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Interactive computerized morphometric analysis for the differential diagnosis between dysplasia and well differentiated adenocarcinoma of the prostate*

F. Aragona[1], V. Franco[1], V. Rodolico[1], G. Dardanoni[2], D. Cabibi[1], D. Melloni[3], C. Pavone[3], G. Campesi[1], and M. Pavone-Macaluso[3]

Institutes of [1]Pathologic Anatomy and Histology, [2]Hygiene and Urology, and [3]Interdepartment Centre for Research in Clinical Oncology, University of Palermo, Palermo, Italy

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Summary. To distinguish prostatic dysplasia (or adenosis) from well differentiated adenocarcinoma on transrectal needle biopsy, a morphometric study was conducted on 20 cases of adenosis and 20 cases of well differentiated adenocarcinoma of the prostate. About 100 cells for each patient were analyzed by means of a computerized image analyzer, and mean nuclear diameter, mean nuclear area, mean form factor and number of cells in eight classes of nuclear diameter were studied. The best predictors of malignancy (evaluated by means of Receiver Operating Characteristics curves) were mean nuclear area $>28\,\mu^2$, presence of more than 5% of cells with nuclear diameter $>6.15\,\mu$, and mean nuclear diameter $>5\,\mu$. Using these diagnostic criteria the probability of malignancy for a positive specimen rises from 14% (pre-test) to 75% (post-test).

Key words: Prostate – Dysplasia – Adenosis – Well differentiated adenocarcinoma – Morphometry – Histologic diagnosis

Introduction

The interpretation of the histologic aspects of dysplasia of the prostate is prone to subjectivity, because of limited information about the biology and morphology of prostatic dysplasia [7, 8] and because of the difficulty in distinguishing these lesions from well differentiated adenocarcinoma.

All organs of the body have lesions that may be diagnosed as benign by one pathologist and malignant by another. However, as Brawn points out [2], the histology of no organ has such a wide difference of interpretation as the prostate. A variety of aspects, including dysplasia, atypical hyperplasia and even occasional forms of benign hypertrophy may be mistaken for carcinoma, expecially in that the presence of infiltration is difficult to evaluate in an organ in which stroma and glands are normally blended without a linear basement membrane. A special growth pattern of prostatic dysplasia which is most often confused with prostatic carcinoma has been defined under the term of "adenosis" which, in turn, has been classified under three grades: mild, moderate and severe. According to Brawn, adenosis is a dysplastic lesion characterized by mild nuclear pleomorphism, including the presence of nucleoli, in a circumscribed area of glandular proliferation, or by a growth pattern of the prostate glands which simulates stromal infiltration but is always lined by columnar cells with benign nuclei [1, 2].

Typical examples of adenosis and of well differentiated adenocarcinoma of the prostate are shown respectively in Fig. 1–3.

Such a differential diagnosis may be difficult even when abundand tissue is available from surgical extirpation or transurethral resection (TUR). It becomes even more difficult when the tissue has been obtained by transrectal or perineal needle biopsy. Needle biopsy often yields more significant information insofar as the tissue is obtained from the caudal extrasphinteric zones of the prostate, where cancer is more likely to develop [10].

An easier identification of well differentiated adenocarcinoma from prostatic dysplasia, would not only be of great theoretical importance but would also be of therapeuti importance [1, 3, 4, 9, 11].

Materials and methods

Twenty cases of benign prostatic hyperplasia with areas of adenosis and twenty cases of well differentiated prostatic adenocarcinoma were randomly selected.

