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Morphometrically estimated variation in nuclear size

A useful tool in grading prostatic cancer

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Summary. At present there are several grading systems for prostatic carcinoma. Most are difficult to reproduce. An objective method of grading seems to be necessary and could make comparisons between various groups of patients easier and grading more reliable.

In the present study morphometrically estimated nuclear size and variation in nuclear size are matched with the survival rates of 207 patients who underwent total perineal prostatectomy for cancer. On the basis of morphometrically estimated variation in nuclear size the patients could be divided into two groups with significantly differing survival rates. In this way it was possible to split the group of patients with grade 2 carcinoma (Mostofi's grading system) into two groups of patients with significantly different survival rates. The survival rates in these two groups did not differ significantly from those in the patients with Grade 1 and Grade 3 tumors respectively.

The results are discussed in the light of the recent literature on the subject. Morphometry seems to be a valuable tool in grading prostatic cancer.

Key words: Prostate cancer – Cell morphometry – Patient survival

Since Broders' first report on grading epitheliomas of the lip in the early 1920's [5] many investigators have tried to correlate the histological picture of prostatic carcinomas with the clinical course of the disease [9, 13]. This has resulted in the introduction of many grading systems for prostate cancer, but only few of them found wide acceptance. Several reasons can be indicated for this phenomenon:

First prostatic carcinoma often presents in various histological patterns and several of such patterns can be found in the same tumor and even in the same slide. The different patterns can vary considerably in appearance, ranging from well differentiated parts, almost resembling normal prostatic glandular tissue, to undifferentiated parts in which absolutely no features of the original prostatic tissue are recognizable. Within these different patterns or "tumor formations" cytological characteristics may vary in the same way from regularly arranged cuboidal cells without any nuclear pleomorphism to disorderly arranged cells with nuclei that show considerable variation in size, shape and staining. Furthermore it is not uncommon that in rather well-differentiated parts of a tumor cytological characteristics show marked abnormalities, suggesting a very malignant tumor, while on the other hand hardly any nuclear pleomorphism may be found in tumors with a solid pattern of growth. It is difficult to take account of all these variable features in one grading system.

A second reason for the poor acceptance of grading systems is their poor reproducibility. Most grading systems produce the best results in the hands of the person who developed the system, while in other hands the reproducibility is rather disappointing [10, 12, 14].

A third reason is the subjectivity in interpreting the results of the various grading systems. Generally there is no problem in identifying the low grade and high grade tumors, whatever system is used. The problem lies in the large group of patients that neither have clear high grade nor evident low grade tumors and are by exclusion placed in the poorly defined intermediate group of patients whose prognosis apparently is not clearly defined. This is the truly problematic group.

In 1975 Mostofi [13] proposed a grading system that seemed to be quite easy to apply. In the first place he clearly defined differentiation as the tendency of a tumor to form glands and the characteristics of these glands as compared to normal prostatic glands. Anaplasia was defined as a scaled assessment of nuclear characteristics such as nuclear size, hyperchromatism, pleomorphism, presence of nucleoli and mitoses. This system seemed to solve the problem of classifying tumors that on one hand may grow in solid sheets with no gland formation and with a slight cellular atypia as opposed to the cytologically more anaplastic tumors forming well developed glands.

Table 1. Number of tumor formations

1 formation	113 patients	113 formations
2 formations	152 patients	304 formations
3 formations	73 patients	219 formations
4 formations	8 patients	32 formations
Total	346 patients	668 formations

Table 2. Tumor stage in 207 patients

Stage	No. patients	%
A	28	13.5
B	112	54.1
C	64	30.9
unknown	3	1.5

In an extensive study Schroeder and co-workers [16] evaluated the prognostic weight of each of the parameters in Mostofi's grading system and they came to the conclusion that only glandular differentiation, nuclear pleomorphism and amount of tumor seen in the slide were important parameters in relation to the prognosis of the disease. The presence of mitoses also showed importance, but the vast majority of prostatic carcinomas contain no or very few mitoses. Schroeder and co-workers proposed a simplification of Mostofi's grading system, showing its application in a large series of 346 cases of prostatic carcinoma, all graded by Mostofi [17].

