ORIGINAL ARTICLES

Diagnostic utility of immunohistochemical marker 34βe12 (keratin 903) in prostate pathology.

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ABSTRACT: Cytokeratin clone 34βE12 (keratin 903) is a high molecular weight monoclonal antibody which is immunoreactive against a variety of epithelial tissues and is widely used as a basal cell specific marker. Our aim was to highlight the importance of 34βE12 immunohistochemical detection over the diagnostic evaluation of prostatic lesions. A total of 15 prostatectomy specimens were reviewed, 5 with Benign Hyperplasia (BH), 5 with carcinoma and 5 with high grade Prostatic Intraepithelial Neoplasia (PIN). 34βE12 imunnohistochemistry was performed on representative sections. The specimens were retrieved from «G. Gennimatas» General Community Hospital Laboratory of Pathology. Normal staining pattern was observed in all 5 cases of BH. PIN showed positive staining in a limited number of basal cells whereas all cases of carcinoma were negative. Cytokeratin 34βE12 is a basal-cell specific antibody. It shows no reactivity neither for the secretory nor for the stromal cells of the prostate. The basal cell layer appears to be continuous in BH whereas it is discontinuous in PIN and totally absent in carcinoma. Thus differential diagnosis between BH, PIN and prostatic carcinoma can be based on the discrete staining pattern of basal cells with 34βE12 and is mostly appreciated in cases where basal cells are hard to distinguish in standard Hematoxylin - Eosin sections.

Key Words: 34βE12, Keratin 903, Cytokeratin, Basal cells, Prostate.

INTRODUCTION

Monoclonal antibody 34βE12 recognizes the high molecular weight cytokeratins 1,5,10 and 14 (MW 68,58,54,5,50 Kd) which are expressed by basal cells of prostatic epithelium[1]. It is also immunoreactive against squamous, urothelial, bronchial/pneumocyte, thymic, some intestinal and ductal epithelium (breast, pancreas, bile duct, salivary gland, sweat duct, renal collecting duct), and mesothelium[2]. On the contrary the antibody does not label prostatic secretory cells which are the origin of adenocarcinoma[3,4,5,6]. It is known that whereas basal cells are preserved in benign prostatic lesions they are absent in adenocarcinoma. However on occasion benign lesions fail to provide a clear view of basal-cell layer in simple Hematoxylin - Eosin (H-E) sections. Immunohistochemical labeling of basal cells with 34βE12 in such ambiguous cases is fundamental for excluding malignancy[7,8]. On the other hand the credibility of this antibody has been challenged by the reported presence of positive cells in otherwise typical cases of carcinoma[9,10,11]. It is therefore of great importance that immunohistochemical evaluation of antikeratin 34βE12 be always carried out in association with standard morphological findings on routine H-E sections before establishing a definite diagnosis. This is a retrospective study of archival material aiming to highlight the diagnostic value of immunohistochemical marker antikeratin 34βE12 in prostate pathology.

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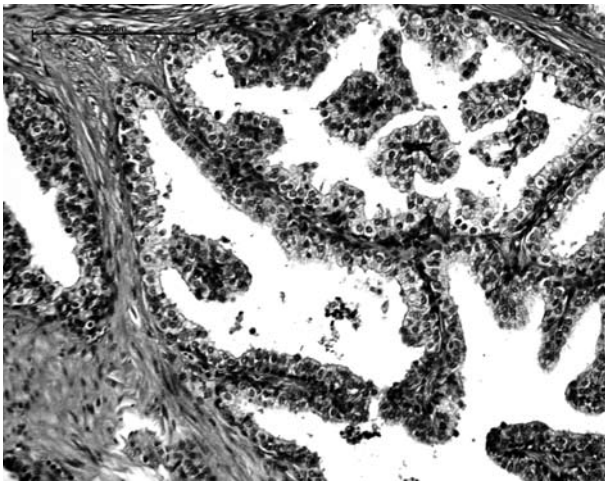

Figure 1. Benign Hyperplasia (BH).Hematoxylin-Eosin stain(H-E).

