Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 2, 2010, pp. 11-16

IMMUNOHISTOCHEMISTRY METHOD PRECEDED BY TISSUE TRANSFER - A RELIABLE ALTERNATIVE TO CURRENT PRACTICE OF PROSTATE PATHOLOGY

Denisa ANDERCO[1]*, Elena LAZAR[2], Angela BORDA[3], Sorina TABAN[2], Felix MIC[1], Andrada LOGHIN[3], Alis DEMA[2]
[1]Department of Pathology, Emergency County Hospital, Timisoara, Romania
[2]Department of Pathology, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania
[3]Department of Histology, University of Medicine and Pharmacy Targu Mures, Romania

ABSTRACT. In the context of increased incidence of the prostate cancer, the pathologists are more frequently faced with the task to establish a diagnosis on quantitatively reduced biopsy specimens. Sometimes the usual diagnostic methods do not allow a definite morphological diagnosis due to the limited nature of suspicious lesions and inability to evaluate its architecture. In addition, the loss of these atypical foci from the paraffin blocks during repeated sections is not an isolated phenomenon. In this regard, we reassessed the cases with equivocal diagnoses established on prostate biopsy specimens in our hospital over the past 2 years. We performed the tissue transfer on charged slides, followed by AMACR/p63 cocktail immunostaining in order to establish a certain diagnosis. Following this procedure we established a final diagnosis in 17 of the 19 cases (89.47%). We claim that this method is a reliable alternative to the classical processing of minute atypical glandular lesions, when minimum two H&E–stained sections that harbour suspicious foci are available.

Keywords: prostate biopsy; diagnosis; immunohistochemistry; AMACR/p63 cocktail

INTRODUCTION

With the widespread use of the prostate specific antigen (PSA) screening there has been a remarkable change in prostate cancer detection and staging, toward an increased incidence of the low-volume, low-stage diseases [Shah, 2009; Mancuso et al., 2007; Paner et al., 2008; Cheng et al., 2005; Epstein et al., 2008; Bostwick et al, 2008]. Although lately pathological evaluation of prostate biopsy (PB) specimens has achieved a real progress in terms of diagnosis accuracy and comprehensiveness, diagnosis certainty can sometimes be a challenge task when it is carried on PB fragments, especially in cases with very limited atypical foci. In these situations, immunohistochemistry (IHC) using single antibodies, but especially in antibodies cocktails may be useful to confirm the presence of a minimal cancer and to exclude the cancer's mimickers. Prostate cancer is morphologically characterized by the absence of basal cell layer highlighted by specific antibodies against basal cells (HMWCK, p63) [Paner et al., 2008; Epstein et al., 2008] and, more recently, by concomitant and selective alpha-methylacyl-CoA racemase (also known as AMACR/P504S) overexpression [Epstein et al., 2008; Browne et al., 2004; Jiang et al., 2004; Zhou et al., 2004; Carswell et al., 2006]. Compared to the use of individual antibodies, the use of the antibody cocktails led to increased diagnostic efficiency [Paner et al., 2008; Hameed et al., 2005; Molinié et al., 2004; Sanderson et al., 2004], with the decrease of false negative and false positive results. Not infrequently, however, the minute suspicious lesions are lost during successive sections from the paraffin blocks and additional IHC methods become useless. For this particular situation, the immunostaining of previously H&E-stained section can be a useful approach to establish a certain diagnosis.

The aim of this study is to evaluate the diagnostic usefulness of AMACR/p63 cocktail performed on the initial H&E-stained sections, transferred on adhesive slides.

MATERIALS AND METHODS

From the files of Department of Pathology, Timisoara Emergency County Hospital, we selected a group of 21 cases with equivocal diagnoses established on PB specimens over the past 2 years, in which the tissue material containing limited atypical glandular lesions was lost during successive sections from the block. Among them, the cases which had a single H&E-stained section on the slide were excluded (n = 2).

