Article is available online at http://www.webio.hu/por/2005/11/2/0103

ARTICLE

# Proliferative Lesions of Prostate: a Multivariate Approach to Differential Diagnosis

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**Prostatic needle biopsies from 142 patients were studied: 61 cases were "benign", 19 atypical small acinar proliferation, 31 high-grade prostatic intraepithelial neoplasia, and 31 adenocarcinoma. Using univariate analysis of 46 previously described morphological features, 16 variables were selected, which were followed by multivariate discriminant analysis. Of these parameters, seven (glandular fusion, crystalloids, nucleolomegaly, papillary architecture, visibility of basal cell layer, areas of normal luminal cell nucleus/cytoplasm ratio and areas of high luminal cell nucleus/cytoplasm ratio) remained significant in discriminating the groups. Multivariate analysis selected a small panel of histological features as those most helpful in the differential diagnosis of proliferative lesions in prostate biopsies.** (Pathology Oncology Research Vol 11, No 2, 103–107)

*Key words:* prostatic hyperplasia, prostatic intraepithelial neoplasia, atypical small acinar proliferation, prostate cancer, histological features

## *Introduction*

Prostate cancer is the most frequent internal malignancy, and the third leading cause of cancer-related death in men in Brazil.[1] In the last few decades, transrectal needle biopsy has become widely used, revealing to the pathologist a wide array of prostate disorders: benign proliferation, atrophy, inflammation, prostatic intraepithelial neoplasia and carcinoma. However, the often scarce material in needle biopsies may pose major challenges for the histopathologist.[2]

The purpose of the present study was to select, through a multivariate analysis, the most important morphological features in the differential diagnosis of prostatic epithelial proliferations in biopsy specimens.

## *Materials and Methods*

Formalin-fixed paraffin-embedded prostatic transrectal needle biopsy samples from 142 patients were selected from the files of the Hospital das Clínicas – São Paulo University School of Medicine (HCFMUSP), from March 1996 to December 1997, in order to assure at least 15 cases for each diagnostic group. No patient had previously received either hormonal or radiotherapy. The cases were divided in four groups:

*Group 1*: "Benign lesions": 61 cases (22 with well-defined usual prostatic hyperplasia, 24 with post-atrophic hyperplasia and 15 with basal cell hyperplasia)

*Group 2*: 19 cases with atypical small acinar proliferation (ASAP)

*Group 3*: 31 cases with high-grade intraepithelial neoplasia (PIN)

*Group 4*: 31 cases with adenocarcinoma (Gleason 4-6: 19, Gleason 7-9: 12)

The following morphological features, previously described in the literature, were assessed as being present or absent in each case:

• *Glandular architecture*: small and round, small and angulated, gland fusion, cribriform, papillary, trabecular, macroglands with epithelial infoldings, dilated glands, solid pattern, isolated cells.

• *Epithelial stratification*: one cell layer, double cell layer, irregular stratification.