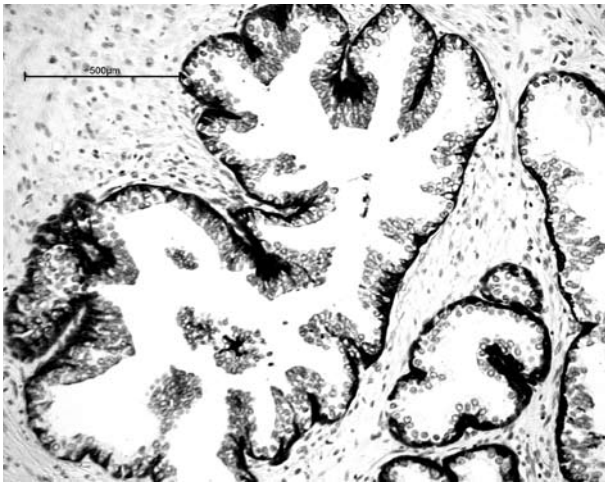

Figure 2. BH (same focus): the basal cell layer is uniformly labeled (antikeratin 34βE12 immunohistochemical stain).

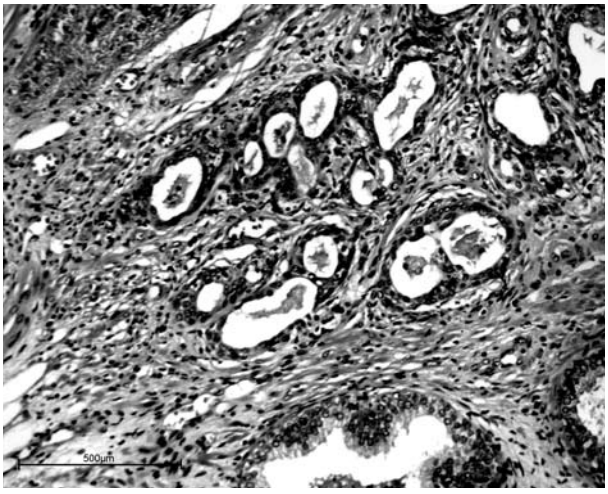

Figure 3. Atypical adenosis: small cluster of glands with «atypical morphology» (H-E).

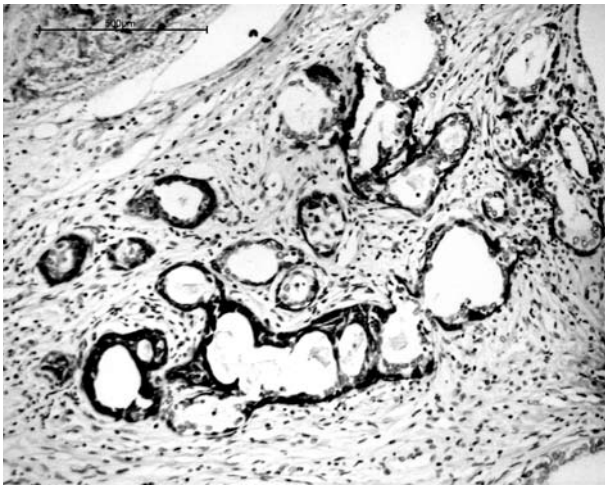

Figure 4. Atypical adenosis (same focus): decoration of the basal cell layer rules out malignancy (antikeratin 34βE12 immunohistochemical stain).

MATERIALS AND METHODS

Surgical resection specimens included in this study come from 15 patients aged 51-79 years old. The cases were retrieved from the surgical files of the Laboratory of Pathology of General Community Hospital «G. Gennimatas». 5 cases of simple prostatectomy, 5 cystoprostatectomies and 5 radical prostatectomies were examined. It should be noted that urothelial tumor present in cystoprostatectomy specimens did not extend towards the prostatic urethra nor did it infiltrate the prostate gland. All specimens were fixed in formalin and embedded in paraffin. 3 blocks were chosen from each specimen and 2 4-μm thick sections were obtained. One was stained with H-E and the other was deparaffinized and pretreated with pepsin for 20 min at 37°C. The slides were stained on a Optimax (Menarini Diagnostics) Automatic Immunostainer with antibody 34βE12 (Biogenex) and appropriate buffer solutions.