The other 19 cases underwent tissue transfer from the H&E - stained slides on silanized slides (Dako Silanized Slides, Code 3003), following the protocol described by Hameed & Humphrey [Hameed et al., 2005] (figure 1):

1) H&E - stained slides were marked to delineate the section which requires transfer;

2) the coverslips were removed by soaking in xylene at room temperature;

3) then slides were rinsed in fresh xylene to remove all traces of mounting medium;

***Correspondence:** Anderco Denisa, Department of Pathology, Timisoara Emergency County Hospital, Iosif Bulbuca Street no. 10, Timisoara 300736, Timis, Romania, email: denisa_dobre@yahoo.com*
Article received: December 2009; published: May 2010

4) the section that required transfer was completely covered with liquid mounting medium;

5) slides were placed for 2 h at 600C oven until the mounting medium hardened and formed a meniscus;

6) slides were immersed in hot water for minimum 2 hours, so that the mounting medium could be easily detached;

7) with a scalpel blade, the meniscus including mounting medium and tissue fragments was carefully split and then transferred on a silanized slide, moistened with water for a better adhesion;

8) slides were left to dry for 2 hours in the 600°C oven;

9) slides were then dewaxed in xylene and rehydrated through successive baths of absolute alcohol to distilled water.

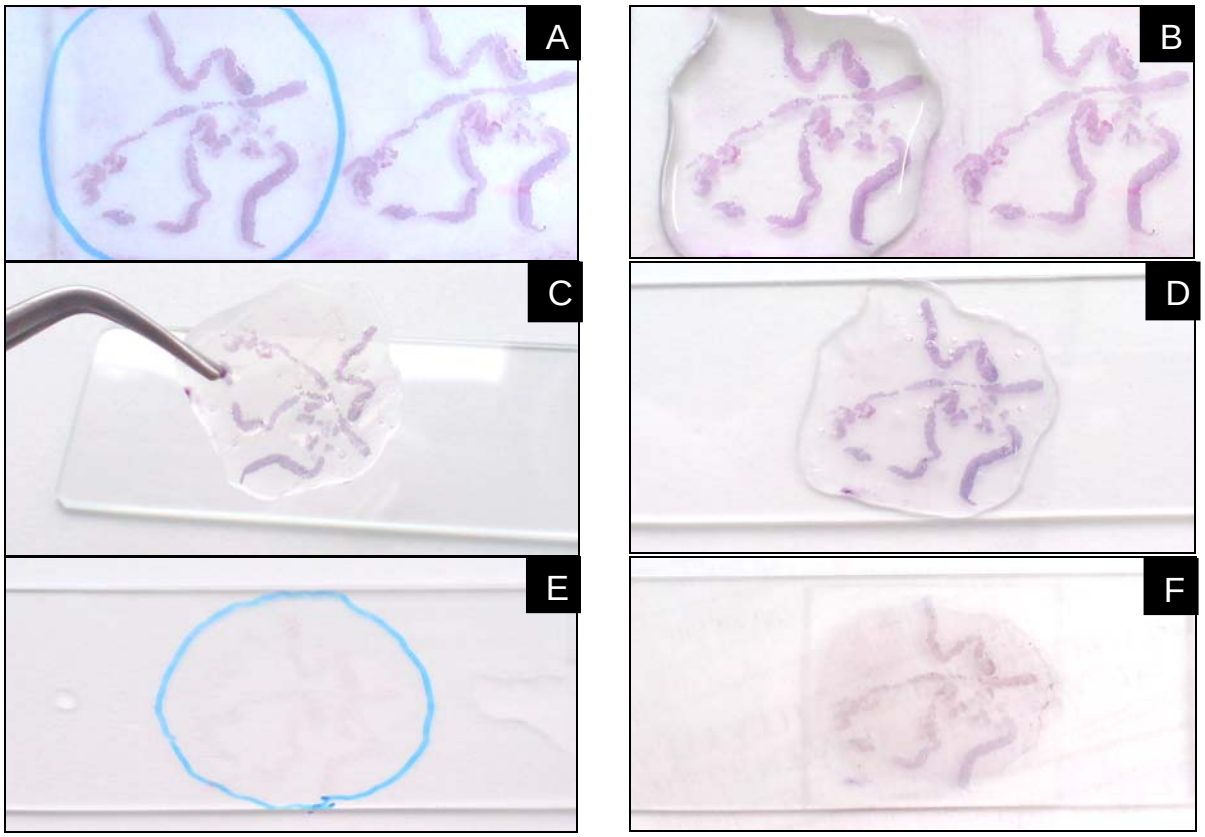

Fig. 1 A. Gross appearance of the H&E-stained slide (prostate biopsy specimen). B. The liquid coverglass medium covers the left section that requires transfer. C. The meniscus including mounting medium and tissue fragments is carefully split and then transferred on a silanized slide. D. The section is already mounted on the silanized slide. E. The section is now completely destained after HIER procedure. F. Final gross appearance of immunostained slide.