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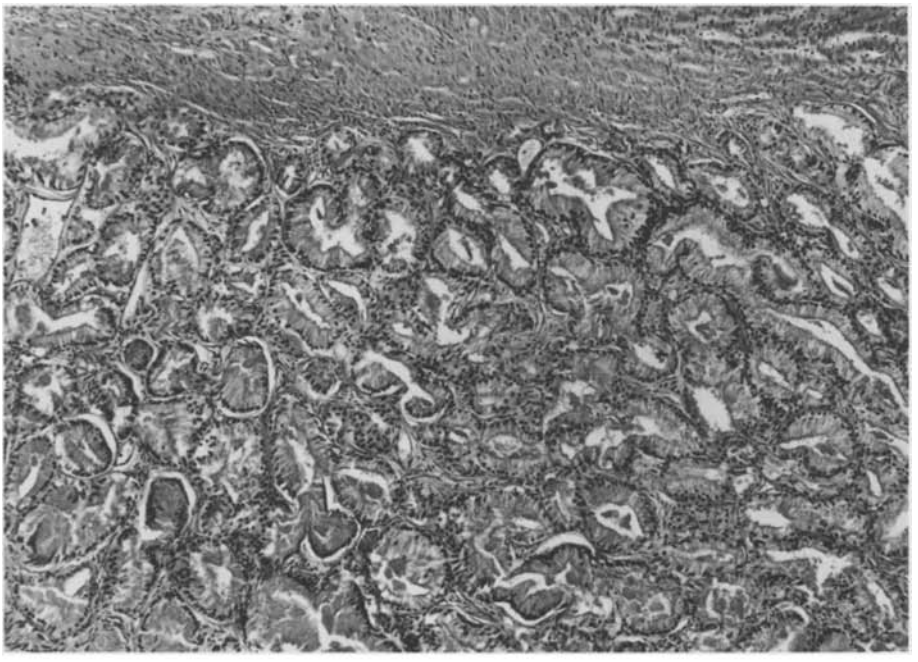

Fig. 1. Adenosis with confinded glandular proliferation

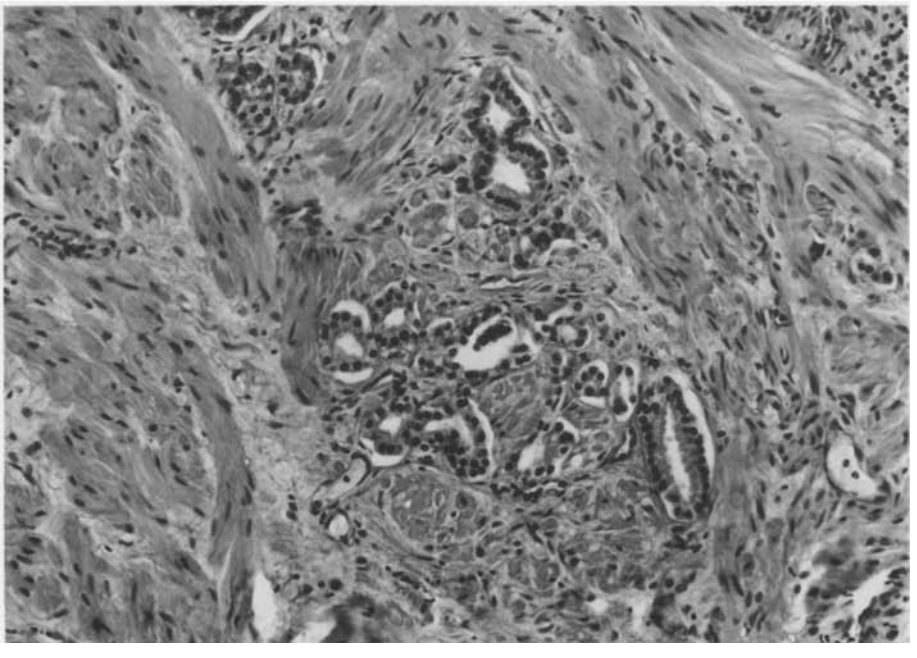

Fig. 2. Infiltrating adenosis

For this purpose only tissues obtained by TUR or open adenomectomy were employed.

The specimens were fixed in 4% buffered formaldehyde solution and embedded in paraffin. Five micron sections were stained with hematoxylin and eosin.