*Received:* July 26, 2004; *accepted:* Jan 3, 2005

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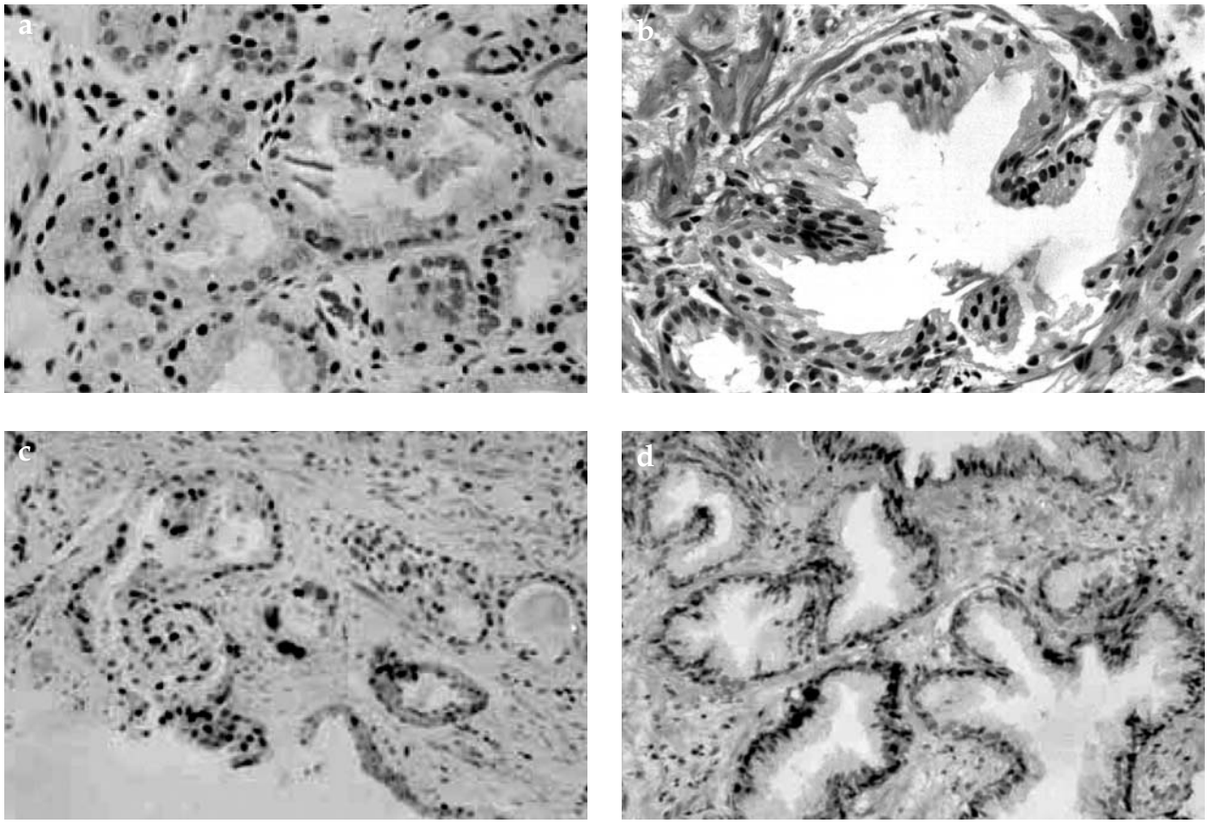


***Figure 1.*** *Major features in proliferative lesions: adenocarcinoma with crystalloids and prominent nucleoli* ***(a);*** *PIN with papillary architecture* ***(b);*** *adenocarcinoma with high N/C ratio and fusion* ***(c);*** *positive immunostaining for high-molecular-weight cytokeratins in hyperplasia* ***(d).***

• *Cytoplasm of secretory cells*: clear eosinophilic, dark eosinophilic, granular or homogeneous.

• *Nucleus of secretory cells*: condensed chromatin, fine granular chromatin, high nucleus/cytoplasm (N/C) ratio and normal nucleus/cytoplasm (N/C) ratio (these variables were assessed individually, as they frequently co-existed in the same lesion).

• *Nucleoli of secretory cells*: not visible, prominent (considered in this study as visible at 100x magnification) or inconspicuous (considered in this study as visible only at 400x magnification).

• *Mitosis* in secretory cells and basal cells.

• *Basal cell layer*: intact, fragmented, peripheral palisading of cells, absent.

• *Nucleus of basal cells*: fine granular or condensed chromatin, visible nucleoli.

• *Luminal content*: exfoliated cells, crystalloids, collagenous micronodule, corpora amylacea, eosinophilic secretion, blue mucin, absence of luminal content.

• *Stromal features*: elastosis, sclerosis, muscle fiber atrophy.

• *Inflammation*: absent, lymphocytes, neutrophils.

• *Nerves and vessels*: neoplastic infiltration.