The slides were further immunostained with AMACR/p63 cocktail, following the usual procedure in our laboratory:

1. Blocking the endogenous peroxidase activity with 0.3% hydrogen peroxide for 10 minutes.

2. Antigen unmasking using HIER method (Heat Induced Epitopes Retrieval – this step also allowed destaining of uncovered section) and buffer solution (DakoCytomation Target Retrieval Solution, Citrate pH 6, code S2369, 1:10 dilution).

3. Incubation with AMACR/p63 cocktail (Abcam AMACR + p63 antibody [4A4(p63)] cocktail of mouse monoclonal and rabbit polyclonal, code ab14202, 1:80 dilution) for 30 minutes.

4. Application of secondary HRP-conjugated antibody (Abcam Mouse IgG + IgM + IgA secondary antibody, prediluted, code ab2891) for 30 minutes.

5. Incubation with 3, 3' - diaminobenzidine tetrahydrochloride (DAB) solution for 5-15 minutes.

6. Post-chromogenic enhancement with 4 % copper sulfate solution; 7. Nuclear counterstaining with hematoxylin; 8. Dehydration of the sections; 9. Coverslips mounting.

RESULTS

Of the 19 cases that initially had an equivocal diagnosis, the immunostaining with AMACR/p63 cocktail established a precise diagnosis in 17 cases (89.47%). Of the latter, the malignant nature was

certified in 6 cases (32.59%). The rest of 11 cases showed benign/nonmalignant features (64.70%). Only 2 of the 19 sections (10.5%) fell off the slides during the transfer procedure. IHC reaction was performed in optimal conditions in all other cases. AMACR expression was considered positive when intense, granular, circumferential, cytoplasmic, apical stronger signal was identified (figure 2). Positive reaction for p63 was easily recognizable as nuclear signal of moderate / high intensity, in frankly benign or non-malignant glands. A few cases showed a nonspecific background staining, most likely caused by chromogen spreading for p63 in the neighboring structures, but without compromising the interpretation of the reaction. A slight variation of the intensity of IHC reactivity was observed between different cases and it was correlated with slides aging. At the very beginning we have also noticed a lower intensity of immunostaining but still satisfactory for interpretation, for the slides where the water was probably not completely removed from the tissue (we have corrected this issue leaving the slides in xylene for a good amount of time after transfer). The IHC interpretation was performed in conjunction with H&E morphology, as we usually proceed.

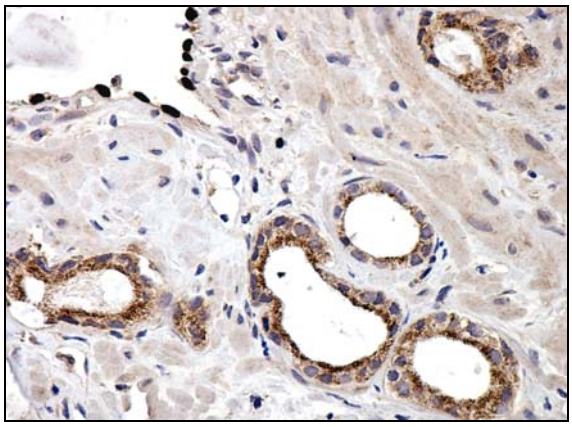

Fig. 2 AMACR/p63 cocktail immunostaining showing a focus of limited carcinoma with strong expression for AMACR adjacent to a benign prostatic gland positive for p63

