<0.001
Aneuploid	40 (34.8)	28	3.07	

one of 5 μm. The 5-μm sections were stained with hematoxylin-eosin, and the presence of tumor cells was assessed. In the first section, the Gleason score was determined. For statistical purposes, tumors were classified as Gleason score 2 to 6, 7, and 8 to 10.

DNA Analysis

The 50-μm sections were used for the DNA ploidy study. Nuclear suspensions from paraffinized samples were made following the technique described by Hedley.[11] The cells from deparaffinized samples were obtained using a previously described technique.[2] The nuclear sediment obtained was processed with the commercial kit DNA-Prep (Coulter Reagents Kit) consisting of two preparations: DNA-Prep LPR and DNA Prep Stain (propidium iodide). The nuclear suspension was analyzed using Cytoronabsolute Flow Cytometry (Ortho Diagnostic Systems). Only histograms with a variation coefficient lower than 10 were accepted. Tumors were considered diploid, aneuploid hyperdiploid, and tetraploid when the DNA index was equal to 1, 1.05 to 1.89, and 1.9 to 2.1, respectively.[2]

Nuclear Morphometry

The cytomorphometry study was performed using 5-μm sections stained with hematoxylin-eosin. Measurements were made using a 100× objective with oil immersion in an Nikon optical microscope, providing 1400× magnification. The microscope was connected to a video camera that was also connected to a computer. Measurements were performed in an interactive manner with a planimeter composed of a digital panel (Synoptics) connected to a personal computer programmed with the INSIGHT-PC software model. Ninety nuclei were measured per biopsy. Only well-defined nuclei were studied. Morphometric descriptors were calculated automatically. The system provides 16 nuclear morphology descriptors, and up to 17 statistical measurements can be described for each of them, accounting for 272 morphometric tests per nucleus.[12] However, they are highly correlated and to avoid redundant information, we selected the following descriptors: nuclear area, form factor, orientation, X gravity center, and Y gravity center. Nuclei were selected in a random manner. The number of nuclei to be studied was calculated by the running mean procedure. A variation coefficient of less than 5% between and among investigators demonstrated the accuracy and reproducibility of the measurements. A total of 70 nuclei were enough to keep the mean invariable; however, to ensure reproducibility, 20 more nuclei were counted.

Statistical Analysis

For univariate analyses, the survival of the different groups of patients was evaluated using the Kaplan-Meier method, calculating the relative risk in each group. The log-rank test was used to compare two or more groups. The Cox proportional hazards model was used to test the effect of the explanatory variables on the time of survival. A 5% level of significance was used for all statistical testing.

RESULTS

The mean age of the sample was 70 ± 7 years. The survival median of the global sample was 51 months, and the survival likelihood at 5 and 10 years was 47% and 28%, respectively. Table I shows the distribution of the cases according to age, stage, and histologic findings (Gleason score). In the univariate analysis, the T category, presence of metastasis, and histologic grade were prognostic factors.

In flow cytometry, 12 samples were not valid for the study (4 had a variation coefficient greater than 10%, and in 8, the biopsy material was deteriorated). Of the valid results, 75 tumors were diploid (65%), 32 hyperdiploid aneuploid (28%), and 8 tetraploid (7%). Data were analyzed by relating the tetraploidies with the aneuploidies. Survival was significantly lower in patients with aneuploid tu-

TABLE II. *Multivariate analysis: regression model obtained using the parameters age, T category, presence of metastasis, histologic grade (Gleason), DNA ploidy, and nuclear area*

Variable	B Coefficient	Relative Risk (95% CI)	*P* Value
Nuclear area	0.65	1.93 (1.09–3.41)	0.024
DNA ploidy	0.83	2.31 (1.32–4.03)	0.031
Presence of metastasis	0.81	2.25 (1.33–3.79)	0.002
Gleason score			<0.001
7	0.13	1.14 (0.61–2.11)	0.672
8–10	1.13	3.11 (1.63–5.94)	0.006