RESULTS

Benign Hyperplasia (BH) was diagnosed in all 5 simple prostatectomies showing typical staining of

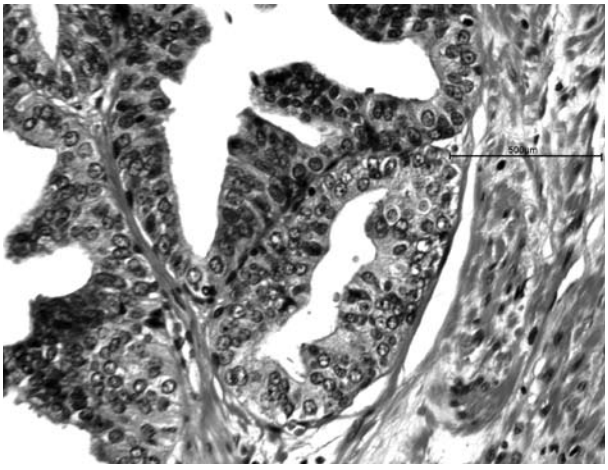

Figure 5. High Grade Prostatic Intraepithelial Neoplasia (PIN). (H-E).

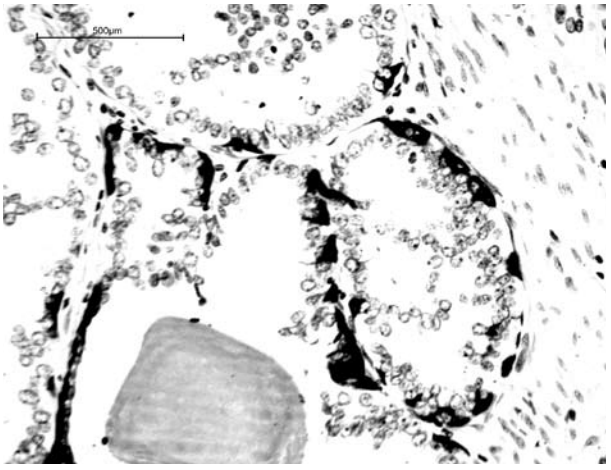

Figure 6. High Grade PIN (same focus): basal cell layer appears discontinuous but prominent (antikeratin 34βE12 immunohistochemical stain).

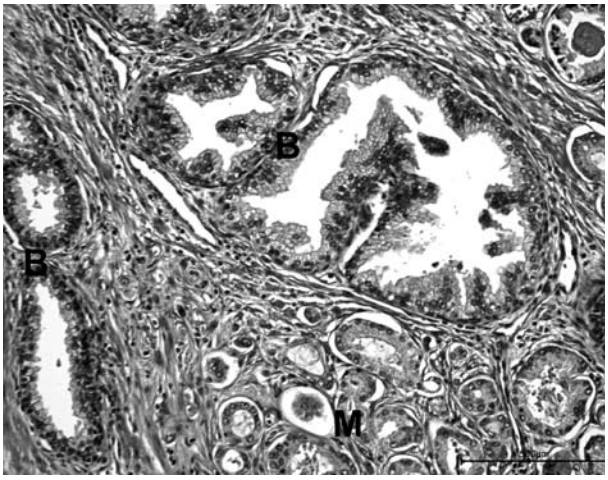

Figure 7. Malignant glands (M) bottom right, adjacent to normal, benign (B) glands top and left (H-E).