The data were analyzed using SPSS-PC (Version 8.0). Initially, all 46 morphological features were submitted to univariate analysis examining associations with diagnoses through Chi-square tests and Spearman's correlation coefficient. The sixteen variables with best diagnostic performance were then submitted to multivariate comparisons through Multiple Discriminant Analysis (MDA) that analyzes the combinations of predictor variables (morphological features) in mathematical functions and categories of a given dependent variable (diagnostic groups).[3,4]

This analysis can be graphically observed in a territorial map projecting boundaries of diagnosis categories. A rotated correlation matrix of functions and variables adds information on how each variable loads each function.

### *Results*

Sixteen histological criteria achieved a level of association with diagnosis of at least 0.30 for Spearman's correlation coefficient, and thus were selected to enter MDA: glandular fusion (0.510), prominent nucleoli (0.779), crystalloid (0.349), delicate chromatin in secretory cells

(0.426), solid arrangement (0.300), eosinophilic secretion (0.464), stromal sclerosis (0.350), absence of luminal content (-0.450), PMN (-0.304), nerve invasion (0.300), papillary arrangement (0.300), two cell layers (-0.315), basal cell visualization (0.708), high N/C ratio (0.592), normal N/C ratio (-0.383) and granular cytoplasm of secretory cells (0.301).

MDA identified seven variables that had statistical significance to build three mathematical functions ($p<0.001$), able to discriminate the major diagnostic groups. Function 1 discriminates carcinoma and is best correlated to variables glandular fusion (*Figure 1c*), prominent nucleoli (*Figure 1a*) and crystalloids (*Figure 1a*) (*Table 1*). Function 2 discriminates PIN, and is correlated to variable papillary arrangement (*Figure 1b*) (*Table 1*). Finally, function 3 discriminates ASAP and PIN, thus excluding benign lesions, and is correlated to variables high N/C ratio (*Figure 1c*), normal N/C ratio, and evident basal cells (*Figure 1d*) (*Table 1*).

The first and second functions explain 95.7% of the variations in original variables selected for the model, and their canonical correlations were high (0.907 and 0.855 respectively). The third function is less informative, explaining only 4.3% of variations. Its canonical correlation is lower (0.50).

The bi-dimensional territorial map of groups 1 to 4, using functions 1 and 2 is depicted in *Figure 2*.

*Table 1* presents the relationship between functions and all variables considered. Seven variables with statistical significance to make up the mathematical model are highlighted, and an asterisk marks the highest loads.

***Table 1.*** **Rotated structure matrix**

| | *Function* | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| glandular fusion | .546(*) | –.169 | –.304 |
| nucleoli | .544(*) | –.011 | .211 |
| crystalloid | .327(*) | –.121 | –.185 |
| fine chromatin (luminal cell)(a) | .311(*) | .036 | .016 |
| solid pattern(a) | .167(*) | –.125 | .060 |
| eosinofilic sec.(a) | .139(*) | .049 | .071 |
| sclerosis(a) | .136(*) | .041 | –.049 |
| lum. content absent(a) | –.132(*) | –.004 | –.085 |
| PMN(a) | .071(*) | –.019 | .012 |
| nerve infilt.(a) | –.038(*) | –.005 | –.007 |
| papillary gland | .157 | .935(*) | .121 |
| double cell layer(a) | .000 | .153(*) | –.123 |
| basal cell visibility | .317 | –.413 | .638(*) |
| high N/C | .119 | .020 | .518(*) |
| normal N/C | .008 | .047 | –.428(*) |
| granular cytoplasm (luminal cell)(a) | –.018 | -.009 | .231(*) |

Rotated pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. Variables ordered by size of correlation within function.
* Largest absolute correlation between each variable and any discriminant function.
a This variable was not used in the analysis.

## *Discussion*

A variety of prostatic lesions can mimic prostate cancer in needle biopsies.[5] Many morphological features, as well as immunostaining with high-molecular-weight cytokeratins have been claimed as "definite criteria" or as "clues" for the differential diagnosis.