Morphometric analysis was performed by means of a computerized image analyzer, Leitz TAS Plus.

Ninety to 110 nuclei of epitelial cells, were analyzed for each patient. The methodology used was based on the following steps:

- display live image on the monitor;
- close holes of all particles;
- load displayed image;
- erode displayed image;
- reconstruct loaded image;
- verify the correspondence between real and displayed image;
- interact eventually with light pen;
- measure mean nuclear diameter, mean nuclear area, form factor of particles and number of cells in each of eight classes of nuclear diameter (see Table 2) for each patient.

The mean nuclear diameter was calculated as the mean of the equivalent diameters of all nuclei analyzed. The equivalent diameter of a nucleus is defined as the diameter of a circle which has the same area as the nucleus. Form factor (or roundness factor) is defined as the degree to which the nucleus in cross section approximates a perfect circle [5]; it was automatically calculated according to the formula:

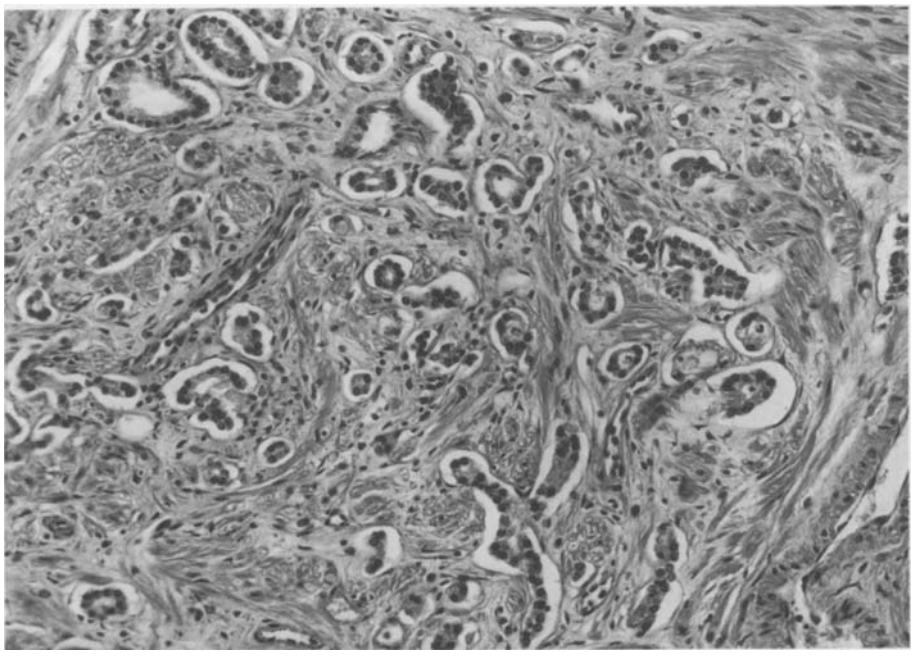

Fig. 3. Well differentiated adenocarcinoma

Table 1. Mean diameter, mean standard deviation, mean area and mean form factor of cellular nuclei

	Well differentiated adenocarcinoma	Adenosis	*P*
Mean diameter (μ)	5.59	4.62	<0.01
Mean SD (of diameter)	0.91	0.69	<0.01
Mean area (μ^2)	34.6	22.9	<0.01
Mean form factor	0.87	0.90	<0.01

$$\text{Form factor} = \frac{4\pi * \text{area}}{(\text{mean perimeter})^2}$$

The significance of difference in mean diameter, standard deviation, mean area and mean form factor between cancer and adenosis was tested by means of non parametric Mann-Withney's U test; chi square and chi square for trend were used to test the significance of difference between the two groups in the distribution of number of cells in the eight classes of diameter.

Correlation between the variables was tested by means of non parametric Spearman's R test.