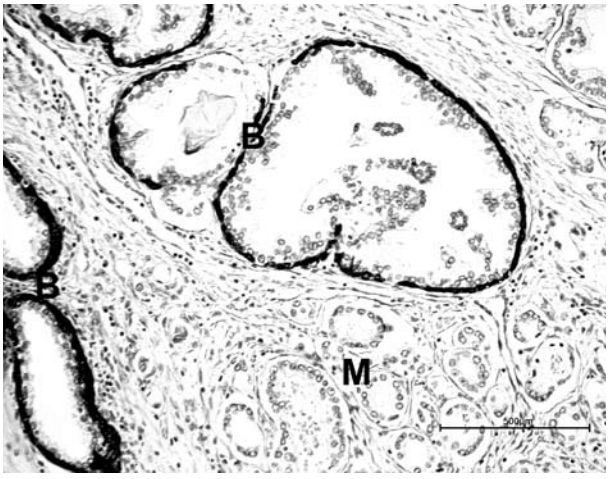

Figure 8. Malignant glands adjacent to normal glands (same focus): malignant glands show no reactivity (antikeratin 34βE12 immunohistochemical stain).

basal cells with 34βE12 (Figure 2). In 3 of the cases additional foci of atypical adenosis were observed in H - E sections (Figure 3). 34βE12 labeling of basal cells ruled out the possibility of malignancy (Figure 4). Multiple high grade Prostatic Intraepithelial Neoplasia(PIN) foci were present in the 5 cystoprostatectomy specimens (Figure 5) showing characteristic discontinuous distribution of basal cells as marked by 34βE12 (Figure 6). Finally all 5 radical prostatectomy specimens included multiple foci of carcinoma (Figure 7) as well as PIN. As expected malignant glands showed no reactivity for 34βE12 (Figure 8). Evidently Antikeratin 34bE12 confirmed our initial diagnosis in all 12 cases and was the key to the differential diagnosis of the 3 atypical lesions.

CONVERSATION

The normal prostate is composed of glands and stroma[3,4]. The former consists of acini and ducts, both of which are lined by secretory, basal and occasional neuroendocrine cells. Secretory cells are tall, columnar cells placed towards the luminal surface. They are known to synthesize prostatic acid phosphatase and prostatic specific antigen, two organ specific proteins which play a major role in prostate diagnostics as they

can be detected by immunohistochemistry[12]. Basal cells form up a thin, continuous layer between secretory cells and basal lamina. They are elongated, flat cells situated parallel to the basal lamina, with relatively scarce, mostly inconspicuous cytoplasm and a small deeply basophilic nucleus. Proliferation and maturation of these cells towards the luminal surface leads to the regeneration of the secretory cell layer. Among other cytokeratins expressed in the cytoplasm of basal cells 34βE12 is immunohistochemically detected by using a monoclonal antibody keratin 34βE12. This positive staining reaction facilitates the detection of basal cells in cases they are morphologically not discernible in H-E sections, thus clarifying the benign or malignant nature of ambiguous glands.

BH is a common benign condition typically presenting with swelling of the prostate which if intense can lead to urethral obstruction and dysouria. Microscopically both glands and stroma are hyperplastic[3]. The epithelium ranges from flat to columnar and formation of papillary structures is a common feature. The basal cell layer is retained just like in normal glands and basal lamina is well formed. All cases of BH included in our study corresponded to the morphology fore mentioned. Occasionally small foci of adenosis can be observed[3] among hyperplastic glands and as shown in 3 of our cases with such "atypical" morphologic features, positive staining with 34βE12 substantially helps rule out the possibility of malignancy.