DISCUSSIONS

Prostate cancer may be diagnosed at an early stage through PSA testing followed by PB sampling, and patients with organ-confined disease may be cured by radical prostatectomy or radical radiotherapy [Bryant et al., 2009]. Discrimination of prostate cancer from its benign mimickers on small PB specimens is a real challenge for the general pathologist and the uropathologist, too. In order to facilitate the morphological diagnosis, precise criteria were defined for limited/minimal carcinoma and atypical small acinar proliferation suspicious but insufficient for malignancy (ASAP). The limited/minimal carcinoma is defined as affecting less than 5 % of the examined area or a length of less than 0.5 mm of PB fragment [Epstein et al., 2008; Bostwick et al., 2008; Hameed et al., 2005; Epstein, 2004]. ASAP term denotes the presence of minute focus showing atypical glands, but which do not meet all/enough cytological or architectural criteria to support a diagnosis of malignancy [Mancuso et al., 2007; Epstein et al., 2008; Bostwick et al., 2008; Bostwick et al., 2006]. Compared with minimal carcinoma, ASAP is characterized by smaller size (average length of atypical foci is 0.4 mm in ASAP vs. 0.8 mm in minimal cancer), reduced number of involved acini (on average 11 suspicious glands in ASAP vs. 17 acini in limited cancer), lack of infiltrative growth pattern (a criteria difficult to estimate on biopsy cores anyway), absence of mitotic figures (even if this is not a frequent feature in prostate cancer neither), prominent nucleoli in less than 10% of cells, a lower incidence of hypercromasia, large nucleoli and absence of acidic mucins secretion [Bostwick et al., 2008]. The ASAP term denotes not an individual entity, but rather brings together various prostatic lesions (atrophy, basal cell hyperplasia, adenosis, reactive atypia or a marginally biopsied cancer) [Mancuso et al., 2007; Paner et al., 2008; Bostwick et al., 2008; Epstein et al., 2008; Flury et al., 2007]. So ASAP represents an equivocal pathological diagnosis and a signal for the urologist concerning the existence of an increased risk of subjacent cancer [Mancuso et al., 2007; Bostwick et al., 2008; Lopez, 2007; Schlesinger et al., 2005; Iczkowsky, 2006]. This is why the ASAP diagnosis requires to repeat the biopsy in 3 to 6 months [Mancuso et al., 2007; Bostwick et al., 2008; Bostwick et al., 2006; Iczkowsky, 2006] with the recommendation to extend the PB sampling to both prostatic lobes [Mancuso et al, 2007; Epstein et al., 2008; Iczkowsky, 2006], for a better “mapping” of the whole gland.

Many ASAP lesions can be elucidated by IHC. Several markers are used as ancillary methods to morphological diagnostic criteria: antibodies against basal cells (HMWCK and p63) in conjunction with an antibody specific for the prostate cancer (AMACR/p504S). Additionally, the recently introduction of antibody cocktails has been used to establish a certain diagnosis in minute suspicious foci. Some studies proved the malignant nature of the lesion in up to 47 % of initially ASAP dignoses after AMACR/p63 cocktail immunostaining (Molinié et al, 2004). However IHC cannot be performed when atypical glands dissappear during successive sections, a rather frequent issue in these cases with minute suspicious lesions identified on PB specimens [Bostwick et al., 2008; Hameed et al., 2005]. Several methods have been proposed during the last years hoping to solve these issues.

In 1999, Green and Epstein made a comparison between the utility of saving intervening unstained sections versus cutting new sections from the paraffin blocks in a group of 94 PB. HMWCK immunostaining

was done in both conditions and in 31 cases; the lesion identified on the intermediate levels has disappeared in subsequent levels. Thus a precise diagnostic could be established in only 31 of the 94 biopsies (32.98%) through saving intervening sections. On the same topic, the latest papers (Hameed et al., 2009; Paner et al., 2008; Epstein et al., 2008) encourage the practice of saving unstained sections of PB specimens for potential IHC evaluation.

Another approach belongs to Dardik and Epstein, 2000, who destained and then marked with HMWCK a group of 105 PB with minute atypical lesions that were initially H&E-stained. Working in this manner, they established a definite diagnosis in 58 % of the cases. In 19 % of the cases the immunostaining failed and in another 9% the suspected lesion was lost during processing, even if charged slides were used.

Tissue protection immunohistochemistry (TPI) is another technique conceived by Miller & Kubier in 2002, which consists in IHC staining of an initially H&E-stained section, without any prior destaining. The method requires from the beginning the use of adhesive slides to ensure a good adhesion of tissue sections, eliminating the risk of their detachment during HIER (heat induced epitope retrieval) processing. As we claimed in a previous work [Dema et al., 2010], the TPI allows the simultaneous examination on the same slide, in optimal conditions, of the H&E - stained section and of the immunostained one.

The most recent approach on this topic belongs to Hameed & Humphrey, 2005, and implies the tissue transfer of limited suspicious proliferations on charged slides followed by immunostaining with antibodies cocktail. The technique was initially described for the difficult cytological smears with the purpose of establish a precise diagnosis [Miller et al., 2002; Gong et al., 2005; Hunt et al., 1998]. This situation involves processing a cell block to allow further testing of several antibodies reactivity [Miller et al., 2002]. Hameed & Humphrey have adapted this technique for PB specimens including small foci of atypical glands, then they performed IHC using AMACR/p63 cocktail. The immunostaining has been successful in 97% of the cases. The study aimed a comparison between IHC performed on stored sections transferred on adhesive slides and new cut sections from the block. The results were excellent for those sections not older than a month, which had a very similar signal with the new cut ones. In the case of older sections, the authors have noticed a much decreased intensity of p63 staining, and only a slightly reduced expression for AMACR. Other authors reported similar results in older stored tissues [Burford et al., 2009; Ali et al., 2008; Vanguri et al., 2006].