Sensitivity and specificity for several levels of mean diameter, mean area, mean form factor and percentage of cells in the classes from 5 to 8 of diameter were calculated, and four Receiver Operating Characteristic (ROC) curves (percentage of false positives against percentage of true positives) were plotted to identify the best diagnostic cutoff point. Likelihood ratio (LR) (ratio of percentage of true positive and percentage of false positive) and post-test probability were also calculated in this cutoff point [12].

Results

Twenty cases of adenosis and twenty of well differentiated adenocarcinomas were studied. In Table 1 mean nuclear diameter and mean standard deviation, mean nuclear area and mean form factor of the two groups are reported.

Adenocarcinoma showed a mean diameter, mean standard deviation and mean area significantly higher than adenosis, and a mean form factor significantly lower ($P<0.01$).

Distribution of the number of cells in the eight classes of diameter was significantly different, and linear trend was present ($P<0.01$) (see Table 2).

In Fig. 4–7 the ROC curves for mean diameter, mean area, mean form factor and percentage of cells in the classes of diameter from 5 to 8 are reported.

It can be seen that the best diagnostic cutoff points are respectively mean diameter greater than 5.00 μ, mean area greater than 28 μ^2, mean form factor less than 0.88 and more than 5% of cells in the classes of diameter from 5 to 8. The best index seems to be mean area greater than 28 μ^2, with a sensitivity of 90% and a specificity of 95%; the LR is 18, and the probability of cancer rises from 14% to 74%.

While percentage of cells in the highest classes of diameter is also quite good as a diagnostic test (sensitivity 85%, specificity 95%, LR 17), mean diameter is less valid (sensitivity 90%, specificity 85%, LR 6) and mean form factor is unable to discriminate between cancer and adenosis (sensitivity 75%, specificity 70%, LR 2.5) (see Table 3).