PIN refers to the neoplastic change of prostatic acinar and ductal epithelium[13] and is believed to be a precancerous condition[14]. Morphologic features of PIN are observed within preexisting almost normal sized glands and involve the secretory cell layer. In summary they consist of cellular crowding and stratification, nuclear enlargement (pleomorphism and chromatin pattern alterations), and prominent nucleolus. PIN is divided into two grades (low-grade and high-grade). The differential diagnosis between the two grades is mainly based on nuclear features and in particular the size of the nucleolus. Fragmentation of the basal cell layer is a distinctive finding but often requires immunohistochemical studies with 34βE12 as shown in our cases. The incidence of PIN is related to the patients' age in the sense that it increases along with age progression. In our study PIN was present in all 5 cystoprostatectomy specimens coming from elderly patients with urothelial cell carcinoma in accordance to the age-related incidence stated above. The relationship between high-grade PIN and carcinoma has been well documented given that the former is identified in 60% up to 100% of radical prostatectomy specimens[3], a statistical correlation also expressed in our study. All 5 specimens with carcinoma had foci of co-existent PIN. This correlation makes PIN a highly sensitive predictive marker for concomitant or successive carcinoma and if found in biopsy, patient follow-up is necessary[15]. Low-grade PIN on the other hand is a relatively common finding in young adults and does not seem to be directly related to carcinoma[16]. It is for this reason that low-grade PIN can be excluded from the pathology report[17].

Prostatic adenocarcinoma is an invasive, epithelial, malignant neoplasm arising from the secretory cells of the prostate. It is quite frequent and mostly affects men over the age of 50. On morphologic grounds it presents with a variety of architectural patterns depending on the level of the tumor differentiation. One side of the spectrum is occupied by well differentiated morphologically benign-looking glands the malignant nature of which is often difficult to assert. The other side includes poorly differentiated tumors not at all indicative of their histological origin. The only similarities these two extreme phenotypes share are the presence of a single cell type and the loss of the basal lamina. The majority of carcinomas however are moderately differentiated. The general morphology is associated with crowding of minor to normal size glands lined by a single cell layer with enlarged nuclei, prominent nucleoli and amphophilic cytoplasm. In most cases loss of basal cell layer is detected on routine H-E sections but can also be confirmed by 34βE12 immunohistochemical staining. The latter is indispensable for the diagnosis of well differentiated tumors that mimic hyperplasia. 34βE12 is also useful in cases of so called «minimal cancer» in which tumor biopsy sampling is insufficient for establishing a definite diagnosis as well as in borderline lesions known as Atypical Small Acinar Proliferation(ASAP)[18,19].

Basal cells are not always easy to distinguish on simple H-E sections. Conditions such as atrophy and adenosis which are benign in their nature are well known morphological mimics of carcinoma, as it is

often impossible for the pathologist to confirm a definite view of the basal cell layer. This is a serious diagnostic pitfall given that lack of basal cells in small, newly formed prostatic acini is a major criterion of invasive carcinoma. In such cases 34βE12 plays a key role in the differential diagnosis[7,20]. In our study 3 foci of glands mimicking cancer were finally rendered a non-malignant diagnosis (atypical adenosis) after positive staining of basal cells with 34βE12.

In recent years new markers of basal cells such as CK5/6[21] and p63[22,23,24] have been introduced, however as is the case with 34βE12, they are negative markers for carcinoma a fact that limits their diagnostic liability. Alpha-methylacyl-CoA racemase (AMACR) is a newly discovered positive marker of prostatic adenocarcinoma with a reported sensitivity of 97%-100%[25,26]. An immunohistochemical cocktail of AMACR with p63 has already been used in ambiguous lesions showing even better results[27]. However the use of these new markers is still limited and so is the experience of pathologists to appreciate them.

Continuous efforts are being made towards the improvement of diagnostic comprehension of prostatic specimens. Research still aims at the elimination of false positive or negative results especially concerning biopsies in which diagnostic accuracy is restricted by the small amount of tissue submitted for examination.

Η χρησιμότητα του ανοσοϊστοχημικού δείκτη 34βE12 (keratin 903) στη διαγνωστική του προστάτη.