In the present study, major epithelial proliferative lesions, some of them recently defined in needle biopsies,[6-17] were evaluated in 142 needle biopsies, re-assessing the discriminant validity of each histological variable through a multivariate analysis.

Among the 46 histological criteria the seven with best discriminant performance were as follows:

The presence of an enlarged nucleolus (*Figure 1a*) in secretory epithelium is a marker for malignancy. However, the limits of normal size are not well-defined. The measurement of nucleolus is considered to be precise but not practical for routine use.[3] In the present study, cases could be reliably separated into those with nucleoli visible at 100x magnification (considered "prominent"), and those with nucleoli evident only at high power view (400x magnification, considered "inconspicuous"). According to this definition, no benign lesion had prominent nucleoli, which were, however, found in 28 out of 31 carcinomas. This result could be confirmed by MDA, which selected this variable as the one best discriminating "benign lesions" from carcinomas. Varma et al[17] found "prominent nucleoli" as the most frequent histological feature (94% of 150 cases) in prostatic adenocarcinoma in needle biopsies.

Iczkowski et al[12,13] found 72% of ASAP cases with prominent nucleoli. Cheng et al[18] found prominent nucleoli in 79% of carcinomas and 50% of benign prostate tissue in postirradiation needle biopsies. Bostwick et al[19] found a nucleolar diameter of >1 mm in 17.6% of atypical adenomatous hyperplasia, 58.1% of ASAP and 77.5% of adenocarcinomas. Helpap[20] described usual prostatic hyperplasia without nucleolar enlargement, postatrophic hyperplasia with small to medium size nucleoli, ASAP with mild enlargement, PIN with mostly prominent nucleoli, and low-grade adenocarcinoma with prominent nucleoli (1.0-3.0 mm).

Morphological evaluation of basal cells as "absent", "fragmented", or as an "intact layer" (*Figure 1d*) was found useful to discriminate among the groups. This was confirmed as an important variable in MDA, composing function 3 that best discriminates ASAP from carcinoma.

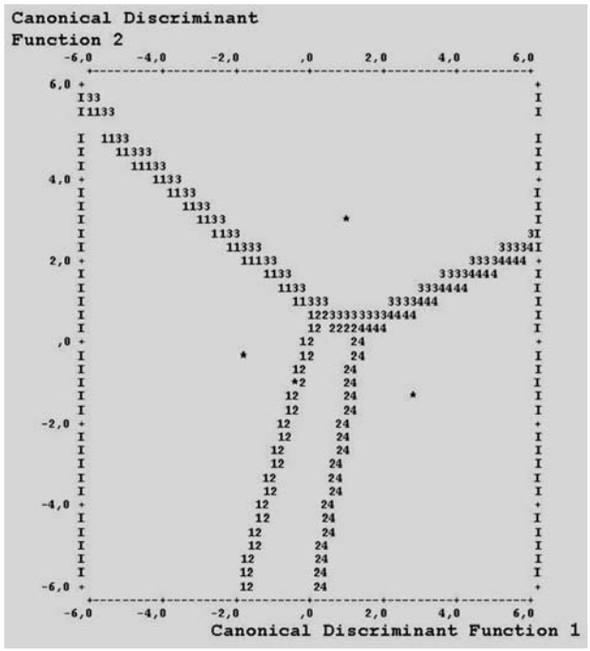


***Figure 2.*** *Bi-dimensional territorial map*

In our study we also searched for high-molecular-weight cytokeratins with monoclonal antibody 34bE12; this approach yielded clear-cut staining of basal cells, further clarifying the presence of a continuous layer, dispersed cells or absence of basal cells in carcinomas. Although in univariate analysis this proved to be a useful variable to distinguish ASAP from adenocarcinomas, it was not found to be better than the morphological evaluation of basal cells in the MDA model.