In 1979, when the present study started, the question came up whether the parameter variation in nuclear size and shape (nuclear pleomorphism) could be objectivated in some way. It has been shown for other tumors and benign tissues [15] that with morphometry, using a planimeter in combination with a computerized evaluation of the measurements, structures can be quantified for several parameters such as surface area, circumference (perimeter), relative volumes, shape descriptions etc. It was hoped that with such an image analysing system it could be possible to have an objective tool in grading carcinoma and to diminish the subjectivity and variability resulting from the use of the conventional grading systems. The initial results were reported in 1982 [3] and 1983 [2, 4].

Independently from our work a similar project was carried out at the Brady Urological Institute in Baltimore. It was shown that the so-called "nuclear roundness factor" correlated very well with prognosis [6, 7, 8].

In the present study nuclear variation in size and shape has been estimated in 207 cases of prostatic carcinoma with a computerized semi-automatic image analysing system. The results have been correlated with survival and Mostofi's grading system.

Material and methods

Patients

In a series of 484 patients on whom the late Dr. Elmer Belt performed a total perineal prostatectomy for cancer the patient charts were reviewed retrospectively. In 346 cases histological slides from the prostatectomy specimens were available for review. These tumors were all regraded by Dr. Mostofi without knowledge of the follow-up of the patients. Most of the tumors consisted of a varying number of morphologically different formations (e.g. tubular, cribriform, solid). As Table 1 shows their number varied from one to four per patient and a total of 668 tumor formations have been matched with the clinical data of 346 patients. This has been reported elsewhere [16].

For various reasons not all of the 346 sets of histological slides were suitable for morphometry. Fourty-six patients received hormonal treatment before total prostatectomy, causing squamous metaplasia to a greater or lesser extent. In most cases the presence of metaplasia was no problem for conventional grading, but these patients were excluded from morphometry. The quality of the histological slides of 20 patients was too poor for morphometrical purposes. The slides of 10 patients had been lost during the last years. In five slides there was a significant squamous metaplasia, suggesting the use of hormones, although there was no note in the patient chart on the use of hormonal treatment. Fifty-eight slides could not be analyzed for various reasons, for instance because the amount of tumor in the slides was too small to obtain enough nuclei to process or the contours of the nuclei were too vague for accurate tracing, or the slides that were at our disposal did not contain tumor at all. This resulted in 207 cases that were available and suitable for morphometry. The number of slides per patient varied from 1 to 24 with an average of 3 slides per case. The slides were almost all from the same institution (Good Samaritan Hospital, Los Angeles, Ca). A few slides came from another hospital (Hollywood Presbyterian Hospital, Hollywood, Ca).

The clinical stages of carcinoma of these 207 patients are given in Table 2.

Patient identification on the slides was only by number and morphometry was therefore carried out in a blind fashion.

Morphometry

The morphometrical analysis was performed with a semi-automatic computerized image analysing system (Videoplan, Kontron). Basically this system consists of three components:

1. a graphic tablet
2. a cursor or a pen, and
3. a desk computer

Both the graphic tablet and the cursor or pen are connected to the computer. Besides these, a printer/plotter is connected to the computer.

The graphic tablet and cursor

The Videoplan graphic tablet (or digitizer tablet) operates on the magnetostrictive principle. The area of the tablet is divided in a horizontal and vertical way by a mesh of ferromagnetic wires, laid on a substrate beneath the tablet surface, spaced at regular intervals in X and Y direction. This mesh of wires provides a permanent magnetic field. In addition the wires conduct electronically induced magnetic pulses in both directions. These pulses are emitted at a constant frequency and travel at a constant speed, unaffected by environmental conditions. The cursor has two crosswires, indicating the exact point on the tablet. In the centre of these crosswires a light-emitting diode (LED) is mounted, to make the centre of the

Table 3. Effect of magnification on accuracy of digitizing (each nucleus is digitized at least 50×)

Magnification	mean area (μ^2)	standard deviation	coefficient of variation
400×	76.65	4.55	5.94
	25.32	2.69	10.62
	59.89	4.15	6.93
	10.31	0.49	4.75
	22.45	2.12	9.44
	5.73	0.25	4.36
630×	78.58	3.13	3.98
	23.84	1.36	5.70
	5.93	0.19	3.20
	10.10	0.34	3.37
	56.18	3.19	5.68
	78.76	3.30	4.19

Table 4. Lymphocyte measurements during several years (the maximum deviation from the mean is 5%)