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ΠΕΡΙΛΗΨΗ: Ο κλώνος 34βE12 της ανθρώπινης κυττοκερατίνης είναι ένα υψηλού μοριακού βάρους μονοκλωνικό αντίσωμα που ανιχνεύει μια ποικιλία επιθηλιακών ιστών και χρησιμοποιείται ευρέως ως δείκτης βασικών κυττάρων. Με τον κλώνο 34βE12 αποδίδεται θετική χρώση στο κυτταρόπλασμα του πλακώδους επιθηλίου του δέρματος, σε μερικά πνευμονοκύτταρα, στο βρογχικό επιθήλιο και στους χοληδόχους πόρους του φυσιολογικού ήπατος. Αντιδρά επίσης με κύτταρα των πόρων του φυσιολογικού παγκρέατος, του μαστού, του ουροθηλίου και βέβαια με τα βασικά επιθηλιακά κύτταρα του προστάτη. Σκοπός της παρούσης εργασίας είναι η ανάδειξη της χρησιμότητας του ανοσοϊστοχημικού δείκτη κερατίνη 34βE12 στη διαγνωστική του προστάτη. Το υλικό της εργασίας προέρχεται από το Παθολογοανατομικό Εργαστήριο του Γ.Ν.Θ. «Γ. Γεννηματάς». Μελετήθηκαν συνολικά 15 παρασκευάσματα προστατεκτομής εκ των οποίων 5 με υπερπλασία (ΚΥ), 5 με καρκίνωμα και 5 με ενδοεπιθηλιακή προστατική νεοπλασία (PIN) υψηλού βαθμού, σε αντιπροσωπευτικές τομές των οποίων εφαρμόσθηκε ο δείκτης κυττοκερατίνη 34βE12. Η κερατίνη 34βE12 είχε φυσιολογική κατανομή σε όλες τις περιπτώσεις με καλοήθη υπερπλασία, έδωσε θετική χρώση σε μειωμένο αριθμό βασικών κυττάρων σε όλες τις περιπτώσεις με PIN, ενώ αντίθετα ήταν αρνητική σε όλες τις περιπτώσεις με καρκίνωμα. Η κερατίνη 34βE12 δίνει θετική χρώση μόνο στα βασικά κύτταρα το οποία βρίσκονται σε συνεχή γραμμή πάνω από τη βασική μεμβράνη, ενώ αποδίδεται αρνητική στα εκκριτικά κύτταρα του προστάτη ή τα στρωματικά κύτταρα. Στην ΚΥ η στιβάδα των βασικών κυττάρων αναδεικνύεται συνεχής, στην PIN εμφανίζεται διακεκομμένη, ενώ στο καρκίνωμα απουσιάζει εντελώς. Έτσι λοιπόν στις περιπτώσεις όπου δεν είναι δυνατόν να τεθεί η διάγνωση με την κλασσική χρώση αιματοξυλίνης - εοσίνης, μπορεί να γίνει διαφορική διάγνωση μεταξύ καλοήθους υπερπλασίας, PIN και καρκινώματος προστάτη, με βάση τη διαφορετική έκφραση της κερατίνης 34βE12 στα βασικά κύτταρα του προστάτη .

Λέξεις Κλειδιά: 34βE12, Κερατίνη 903, Κυττοκερατίνη, Βασικά κύτταρα, Προστάτης.