The presence of basal cells, particularly in the non-small acinar type of adenocarcinoma and using immunohistochemistry for high-molecular-weight cytokeratins, has been described, with variation in intensity. Oliai et al described 36 out of 3198 cases of prostatic adenocarcinoma at least focally stained with 34bE12.[21] Googe et al showed positivity in 43% of cases of metastatic prostate cancer and 54% of primary carcinomas,[22] whereas focal positivity in the cribriform pattern of prostatic ductal carcinoma was described by Amin et al[23] and Millar et al.[24] Although usually described as "fragmented" in at least part of PIN cases,[6,25-28] in the present study the basal cell layer was found "fragmented" only when PIN was associated with carcinoma, while it was usually morphologically "intact" in cases with PIN only.

These findings are similar to studies of Brawer et al,[29] Cheville and Bostwick[7] and Wojno and Epstein[30] who found positivity in PIN and several benign conditions and negativity in cases of adenocarcinomas. It is especially useful in discriminating ASAP versus well-differentiated carcinoma as found in a study of Kahane et al[31] where 336 ASAP cases were submitted to immunostaining with 34bE12, yielding a final diagnosis in 321, only 15 (0.4%) remaining as "atypical", without distinction of the benign or malignant nature of the lesion. In the present study, 21.1% of ASAP cases were widely positive and, therefore, could be diagnosed as benign after the immunostaining.

Positivity for 34bE12 in small acinar lesions has, until now, been considered almost certain of benign or non-invasive lesion. O'Malley et al[32] studied 21 cases of small acinar adenocarcinoma and 47 different benign lesions. All adenocarcinomas were negative for high-molecular-weight cytokeratins, and the benign lesions, especially basal cell hyperplasia and atypical adenomatous hyperplasia, although always positive, were sometimes weak. Ximing et al[33] studied 100 cases of metastatic and locally advanced prostate cancer, and found two cases of weak and diffuse positivity and two cases of strong and focal positivity. On the contrary, negative staining within a suspicious lesion, although suggestive of malignancy, should not be interpreted as diagnostic of carcinoma, as this may represent a false negative either due to the small dimension of the sample, or to the conditions of the immunohistochemical procedures, especially fixation.[13,33]

Nuclear enlargement or high N/C ratio is a very common finding in prostatic neoplasia. In this study this feature was found a marker for ASAP. In the literature, the most common benign condition with nucleomegaly is postatrophic hyperplasia.[7,34] Indeed, in the present study, 21 out of 22 of the benign cases with nuclear enlargement were of postatrophic hyperplasia. Troxel and Sabella,[35] in a study of problem areas in pathology practice, observed that nucleomegaly, associated with prominent nucleoli, was the cause of one of the most common malpractice claims in pathology, where the diagnosis of carcinoma was made in postatrophic hyperplasia. Ruska et al described the cellular kinetics of postatrophic hyperplasia and showed more proliferative activity than in benign, non-atrophic glands.[36] Recently, Leroy et al[37] considered nuclear enlargement as one of the major microscopic criteria for minimal focus of adenocarcinoma in prostate biopsy.

Among the several forms of luminal content,[7,12,13,19,34,38,39] in the present study only the presence of crystalloids (*Figure 1a*) was proved to be a discriminant by MDA. In our study, 25.8% of carcinomas exhibited crystalloids against no cases of ASAP and benign lesions. Cheville and Bostwick,[7] Anton et al[38] and Amin et al[34] did not find crystalloids in postatrophic hyperplasia. Among 60 cases of PIN, Bostwick et al[39] found 3% with crystalloids. Bostwick et al[19] found 13% of cases with crystalloids in ASAP, versus 75% in carcinomas. Iczkowski et al[13] found 0.06% of cases with crystalloids in ASAP. Afterwards, in a larger casuistic study,[12] the same authors could not confirm this feature as being predictive of cancer.

The architecture of the glands, including epithelial stratification, is so important that in many instances it is fundamental to the nature of the lesion, as in the case of complex

glands and fusion (*Figure 1c*) in adenocarcinomas, especially those with Gleason patterns 4 and 5,[9,10] making up function 1. Papillary infoldings (*Figure 1b*) were very specific for PIN, as can be appreciated by MDA (function 2).