Our study also demonstrates the utility of AMACR/p63 cocktail in reducing the incidence of equivocal diagnoses in favor of diagnostic certainty. In our experience the step of tissue transfer from H&E - stained slides on silanized slides was simple, easily reproducible, time saving (it can be performed during regular work of a laboratory technician) and cost-effective. We have tested both methods of epitopes retrieval, through HIER and enzyme digestion, and both of them were successful, with minimum loss of tissue. The reduced rate of tissue loss during IHC recommends the use of this method in the evaluation of atypical minute lesions which are not found any more on deeper sections. Anyway, at least one H&E - stained section must be preserved. We have also estimated the cost of one case processed in the previous manner as being slightly lower than that of a TPI processed section which requires from the beginning the use of charged slide, and much cheaper than regularly saving unstained sections.

CONCLUSIONS

In conclusion, the AMACR/p63 cocktail immunostaining realized on transferred PB sections containing minute atypical lesions is a reliable alternative to current management of prostate biopsy objectified in a decreased number of equivocal diagnoses and the avoidance of the anxiety, costs and discomfort associated with repeated biopsies.

ACKNOWLEDGEMENTS

This work was supported by grant no 42126/2008, National Program of Research, Development and Innovation-Partnerships, Direction 4 Health. *Financing institution:* National Center of Programs Management under the Ministry of Education and Research, Romania.

REFERENCES

Ali TZ, Epstein JI, False positive labeling of prostate cancer with high molecular weight cytokeratin: p63 a more specific imunomarker for basal cells. Am J Surg Pathol, 32(12),1890-1895, 2008.

Bostwick DG, Meiers I, Neoplasms of the prostate. In: Bostwick DG, Cheng L (eds) Urologic Surgical Pathology, 2nd ed. Mosby Elsevier, Philadelphia, 462-473, 2008.

Bostwick D, Meiers I, Atypical small acinar proliferation in the prostate. Arch Pathol Lab Med, 130, 952-957, 2006.

Briant RJ, Hamdy FC, Screening for prostate cancer: un update. Eur Urol,53, 37-44, 2009.

Browne TJ, Hirsch MS, Brodsky G, Welch WR, Loda MF, Rubin MA, Prospective evaluation of AMACR (P504S) and basal cell markers in the assessment of routine prostate needle biopsy specimens. Hum Pathol, 35(12), 1462-1468, 2004.

Burford HN, Adams AL, Hameed O, Effect of storage on p63 immunohistochemistry: a time - course study. Appl Immunohistochem Mol Morphol,17(1), 68-71, 2009.

Carswell BM, Woda BA, Wang X, Li C, Dresse K, Jiang Z, Detection of prostate cancer by alpha-metylacyl CoA racemase (P504S) in needle

biopsy specimens previously reported as negative for malignancy. Histopathology, 48(6), 668-673, 2006.

Cheng L, Poulos CK, Pan CX, Jones TD, Daggy JK, Able JN, Koch MO, Preoperative prediction of small volume cancer (less than 0,5 ml) in radical prostatectomy specimens. J Urol,174 (3), 898–902, 2005.

Dardik M, Epstein JI, Efficacy of restaining prostate needle biopsies with high-molecular weight cytokeratin. Hum Pathol, 31(9),1155-1161, 2000.

Dema A, Anderco D, Taban S, Lazar E, Cornianu M, Lazureanu C, Herman D, Muresan A, Cornea R, Faur A. Useful immunohistochemical techniques for elucidating minute atypical glandular proliferations in prostate biopsy specimens – preliminary results. Biology and therapy of cancer cell. Cluj-Napoca, 28 – 30 april, 2010, pp. 39. Abstract in Official Journal of "Prof. Dr. Ion Chiricuta" Oncology Institute Cluj-Napoca, vol. 2, suppl., ISSN: 2066 – 7558, ISBN: 978-973–75 –308–7.

Epstein JI, Netto GJ (eds), Biopsy interpretation of the prostate, 4th edn. Lippincott Williams & Wilkins, Philadelphia, 2008.

Epstein JI, Diagnosis and reporting of limited adenocarcinoma of the prostate on needle biopsy. Mod Pathol, 17, 307-315, 2004.