KEY: *CI = confidence interval.*

TABLE III. *Cox regression proportional model analysis, for M0 patients, including as explanatory variables classic (age, T stage, and Gleason score) and a combination of classic and experimental prognostic factors (DNA content and nuclear area)*

Variable	B Coefficient	Relative Risk (95% CI)	*P* Value
Classic prognostic factors			
T stage			0.052
T3	0.77	2.16 (0.97–4.79)	0.058
T4	1.13	3.10 (1.21–7.90)	0.017
Gleason score			<0.001
7	0.44	1.55 (0.73–3.30)	0.253
8–10	1.58	4.85 (2.16–10.87)	<0.001
Combination of classic and experimental prognostic factors			
DNA aneuploid	0.91	2.49 (1.26–4.91)	0.008
Gleason score			0.002
7	0.45	1.56 (0.71–3.42)	0.264
8–10	1.56	4.79 (1.98–11.57)	<0.001

KEY: *CI = confidence interval.*

mors than in those with diploid tumors, showing a survival median of 28 and 78 months, respectively (Table I).

The correlation analysis of the different morphometric descriptors showed that most of them contained redundant information and almost all the information obtained from the morphometric study was contained in the five descriptors: mean nuclear area, form factor, cellular orientation, and X and Y gravity centers. For example, the mean nuclear volume correlated closely to area ($r = 0.93$, $P < 0.01$). Thus, only these descriptors were used to analyze the results. The mean nuclear area and the mean nuclear form factor for all patients was $40.5 \pm 12.8\ \mu m^2$ and $87\% \pm 3\%$, respectively. In the study of univariate survival for the different morphometric parameters categorized, only the mean nuclear area was relevant, and thus significant differences were found only in those patients whose tumors had a mean nuclear area of 45 μm^2 or larger. In these patients, the median survival was 23 months; it was 77 months in the group of patients whose mean nuclear area was smaller. The remaining parameters had no prognostic value in the survival analysis.

In Table II, the variables selected by the analysis that supply independent prognostic information with respect to survival time are shown. As can be seen, patients with poorly differentiated tumors (Gleason score 8 to 10), with metastasis at the time of diagnosis, with aneuploid tumors, or with a mean nuclear area of 45 μm^2 or greater had a death risk multiplied by 3.1, 2.2, 2.3, and 1.93, respectively.

For the group of patients free of metastasis (M0), two multivariate analysis by the Cox proportional regression model were performed (Table III). First, a model was constructed in which as explanatory variables were included the classic prognostic factors readily available at diagnosis (age, stage, and Gleason score). Second, to determine whether the experimental variables (DNA content and nuclear

TABLE IV. ***Risk groups created based on Cox regression proportional model, for M0 patients, including as explanatory variables classic (age, T stage, and Gleason score) and a combination of classic with experimental prognostic factors (DNA content and nuclear area)***

Risk Group	Variable	Patients (n)	Median Survival (mo)	95% CI
Classic prognostic factors				
Low	T1–2 and G2–6, G7	24	Not reached	
Intermediate	T3, T4 and G2–6, G7	36	78	49–106
High	T1–2, T3, T4 and G8–10	16	29	23–36
Combination of classic and experimental prognostic factors				
Low	G2–6 and Diploid	28	105	52–159
Intermediate	G7 and Diploid G2–6, G7 and Aneuploid	28	60	17–103
High	G8–10 and Diploid G8–10 and Aneuploid	14	29	23–35