Year	mean nuclear area	S.D.
1939	15.79	2.78
1944	17.23	2.87
1945	17.41	3.47
1955	17.09	3.62
1965	15.47	2.43
1969	16.75	2.60
1970	16.30	3.24
Mean	16.58	

Table 5. Effect of number of nuclei on accuracy

Number of nuclei	Mean nuclear area
25	38.00
50	32.33
75	34.22
100	33.12
125	32.25
150	31.85
200	31.96
275	32.06
400	32.52

crosswires visible in the microscope. When positioned or moved on the surface of the tablet, the cursor intercepts X and Y pulses continuously through a receiver coil to derive coordinate locations. Based on the parameters selected, the microprocessor continuously calculates and updates the individual measurements until terminated. When a line is drawn, for example around a nucleus, the computer can calculate the surface area, the circumference (perimeter) and several other parameters. The resolution of the system is 0.1 mm. However, as the average diameter of a nucleus, projected on the graphic tablet is about 15 mm, this resolution constitutes less than 1% of the total diameter.

The microscope

The microscope is a regular Zeiss microscope. It has 10× wide field eyepieces and plan achromat objectives (magnification: 4×, 10×, 40× and 63×). On the microscope a drawing attachment is mounted (Zeiss 474620), so that the LED in the centre of the cursor can be seen together with the normal field of vision of the microscope. The microscope was arranged in such a way that when the cursor was placed in the centre of the graphic tablet, its LED was seen in the centre of the field of vision of the microscope. In order to see the LED clearly, the light of the microscope had to be adjusted to a convenient level. Also the room illumination had to be dimmed to a lower level.

Accuracy and reproducibility

Before starting the actual morphometric measurements the accuracy and reproducibility of the technique was studied:

1. What is the best magnification of the microscope?
2. Could there be artefacts due to different handling of the material in different laboratories?
3. How many nuclei should be digitized per tumor formation?
4. Should one measure nuclei in all available slides or is limitation to one slide per patient possible?
5. Is one field of vision representative for a given tumor-formation or should one go randomly through the slides?
6. How accurate is the mechanism of tracing nuclei?

1: To establish the best suitable magnification of the microscope we digitized several nuclei of one tumor repeatedly using several magnifications. The results are shown in Table 3.

The largest possible magnification was optimal. Although a higher magnification would probably give better results, the highest power dry system was used for practical reasons. The total magnification of the microscope was $63 \times 10 = 630\times$.

To examine the accuracy of measuring with this magnification a circle in an eyepiece grid was traced several times and a coefficient of variation in surface area of 3.94% was found. This is within acceptable limits.

2: It is a well known fact that fixation and laboratory handling of tissue causes shrinkage of all structures to a certain amount. This is true for fresh and old material. To investigate the effect of tissue handling in the two different laboratories during several years, we digitized lymphocytes in the slides of several patients from each laboratory and representing several years. Slide preparation at various points in time was checked because it is unknown to us whether material handling is still the same now as it was in 1939. Table 4 shows that there is in fact no significant difference between the effect of fixation and tissue handling for the years from 1939 through 1970.

3: To establish the number of nuclei necessary in each tumor formation up to 400 nuclei were digitized in one tumor formation. As shown in table 5, the values for the mean nuclear area did not change significantly above a number of 125 nuclei. On the basis of this result it was decided to use for the routine of this study 150 nuclei per tumor formation.

4 and 5: Regarding the number of slides and the areas in the slides to be digitized an analysis of variance (ANOVA) [1] was used. With this method the tumors of six patients were digitized, three tumors from patients who lived for a long time after total prostatectomy without any evidence of recurrence and three tumors from patients who died very soon after total prostatectomy of metastatic disease. Of each of

Table 6. Analysis of variance Coefficient of variation for nuclear area (V_{area})