### *Conclusions*

A small panel of 7 histological features, selected by MDA in this study, is a potentially useful checklist for the differential diagnosis of prostatic lesions in needle biopsies. The present multivariable approach should be further validated by a prospective study on needle biopsies, with radical prostatectomy specimens as the gold-standard for positive cases.

### *References*


1. IBGE: DPE/DEPIS; MS/InCA/CONPREV/Divisão de Epidemiologia e Avaliação S/SIM- Sistema de Informação sobre mortalidade [http:www.ibge.gov.br/], 2000
2. *Arista-Nasr J, Cortes E, Pichardo R:* Low grade adenocarcinoma simulating benign glandular lesions in needle prostatic biopsy. Rev Invest Clin 49:37-40, 1997
3. *Gilbert ES*: On discrimination using qualitative variables. J Am Stat Assoc 63: 1399-1412, 1968
4. *Moore DH*: Evaluation of five discrimination procedures for binary variables. J Am Stat Assoc 68: 399, 1973
5. *Nasir A, Copeland J, Gillespine JW, et al:* Preneoplastic lesions of the prostate – clinical, pathological and molecular biological aspects. In Vivo 6:557-566, 2002
6. *Brawer MK:* Prostatic intraepithelial neoplasia: A premalignant lesion. Hum Pathol 23: 242-248, 1992
7. *Cheville JC, Bostwick DG:* Postatrophic Hyperplasia of the Prostate. Am J Surg Pathol 19:1068-1076, 1995
8. *Cleary KR, Choi HY, Ayala AG*: Basal cell hyperplasia of the prostate. Am J Clin Pathol 80: 850-854, 1983
9. *Gleason DF* (1992). Histologic grading of prostate cancer. A perspective. Hum Pathol 23: 273-279, 1992
10. *Gleason DF, Mellinger GT, Veterans Administration Cooperative Urological Research Group:* Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111: 58-64, 1974
11. *Grignon DJ, Ro JY, Ordonez NG, et al*: Basal cell hyperplasia, adenoid basal cell tumor, and adenoid cystic carcinoma of the prostate gland: An immunohistochemical study. Hum Pathol 19: 1425-1433, 1988
12. *Iczkowski KA, Bassler TJ, Schwob VS, et al:* Diagnosis of "suspicious for malignancy" in prostate biopsies: predictive value for cancer. Urology 51:749-757, 1998
13. *Iczkowski KA, MacLennan GT, Bostwick DG*: Atypical small acinar proliferation suspicious for malignancy in prostate needle biopsies: clinical significance in 33 cases. Am J Surg Pathol 21:1489-1495, 1997
14. *Moore RA:* Benign hypertrophy of the prostate - a morphological study. J Urol 50: 580-710, 1943
15. *Purnell DM, Heatfield BM, Anthony RL, Trump BF:* Immunohistochemistry of the cytoskeleton of human prostatic epithelium**.** Am J Pathol 126:384-395, 1987
16. *Totten RS, Heineman MW, Hudson PB, et al:* Microscopic differential diagnosis of latent carcinoma of prostate. Arch Pathol 55: 131-141, 1953
17. *Varma M, Lee MW, Tamboli P, et al*: Morphologic criteria for the diagnosis of prostatic adenocarcinoma in needle biopsy specimens. A study of 250 consecutive cases in a routine surgical pathology practice. Arch Pathol Lab Med 126:554-561, 2002
18. *Cheng L, Cheville JC, Bostwick DG:* Diagnosis of prostate cancer in needle biopsies after radiation therapy. Am J Surg Pathol 23: 1173-1183, 1999
19. *Bostwick DG, Sgriley J, Grignon D et al:* Atypical adenomatous hyperplasia of the prostate: Morphologic criteria for its distinction from well differentiated carcinoma. Hum Pathol 24: 819-832, 1993
20. *Helpap B*: Differential diagnosis of glandular proliferations in the prostate. A conventional and immunohistochemical approach. Virchows Arch 433:397-405, 1998