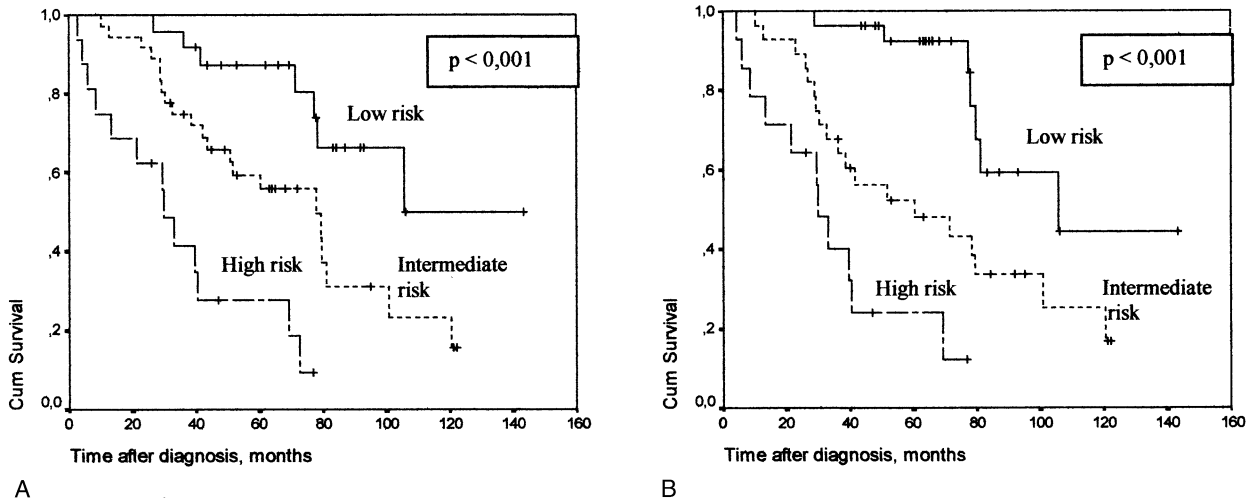

FIGURE 1. *(A) Risk groups created according to results of Cox regression proportional model, including as explanatory variables stage and Gleason score for patients without metastases. Survival plotted as a function of time after the diagnosis for each of three groups: low risk (pT1-2 and Gleason score 2 to 7), intermediate risk (pT3-4 and Gleason score 2 to 7), and high risk (pTany and Gleason score 8 or greater). Statistically significant differences between specific curves are indicated. (B) Risk groups created according to results of Cox regression proportional model, including as explanatory variables Gleason score and DNA content for patients without metastases. Survival plotted as function of time after diagnosis for each of three groups: low risk (Gleason 6 or less and diploid content), intermediate risk (Gleason score 7 or less, and diploid or aneuploid content), and high risk (Gleason score 8 or greater and diploid or aneuploid content). Statistically significant differences between specific curves are indicated.*

area) added prognostic information independent of the classic factors, a model was constructed that included all variables together.

According to the results of the first analysis, three distinct risk groups were created (Table IV). The net result of combining stage and Gleason score was the generation of low, intermediate, and high-risk groups that were all statistically different (Fig. 1A). Also, the results of the second analysis using both classic and experimental variables demonstrated that the created risk group based on Gleason score and DNA content were statistically different (Fig. 1B). However, when comparing Figure 1A and B, one can conclude that the combination of risk factors based on DNA and Gleason score is at least as valuable as the combination of stage and Gleason score in predicting which patient will die.

COMMENT

In the present study, the patients selected were diagnosed before the prostate-specific antigen era. Therefore, local clinical stages were advanced, and

nearly 40% with presented bone metastasis. Our results confirm that the histologic grade is a good predictor of tumor aggressiveness. Nevertheless, tumors of the same grade and stage may behave in a different manner, and, furthermore, other factors such as subjectivity in its identification, variability between and among observers, and tumor heterogeneity must also be taken into account.

The prognostic value of DNA ploidy was determined by means of univariate and multivariate survival analyses. Both analyses revealed that DNA content provided prognostic information about tumor behavior in patients who had undergone palliative therapies, so that those with aneuploid tumors had poorer survival than did those with diploids. Not all studies reported have evaluated the influence of ploidy on survival, but most of the ones that have done this type of analysis report the negative effect of aneuploid population on prognosis.[13]