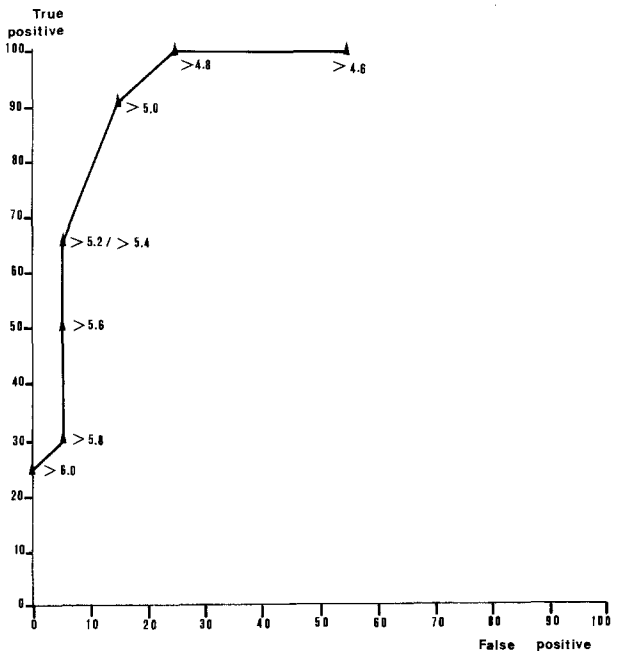

Fig. 4. ROC curve for mean nuclear diameter

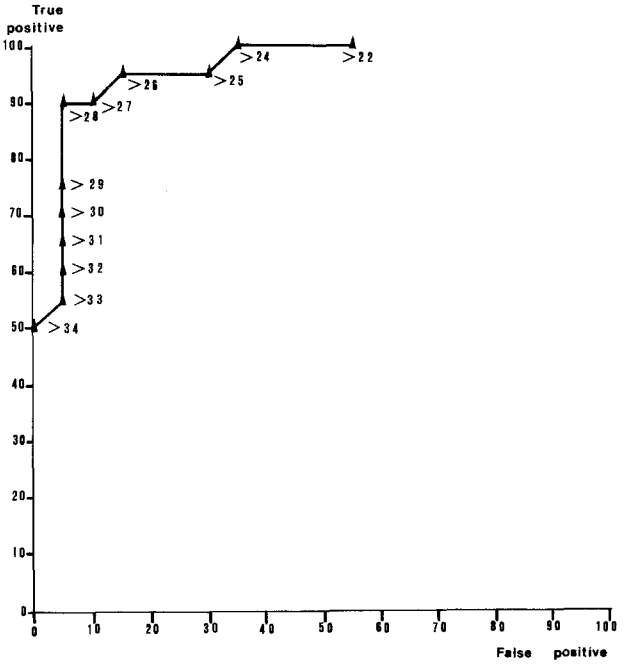

Fig. 5. ROC curve for mean nuclear area

Table 2. Distribution of cells in each class of nuclear diameter (μ)

	Well differentiated adenocarcinoma *N* (%)	Adenosis *N* (%)	*P* trend
1 - <3.50	18 (0.9)	142 (7.1)	
2 - 3.50–4.38	192 (9.6)	608 (30.4)	
3 - 4.39–5.26	575 (28.8)	832 (41.6)	
4 - 5.27–6.14	647 (32.4)	369 (18.4)	
5 - 6.15–7.02	388 (19.4)	48 (2.4)	
6 - 7.03–7.91	139 (7.0)	1 (0.1)	
7 - 7.92–8.79	30 (1.5)	0 (0)	
8 - >8.80	9 (0.4)	0 (0)	<0.01

A multivariate analysis was not performed because all variables, except mean form factor, were highly correlated.

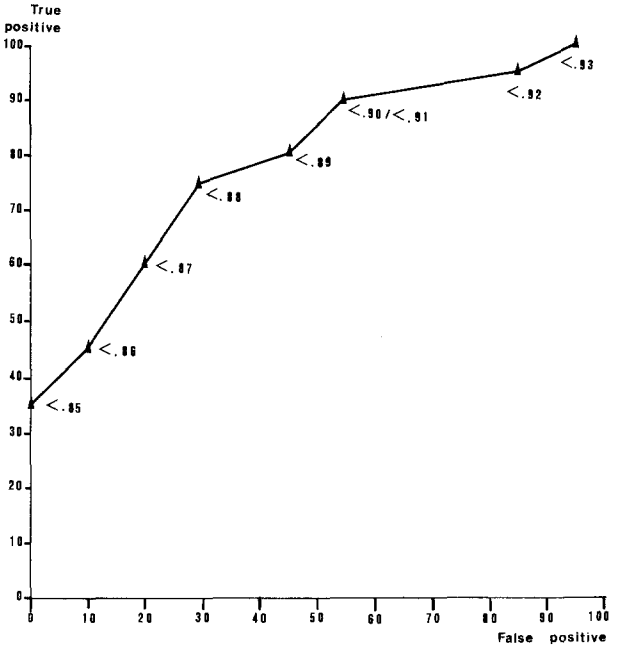

Fig. 6. ROC curve for mean nuclear form factor

Discussion

The data showed significant differences between adenocarcinoma and adenosis for all the morphometric parameters under investigation. In particular, mean nuclear diameter in areas of adenosis was 4.62 μ and mean standard deviation 0.69 μ, versus 5.59 μ and 0.91 μ of that of carcinoma; mean nuclear area was 34.6 μ^2 for malignant nuclei versus 22.9 μ^2 for nuclei of adenosis; very large nuclei (diameter >7 μ) were present only in carcinoma. These data were in close agreement with expected results, if we consider the higher chromatin content of neoplastic cells. The higher mean standard deviation is also in keeping with the greater extent of anisocaryosis present in cancer cells.