21. *Oliai BR, Kahane H, Epstein JI:* Can basal cells be seen in adenocarcinoma of prostate?: an immunohistochemical study using high molecular weight cytokeratin (clone 34bE12) antibody. Am J Surg Pathol 26:1151-1160, 2002
22. *Googe PB, Mcginley KM, Fitzgibbon JF*: Anticytokeratin antibody 34 b E12 staining in prostate carcinoma. Am J Clin Pathol 107: 219-223, 1997
23. *Amin MB, Schultz DS, Zarbo RJ:* Analysis of cribriform morphology in prostatic neoplasia using antibody to high molecular weight cytokeratins. Arch Pathol Lab Med 118: 260-264, 1994
24. *Millar EKA, Sharma NK, Lessels AM:* Ductal (endometrioid) adenocarcinoma of the prostate: a clinicopathological study of 16 cases. Histopathology 29: 11-19, 1996
25. *Bostwick DG*: High grade prostatic intraepithelial neoplasia and early invasion in prostatic carcinoma. Cancer 59:788-794, 1987
26. *Mc Neal JE, Bostwick DG:* Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 17:64-71, 1986
27. *Montironi R, Pomante R, Colanzi P, et al:* Diagnostic distance of high grade prostatic intraepithelial neoplasia from normal prostate and adenocarcinoma. J Clin Pathol 50:775-782, 1997
28. *Rubin MA, de La Taille A, Bagiella E, et al:* Cribriform carcinoma of the prostate and cribriform prostatic intraepithelial neoplasia: incidence and clinical implications. Am J Surg Pathol 22:840-848, 1998
29. *Brawer MK, Peehl DM, Stamey TA, Bostwick DG*: Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 45: 3663-3667, 1985
30. *Wojno KJ, Epstein J*: The utility of basal cell specific anti-cytokeratin antibody (34 B E 12) in the diagnosis of prostate cancer - a review of 228 cases. Am J Surg Pathol 19: 251-260, 1995
31. *Kahane H, Sharp JW, Shuman GB, et al:* Utilization of high molecular weight cytokeratin on prostate needle biopsies in an independent laboratory. Urology 45: 981-986, 1995
32. *O'Malley FP, Grignon DJ, Shum DT:* Usefulness of immunoperoxidase staining with high molecular weight cytokeratin in the differential diagnosis of small acinar lesions of the prostate gland. Virchows Arch Pathol Anat 417:191-196, 1990
33. *Ximing JY, Lecksell K, Gaudin P, Epstein J*: Rare expression of high molecular weight cytokeratin in adenocarcinoma of the prostate gland. A study of 100 cases of metastatic and locally advanced prostate cancer. Am J Surg Pathol 23:147-152, 1999
34. *Amin MB, Tamboli P, Varma M, Srigley JR*: Postatrophic hyperplasia of the prostate gland: a detailed analysis of its morphology in needle biopsy specimens. Am J Surg Pathol 23:925-931, 1999
35. *Troxel DB, Sabella JD:* Problem areas in pathology practice uncovered by a review of malpractice claims. Am J Surg Pathol 18:821-831, 1994
36. *Ruska KM, Sauvageot J, Epstein JI:* Histology and cellular kinetics of prostatic atrophy. Am J Surg Pathol 22:1073-1077, 1998
37. *Leroy X, Aubert S, Villens A, et al*: Minimal focus of adenocarcinoma on prostate biopsy: clinicopathological correlations. J Clin Pathol 56: 230-232, 2003
38. *Anton RC, Kattan MW, Chakraborty S, Wheeler TM*: Postatrophic hyperplasia of the prostate: lack of association with prostate cancer. Am J Surg Pathol 23:932-936, 1993
39. *Bostwick DG, Amin MB, Dundore P, et al:* Architectural patterns of high grade prostatic intraepithelial neoplasia. Hum Pathol 24:298-310, 1993