Patients (prognosis)	field of vision	Slide 1		Slide 2		Slide 3	
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
A	1	27.52	16.97	28.35	24.48	22.96	15.43
(good)	2	25.99	19.71	22.40	18.67	24.45	32.76
B	1	18.57	24.75	25.43	27.39	28.14	19.97
(good)	2	29.21	16.20	21.73	19.24	19.26	18.60
C	1	20.57	20.40	21.67	22.54	23.00	23.96
(good)	2	24.98	22.37	21.46	21.65	19.60	23.80
D	1	42.70	21.52	19.58	19.58	27.13	25.89
(poor)	2	20.27	28.50	29.28	30.94	24.21	27.52
E	1	27.81	24.55	27.61	32.43	29.81	32.12
(poor)	2	17.16	23.71	26.38	25.62	23.99	21.57
F	1	22.48	26.10	32.68	25.08	75.42	67.24
(poor)	2	23.99	24.80	38.76	27.31	43.14	24.67

Table 7. ANOVA Final calculation

Category	DF	diff($\Sigma \times^2$)	Variance diff/DF	F-test
total	71	6,279.05	88.44	
days	1	88.11	88.11	1.20
fields of vision	1	161.79	161.79	2.20
slides	2	333.38	166.69	2.27
patients	1	849.89	849.89	11.58
remainder	66	4,895.88	73.42	

these six tumors 3 slides and in each slide two randomly chosen fields of vision were digitized. The location of the fields of vision was recorded precisely by means of the crosstable of the microscope. Several days later the whole procedure was repeated, digitizing exactly the same fields of vision in the same slides. The results are summarized in Table 6.

From these values variances were calculated for the three different slides, the two fields of vision, the two separate days and the two groups of patients. The results are shown in Table 7.

After entering these values in the F-table [1] it was shown that there were no significant differences between the measurements in the different slides or fields of vision. Also there was no significant difference between measurements on different days. The two groups of patients however, showed a significant difference in variance. Accordingly, the method of morphometry and the coefficient of variation for nuclear area should allow a good differentiation between the various patients.

Our conclusion was that one could digitize anywhere in the slides and use as many or as few fields of vision as needed to obtain the proper amount of nuclei to be digitized.

Methods of measurements (digitizing)

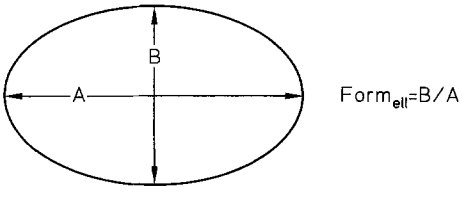
Before the measurements started several parameters were selected in the computer software program. These parameters were:

1. surface area,
2. perimeter, and two so-called form factors:
3. $FORM_{pe}$, also called circularity index. This form factor is given by the equation:

$$FORM_{pe} = \frac{4 \times \pi \times \text{area}}{(\text{perimeter})^2}$$

In the case of an exact circle the value for $FORM_{pe}$ equals 1. In all other cases $FORM_{pe}$ is less than 1. The more the shape of a structure deviates from the circle, the less the value for $FORM_{pe}$ becomes.

4. $FORM_{ell}$, also called ellipticity index. This form factor is given by the equation:



Circles: $Form_{ell}=1$

All other structures: $Form_{ell}<1$

As for $FORM_{pe}$, the value for $FORM_{ell}$ equals 1 in case of a circle. In all other structures the value for $FORM_{ell}$ becomes less than 1.

Both form factors are suitable to objectivate the shape of the nuclei, while the area and perimeter were measures for nuclear size. These four parameters were measured and calculated for 150 nuclei in each tumor formation. When a tumor consisted of only one tumor formation, only 150 nuclei were measured in that tumor. When a tumor consisted of two or three formations, the number of nuclei digitized were 300 and 450 per tumor respectively. From these 150 nuclei a mean value for each parameter and a standard deviation were calculated.

Statistics

The main goal was not to objectivate size and shape of the nuclei, but the *variation* in size and shape. The variation of the form factors was calculated by dividing the standard deviation by the mean value. In this way a coefficient of variation was calculated for each of the parameters in each of the tumor formations. The coefficient of variation is indicated by the capital letter "V".