Table 3. Sensitivity, specificity, LR and post-test probability of the best diagnostic cutoff points

	Sensitivity	Specificity	LR	Post-test probability
Mean diameter $>5\,\mu$	0.90	0.85	6	0.49
Mean area $>28\,\mu^2$	0.90	0.95	18	0.74
Mean form factor <0.88	0.75	0.70	2.5	0.29
$>5\%$ cells in classes of diameter 5 to 8	0.85	0.95	17	0.73

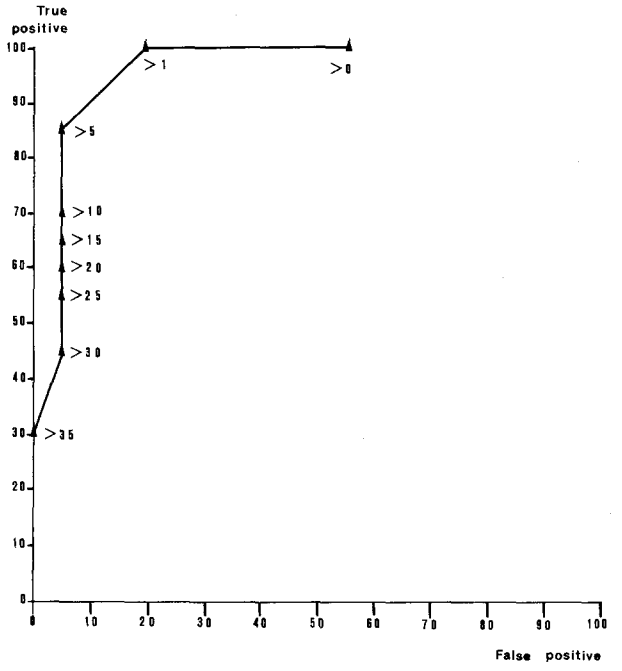

Fig. 7. ROC curve for percentage of cells in classes of nuclear diameter 5 to 8 μ

Form factor has proved to be of lower importance. This appears to be due to the fact that form factor takes in consideration not only irregularities in nuclear contour, which are more prominent in neoplastic cells, but is also influenced by the ellyptical shape of nuclei which, if regular in outline, may be present in non malignant cells [5, 6].

Finally, it has already been stressed that a multivariate analysis was not performed because all the variables under test were highly correlated, except form factor.

These data, albeit significant from the statistical standpoint, are of limited practical value for the pathologist who is more interested in precise cutoff points above which a diagnosis of malignancy can be made with reasonable certainty.

For this purpose the ROC curves for each single parameter under evaluation have been plotted. In particular, it was shown that the following single values, even if individually considered, are good predictors of the malignant nature of a growth:

- mean nuclear diameter greather than 5.00 μ;
- mean nuclear area greater than $28\,\mu^2$;
- presence of more than 5% of cells with nuclear diameter greater than 6.15 μ.

By calculating the LR it emerged that, in particular, the two last parameters had the greatest sensitivity and specificity, with very elevated LR (18 and 17, respectively).

Using LR values in order to assess the probability that a given specimen was a carcinoma, the pre-test probability, which was 14% – which was the proportion of prostatic cancer in our material – rose to about 75% (post-test probability) when cutoff values established for the two last parameters were exceeded.

In conclusion, our results do not enable us to establish parameters of absolute value; therefore the diagnosis is fundamentally based on histological and cytological data. However, morphometric data do represent a very useful aid for the discrimination between adenosis and well differentiated adenocarcinoma. Such differential diagnosis may be rather difficult [7], particularly when transrectal needle biopsy is involved. Since in these cases the available material is often extremely poor and so the growth pattern (circumscribed or infiltrating) is difficult to be characterized, the cytological analysis is the best criterium which can be used.

A further improvement in reliability and accuracy in this field may stem from densitometry (indirect measurement of chromatin in nuclei), and from the evaluation of other shape determinants which are presently under study in our laboratory.

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Dr. D. Cabibi
Istituto di Anatomia Patologica
Policlinico P. Giaccone
I-90127 Palermo
Italy