REFERENCES

1. Brawer MK, Peehl DM, Stamey TA, Bostwick DG. Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 1985;45:3663-67.
2. Gown AM, Voyel AM. Monoclonal antibodies to human intermediate filament proteins. II. Distribution of filament proteins in normal human tissues. Am J Pathol 1984;309-321.
3. Rosai J. Surgical Pathology. Philadelphia. Mosby, 2004:1361-1385.
4. Dabbs D. Diagnostic Immunohistochemistry. Philadelphia. Churchill Livingstone, 2002:409-413.
5. Goldstein NS, Underhill J, Roszka J, Neill JS. Cytokeratin 34βE12 immunoreactivity in benign prostatic aciny. Quantitation, pattern assessment and electron microscopic study. Am J Clin Pathol 1999;112:69-74.
6. Googe PB, McGinley KM, Fitzgbbon JF. Anticytokeratin antibody 34βE12 staining in prostate carcinoma. Am J Clin Pathol 1997;107:219-223.
7. Hedrick L, Epstein JI. Use of keratin 903 as an adjunct in the diagnosis of prostate carcinoma. Am J Surg Pathol 1989;13:389-96.
8. Wojno KJ, Epstein JI. The utility of basal cell-specific anticytokeratin antibody(34βE12) in the diagnosis of prostate cancer. A review of 228 cases. Am J Surg Pathol 1995;19:251-260.
9. Yang XJ, McEntee M, Epstein JI. Distinction of basaloid carcinoma of the prostate from benign basal cell lesions by using immunohistochemistry for bcl-2 and ki67. Hum Pathol 1998;29:1447-1450.
10. Yang XJ, Lecksell K, Gaudin P, Epstein JI. 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 1999;23:147-52.
11. Samaratunga H, Singh M. Distribution patterns of basal cells detected by cytokeratin 34beta E12 in primary prostatic duct adenocarcinoma. Am J Surg Pathol 1997;21:435440.
12. Mills S. Histology for pathologists. Philadelphia. Lipincott Williams & Wilkins, 2007:932-35.
13. Pathology and Genetics. Tumors of the urinary system and male genital organs. Lyon.World Health Organization Classification of Tumours, 2004:172-174.
14. Bostwick D and Qian J. High-grade prostatic intraepithelial neoplasia. Modern Pathology 2004;17:360-379.
15. Brower MK, Bigler SA, Sohlberg OE, Nagle RB Lange PH. Significance of prostatic intraepithelial neoplasia on prostate needle biopsy. Urology 1991;38:103-107.
16. Jakr WA, Hans GP, Cassin BF, Pontes JF, Crissman JP. The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. J Urol 1993;150:379-385.
17. Skjorten FJ, Berner A, Harvei S, Robsahm TE, Tretti S. Prostatic intraepithelial neoplasia in surgical resection: relationship to coexistent adenocarcinoma and atypical adenomatous hyperplasia of the prostate. Cancer 1997;79:1172-1179.
18. Bostwick D και Meiers I. Atypical small acinar proliferation in the prostate. Arch Pathol Lab Med 2006;130:952-957.
19. Cheville JC, Reznicek MJ, Bostwick DG. The focus of «atypical glands suspicious for malignancy» in needle biopsy specimens. Incidence, histologic features and clinical follow up of cases diagnosed in a community practice. Am J Clin Pathol 1997;108:633-640.
20. Young RH, Srigley JR, Amin MB, Ulbright TM, Cubilla AC. Tumors of the prostate gland, seminal vesicles male urethra and penis (fascicle28). Washington DC: 3rd Edition. AFIP, 2000:192.
21. Abrahams N.A., Ormsby A.H., Brainard J. Validation of cytokeratin 5/6 as an effective substitute for keratin 903 in the differentiation of benign from malignant glands in prostate needle biopsies. Histopathology 2002;41:35-41.
22. Signoretti S, Waltregny D, Dilks J et al. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000;157:1769-1775.
23. Kauffman O, Fietze E, Mengs J, Dietel M. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001;116:823-830.
24. Shah RB, Zhou M, LeBlanc M, Snyder M, Rubin MA. Comparison of the basal cell specific markers 34betaE12 and p63, in the diagnosis of prostate cancer. Am J Surg Pathol 2002;26:1161-1168.
25. Xu J,Stolk JA, Zhang X et al. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. Cancer Res, 2000;60:1677-1682.
26. Jiang Z, Wu CL, Woda BA, Dresser K, Xu J, Fanger GR et al. P504S/alpha-methylacyl-CoA racemase: a useful marker for diagnosis of small foci of prostatic carcinoma on needle biopsy. Am J Surg Pathol 2002;26:1169-1174.
27. Molinie V and al. Diagnostic utility of a p63/a-methyl-CoA racemase (p504s) cocktail in atypical foci in the prostate. Modern Pathology 2004;17:1180-1190.

Abbreviations

BH: Benign Hyperplasia
PIN: Prostatic Intraepithelial Neoplasia
H-E: Hematoxylin - Eosin
AMACR: a-methylacyl CoA Racemase