In this way the coefficient of variation for area (V_{area}) was obtained as a standard for the variation in nuclear size and the

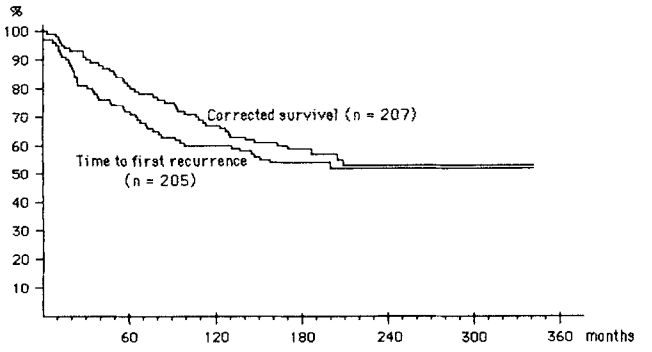


Fig. 1. Comparison between survival and time to first recurrence. The patterns of the curves are identical. The survival curve is corrected for intercurrent or unknown causes of death. In two patients it was not known when they developed metastases

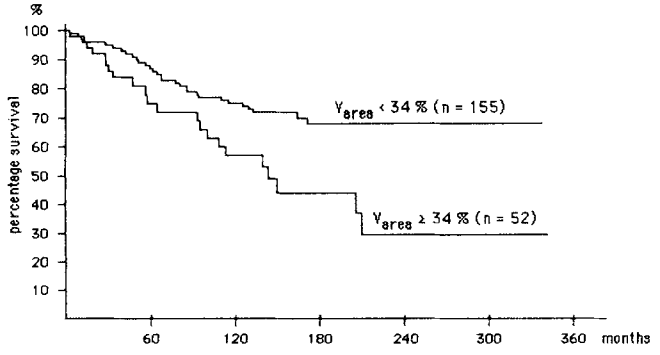


Fig. 2. The patients are divided morphometrically into two groups with significantly differing corrected survival rates ($p < 0.01$)

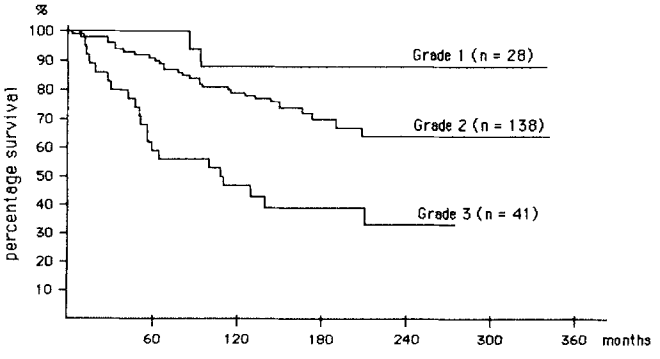


Fig. 3. The patients are divided according to Grade (Mostofi system)

Table 8. Morphometrically estimated variation in nuclear size

V_{area}	N	No. deaths	No. deaths from cancer	% of deaths
<34%	155	140	31	22.1
≥34%	52	48	22	45.8

coefficients of variation for $FORM_{pe}$ and for $FORM_{ell}$ ($V FORM_{pe}$ and $V FORM_{ell}$) as a standard for the variation in nuclear shape.

In each tumor formation 150 nuclei were digitized and for each nucleus the values for area, perimeter, $FORM_{pe}$ and $FORM_{ell}$ were calculated. Furthermore the computer calculated the mean values and the standard deviations. After finishing digitizing the values were all stored on disks and the results were printed out. The procedure was repeated for each tumor formation. At the end the coefficient of variation was calculated by dividing the standard deviation by the mean. In the case of more than one tumor formation per tumor the highest value for V was used for further evaluation.

Most results are presented as survival curves. These curves are calculated according to Kaplan and Meier [11]. The survival curves are corrected for intercurrent, tumor unrelated and unknown causes of death. In this way the curves show the impact of death from carcinoma more clearly without confusing the picture with the relatively high number of intercurrent deaths. For the evaluation of the differences between the curves the Logrank test was used.

We used death as an endpoint of study and not recurence of disease because all patients who had recurrence of their disease were dead at the time of the last review. Most of them indeed died of prstatic carcinoma and only seven (11.8%) died of other causes than prostatic cancer (causes of death in these men were: cardiovascular: 2, cerebrovascular: 1, murder: 1, other cancer: 3). It was shown that the curves did not change in a significant way when time to recurrence was used instead of time to death (Fig. 1). Of course the curve for recurrence of disease is shifted somewhat to the left, but the slopes of the curves are identical.