In general terms and consistent with our results, diploid tumors showed a good and prolonged clinical response to androgen blockade. Tribukait,[14] by means of flow cytometry, reported that ploidy influenced the prognosis of those patients who underwent endocrine therapy, and significant differences were found between diploid, aneuploid, and tetraploid tumors. In the study of local T2 and T3 tumors, Forsslund *et al.*,[15] by static image cytometry, found that aneuploid tumors progress more rapidly, with a mortality rate of 96% at 5 years. Miller *et al.*,[16] in a series of patients with Stage D2 tumors who underwent androgen blockade, reported that DNA content shows a predictive value in terms of survival. Borre *et al.*[17] described the influence of aneuploidy on survival for tumors clinically localized who were followed up, although in the multivariate analysis only the histologic grade was significant and ploidy only had influence in low-grade tumors. The ploidy also had influence on the response to adjuvant androgen blockade after radical prostatectomy in patients with Stage D1 disease.[18]

The prognostic improvement of DNA ploidy could be achieved by image cytometry using Feulgen stain. Also, with this method, it is possible to measure nuclear descriptors at the same time.[19]

Although morphometric analysis changes a subjective and qualitative assessment such as nuclear grade into an objective and quantitative assessment, the prognostic value of nuclear descriptors is still controversial. Among the nuclear descriptors proposed to have prognostic value are ellipticity quartile analysis,[20] variation coefficient of the area,[21] mean nuclear area,[9,21] nuclear roundness variance,[12] or mean nuclear volume.[17,22,23] In our study, mean nuclear area was the only descriptor with prognostic value providing independent predictive information about survival. However, in patients with Stage M0, nuclear area failed as an independent prognostic factor. Some investigators[7,12] have found that the use of nuclear descriptors increases the prognostic value of the histologic grade. Others[8,9] have reported that morphometric descriptors strongly correlate with histologic grade and lose their independent information when analyzed multivariately.

Attempts have been made to improve the predictive value of Gleason score and stage. Veltri *et al.*[24] developed a method based on nuclear shape, size, and DNA content descriptors and markovian chromatin texture features analyzed by logistic regression analysis to predict treatment outcome. Also, the same researchers demonstrated that using artificial neural networks made it possible to improve predictive value.[25] Chiusa *et al.*[26] reported that nuclear and nucleolar area improve the predictive value of histologic grade in those patients treated with hormonal deprivation.

CONCLUSIONS

The results of the statistical analysis revealed that the combination of risk factor based on DNA content and Gleason score is at least as valuable as the combination of stage and Gleason score in predicting survival. However, the determination of DNA ploidy and mean nuclear area did not add enough independent information to improve the predictive value to justify their use.

REFERENCES

1. Bauer KD, Duque RE, and Shankey TV: *Clinical Flow Cytometry: Principles and Applications.* Baltimore, Williams & Wilkins, 1993, pp 1–8.

2. Ruiz Cerda JL, Hernandez M, Gomis F, *et al:* Value of deoxyribonucleic acid ploidy and nuclear morphometry for prediction of disease progression in renal cell carcinoma. J Urol **155:** 459–465, 1996.

3. Shankey TV, Kallioniemi OP, Koslowski JM, *et al:* Consensus review of the clinical utility of DNA content cytometry in prostate cancer. Cytometry **14:** 497–500, 1993.

4. Lieber MM: Practical clinical utility of DNA ploidy for managing patients with prostate carcinoma. Urology **45:** 558–562, 1995.

5. Diamond DA, Berry SJ, Jewett HJ, *et al:* A new method to assess metastatic potential of human prostate cancer: relative nuclear roundness. J Urol **128:** 729–734, 1982.

6. Paulson DF, Stone AR, Walther PJ, *et al:* Radical prostatectomy: anatomical predictors of success or failure. J Urol **136:** 1041–1043, 1986.

7. Veltri RW, Partin AW, Epstein JE, *et al:* Quantitative nuclear morphometry, markovian texture descriptors, and DNA content captured on a CAS-200 image analysis system, combined with PCNA and HER-2/neu immunohistochemistry for prediction of prostate cancer progression. J Cell Biochem Suppl **19:** 249–258, 1994.