Results

With the morphometrically estimated variation in nuclear size (V_{area}) it was possible to split the whole group of 207 patients in two subgroups with a different prognosis. One group of patients with a $V_{area} < 34\%$ and a second group of patients with a $V_{area} \geq 34\%$. The cut-off point of 34% was found empirically. The first larger group consists of 155 patients. In this group there were 31 patients who died of carcinoma. The second group, counting 52 patients, showed death from carcinoma in 22 patients. The difference between the two groups is significant ($p < 0.01$, Table 8)

Graphically the corrected survival rates of the two groups of patients are shown in Fig. 2. As can be seen from this figure even after ten years there is a fair chance of dying of carcinoma. Also here the difference between the two groups is significant (Logrank test, $p < 0.01$).

Figure 3 shows the survival rates of the same 207 patients, divided into three groups according to grade (Mostofi system). As can be expected the patients with a grade 1 tumor had the best prognosis. In the whole group only two patients died of carcinoma and after 93 months there was no death of tumor in this group. Patients with grade 3 tumors do worst, even after 200 months patients died of prostatic carcinoma. The largest group of patients ($n = 138$) have grade 2 tumors and show an intermediate course of disease. However, also in this group after 15 years patients still died of prostatic carcinoma (see Table 9).

When the group of patients with grade 2 tumors was divided according to morphometrical measurements, two groups of patients with significantly differing survival rates ($p < 0.01$) were identified (Fig. 4). However, the

Table 9. Corrected survival of 207 patients, divided according to grade

Grade	No. patients	5-years	10-years	15-years	20-years
1	28	100%	88%	88%	88%
2	138	91%	79%	70%	64%
3	41	59%	47%	39%	33%

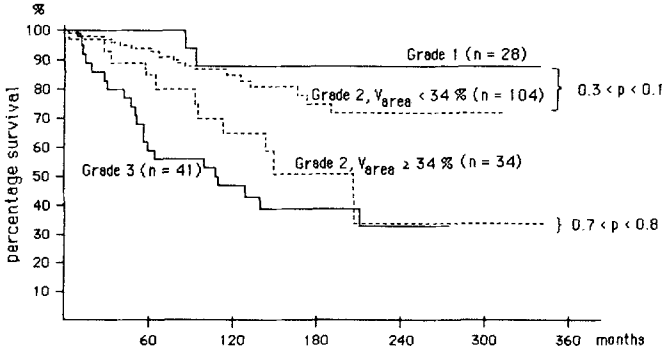


Fig. 4. The group of patients with Grade 21 tumors is divided morphometrically into two groups with significantly differing survival rates

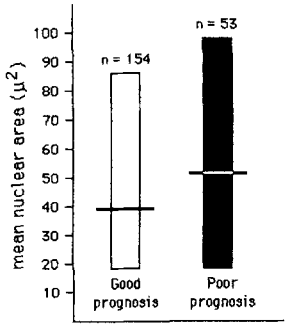


Fig. 5. Mean nuclear area 207 patients are divided into two groups according to prognosis (death from prostatic cancer)

patients with grade 2 tumors and $V_{area} < 34\%$ did not show a significantly differing survival rate from those with grade 1 tumors, while the patients with grade 2 tumors and a $V_{area} \geq 34\%$ had survival rates not differing from the patients with grade 3 tumors. The intermediate group of patients with grade 2 tumors could be divided into two groups: one with a prognosis almost equal to those with Grade 1 tumors and one with a prognosis almost equal to those with Grade 3 tumors.

Besides the variation in nuclear size also the mean nuclear size showed some correlation with the prognosis. In the group of 53 patients who died of prostatic carcinoma the tumors had a mean nuclear surface area of 51.4 μ^2, while the mean nuclear size in the tumors of the remainder of the patients was 39.6 μ^2. The difference is significant, but as Fig. 5 shows there is an almost complete overlap of the two groups.

In the group of 140 patients with a mean nuclear size $< 50\ \mu^2$ twenty-six patients (18.6%) died of prostatic carcinoma. This was the case in 27 patients (40.3%) with a mean nuclear size of $\geq 50\ \mu^2$. This difference is significant ($p < 0.01$).

Neither V $FORM_{pe}$ nor V $FORM_{cell}$ allowed to identify patients with different survival patterns. In now way was it possible to correlate these parameters with prognosis.

Discussion

Besides clinical stage the histopathological grade of a tumor plays an important role in establishing the prognosis of prostatic carcinoma. It is a well established fact that nuclear pleomorphism is one of the most important parameters in grading prostatic carcinoma. Most grading systems, especially those developed in the last two decennia use this parameter besides glandular differentiation and a varying number of other parameters. In an extensive study on 346 cases of prostatic carcinoma, all graded by Dr. Mostofi using his grading system [13], Schroeder and co-workers [16] found that in grading especially glandular differentiation and nuclear pleomorphism play an important role in the evaluation of the malignant potential of the tumor. Only the presence of mitoses may have an additional effect on the prognosis, but all other parameters as for instance the aspect of the cytoplasm, the presence or absence of nucleoli, the presence or absence of nuclear vacuoles, the number of various tumor formations, nuclear size do not have any weight in the prognosis of the tumors and may as well be omitted to simplify the system of grading.

Some reports show that grading of prostatic carcinoma is a somewhat subjective matter. Generally there are little problems in recognizing the true high grade and the true low grade tumors. The problems arise with the tumors that are neither high grade nor low grade. These tumors are by exclusion placed in a large and poorly defined intermediate group. However, in this group there may be large differences in prognosis, indicating that although these tumors all seem to have an intermediate grade, they are not uniform in behaviour. It is mainly the large group of Grade 2 tumors which presents difficulties in predicting prognosis. In this light it is strange that attempts to objectivate grading of prostatic carcinoma have started rather late.

The first investigators who quantitated nuclear characteristics and correlated their findings with tumor grade were Stöber and Schmidt [18] who measured nuclear area morphometrically and found a correlation with nuclear size and tumor grade.

In 1982 Diamond and colleagues [6, 7] presented their results with a new shape descriptor, called Nuclear Roundness Factor, and they were able to identify in a blind way two groups of patients who were cured by radical surgery or who would later die of cancer. Their system was shown to be 100 per cent accurate in this small

series of 27 patients. There were no false positives and no false negatives in the prediction of death from carcinoma of the prostate. In an attempt to reproduce these findings the nuclear roundness factor was calculated using the data of our patients. Surprisingly the values for nuclear roundness did not correlate well with corrected survival. Even an attempt to digitize some of our histological material on Diamond's equipment failed to identify nuclear roundness as a useful parameter in our hands. There was a very good correlation between the calculated nuclear roundness factor and the $FORM_{pe}$, but both failed to show any correlation with the prognosis.

It has not become clear to us why in our hands the nuclear roundness factor was not an important prognostic parameter. In an attempt to resolve this discrepancy one of us (JB) and one of the investigators from the Brady Urological Institute digitized on two occasions some geometrical figures with known sizes and shapes (circles, ellipses, squares, triangles and hexagons). It was noticed that each investigator's own results were easily reproducible, but that it was not possible to reproduce the results of each other. It was also found that the intra-observer variations were largest in digitizing the smaller figures.

In order to evaluate whether a difference in equipment might be the cause of the fact that nuclear roundness was not prognostic in our hands, some of our slides were digitized on Diamond's equipment. Also on Diamond's equipment in our hands nuclear roundness did not predict prognosis. In five patients who did not develop metastases up to a mean time of 147.8 months after total prostatectomy the mean nuclear roundness varied from 1,024 to 1,085 with an average of 1,054.4. A group of 17 patients who developed metastases after a mean interval of 34.9 months after total prostatectomy showed a mean value for nuclear roundness of 1,049.2 (range: 1,020–1,079). In the same groups of patients the mean values for V_{area} were 34.1% in the group with a good prognosis (range:28.3–40.0%) and 36.0% in the group with a poor prognosis (range: 24.9–52.8%).

Digitizing of some of our slides by D. Diamond also failed to show any correlation of nuclear roundness with survival. The reasons for the discrepancy of results is not clear, but it may be concluded that the accuracy of the digitizing equipment does not play as important a role as suggested by Diamond [7].

In conclusion it can be said that objectivation of nuclear size is possible and that the variation of this parameter gives a good correlation with survival. Patients with tumors that show a large variation in nuclear size will have a poorer prognosis than patients whose tumor nuclei do not show large variation in size. Morphometry can help in making a decision whether the patient might have a poor prognosis and may need aggressive therapy. Morphometry cannot replace the conventional grading systems at this time, but it can add objectivity to grading. More work has to be done to standardize the system of morphometry to obtain interchangeable results.

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