8. Vesalainen S, Lipponen P, Talja M, *et al:* Nuclear morphometry is of independent prognostic value only in T1 prostatic adenocarcinomas. Prostate **27:** 110–117, 1995.

9. Chiusa L, Galliano D, Formiconi A, *et al:* High and low

risk prostate carcinoma determined by histologic grade and proliferative activity. Cancer **79:** 1956–1963, 1997.

10. Sobin LH, and Wittekind C (Eds): *UICC: TNM Classification of Malignant Tumours,* 5th ed. New York, Wiley-Liss, 1997. pp 272–280.

11. Hedley WD, Friedlander ML, Taylor IW, *et al:* Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem **31:** 1333–1335, 1983.

12. Partin AW, Steinberg GD, Pitcock RV, *et al:* Use of nuclear morphometry, Gleason histologic scoring, clinical stage and age to predict disease-free survival among patients with prostate cancer. Cancer **70:** 161–168, 1992.

13. Adolfsson J: Prognostic value of deoxyribonucleic acid content in prostate cancer: a review of current results. Int J Cancer **58:** 211–216, 1994.

14. Tribukait B: Nuclear deoxyribonucleic acid determination in patients with prostate carcinomas: clinical research and application. Eur Urol **23**(suppl 2): 64–76, 1993.

15. Forsslund G, Esposti P, Nilsson B, *et al:* The prognostic significance of nuclear DNA content in prostatic carcinoma. Cancer **69:** 1432–1439, 1992.

16. Miller J, Horsfall DJ, Marshall VR, *et al:* The prognostic value of deoxyribonucleic acid flow cytometric analysis in stage D2 prostatic carcinoma. J Urol **145:** 1192–1196, 1991.

17. Borre M, Hoyer M, Nerstrom B, *et al:* DNA ploidy and survival of patients with clinically localized prostate cancer treated without intent to cure. Prostate **36:** 244–249, 1998.

18. Zincke H, Bergstralh EJ, Larson-Keller JJ, *et al:* Stage D1 prostate cancer treated by radical prostatectomy and adjuvant hormonal treatment: evidence for favorable survival in patients with DNA diploid tumors. Cancer **70**(1 suppl): 311–323, 1992.

19. Pindur A, Chakraborty S, Welch DG, *et al:* DNA ploidy measurements in prostate cancer: differences between image analysis and flow cytometry and clinical implications. Prostate **25:** 189–198, 1994.

20. Partin AW, Walsh AC, Pitcock RV, *et al:* A comparison of nuclear morphometry and Gleason grade as a predictor of prognosis in stage A2 prostate cancer: a critical analysis. J Urol **142:** 1254–1258, 1989.

21. Blom JHM, Ten Kate FJW, Schroeder FH, *et al:* Morphometrically estimated variation in nuclear size: a useful tool in grading prostatic cancer. Urol Res **18:** 93–99, 1990.

22. Arima K, Sugimura Y, Hioki T, *et al:* Stereologically estimated mean nuclear volume of prostatic cancer is a reliable prognostic parameter. Br J Cancer **76:** 234–237, 1997.

23. Zhang YH, Kanamaru H, Oyama N, *et al:* Prognostic value of nuclear morphometry on needle biopsy from patients with prostate cancer: is volume-weighted mean nuclear volume superior to other morphometric parameters? Urology **55:** 377–381, 2000.

24. Veltri RW, Miller MC, Partin AW, *et al:* Ability to predict biochemical progression using Gleason score and a computer-generated quantitative nuclear grade derived from cancer cell nuclei. Urology **48:** 685–691, 1996.

25. Potter SR, Miller MC, Mangold LA, *et al:* Genetically engineered neural networks for predicting prostate cancer progression after radical prostatectomy. Urology **54:** 791–795, 1999.

26. Chiusa L, Galliano D, Formiconi A, *et al:* High and low risk prostate carcinoma determined by histologic grade and proliferative activity. Cancer **79:** 1956–1963, 1997.