Flury SC, Galgano MT, Mills S, Smolkin ME, Theodorescu D, Atypical small acinar proliferation: biopsy artefact or distinct pathological entity? BJU Int, 99, 780-785, 2007.

Gong Y, Joseph T, Sneige N, Validation of commonly used immunostainson cell-transferred cytologic specimens. Cancer, 105, 158-164, 2005 .

Green R, Epstein JI, Use of intervening unstained slides for immunohistochemical stains for high molecular weight cytokeratin on prostate needle biopsies. Am J Surg Pathol, 23(5), 567-570, 1999.

Hameed O, Humphrey PA, Immunohistochemical evaluation of prostate needle biopsies using saved interval sections vs new recut sections from the block: a prospective comparison. Am J Clin Pathol,131(5), 683-687, 2009.

Hameed O, Sublett J, Humphrey PA, Immunohistochemical stains for p63 and alpha-metylacyl-CoA racemase, versus a cocktail comprising both, in the diagnosis of prostatic carcinoma: a comparison of the immunohistochemical staining of 430 foci in radical prostatectomy and needle biopsy tissues. Am J Surg Pathol, 29(5), 579-587, 2005.

Hameed O, Humphrey PA, p63/AMACR antibody cocktail restaining of prostate needle biopsy tissues after transfer to charged slides. A viable approach in the diagnosis of small atypical foci that are lost on block sectioning. Am J Clin Pathol, 124, 708-715, 2005.

Hunt JL, van de Rijn M, Gupta PK, Immunohistochemical analysis of gel-transferred cells in cytologic preparations following smear division. Diagn Cytopathol, 18, 377-380, 1998.

Iczkowski KA, Current prostate biopsy interpretation. Criteria for cancer, atypicall small acinar proliferation, high-grade prostatic intraepithelial neoplasia, and use of immunostains. Arch Pathol Lab Med, 130, 835-843, 2006.

Jiang Z, Woda BA, Wu C, Yang X, Discovery and clinical applications of a novel prostate cancer marker. Am J Clin Pathol, 122, 275-289, 2004 .

Kubier P, Miller RT, Tissue protection immunohistochemistry. A useful adjunct in the interpretation of prostate biopsy specimens and other selected cases in wich immunostains are needed on minute lesions. Am J Clin Pathol, 117, 194-198, 2002.

Lopez JL, Prostate adenocarcinoma detected after high - grade prostatic intraepithelial neoplasia or atypical small acinar proliferation. BJU Int, 100, 1272–1276, 2007.

Mancuso PA, Chabert C, Chin P, Kovac P, Skyring T, Watt WH, Napaki S, Prostate cancer detection in men with an initial diagnosis of atypical small acinar proliferation. BJU Int, 99(1), 49-52, 2007.

Miller RT, Kubier P, Immunohistochemistry on cytologic specimens and previously stained slides (when no paraffin block is available). J Histotechnol, 25(4), 251-257, 2002.

Molinié V, Fromont G, Sibony M, Vieillefond A, Vassiliu V, Cochand-Priollet B, Herve JM, Lebret T, Baglin AC, Diagnostic utility of a p63/α - methyl-CoA racemase (p504s) cocktail in atypical foci in the prostate. Mod Pathol, 17, 1180–1190, 2004.

Paner GP, Luthringer DJ, Amin MB, Best practice in diagnostic immunohistochemistry. Prostate carcinoma and its mimics in needle core biopsies. Arch Pathol Lab Med, 132, 1388–1396, 2008.

Sanderson SO, Sebo TJ, Murphy LM, Neumann R, Slezak J, Cheville JC, An analysis of the p63/α-methylacyl coenzyme A racemase immunohistochemical cocktail stain in prostate needle biopsy specimens and tissue microarrays. Am J Clin Pathol, 121, 220-225, 2004.

Schlesinger C, Bostwick DG, Iczkowski KA, High – grade prostatic intraepithelial neoplasia and atypical small acinar proliferation: predictive value for cancer in current practice. Am J Surg Pathol, 29(9), 1201–1207, 2005.

Shah RB, Current perpectives on the Gleason grading of prostate cancer. Arch Pathol Lab Med, 133, 1810-1816, 2009.

Vanguri VK, Woda BA, Jiang Z, Sensitivity of P504S/alpha – metylacyl – CoA racemase (AMACR) immunohistochemistry for the

detection pf prostate carcinoma on stored needle biopsies. Appl Immunohistochem Mol Morphol, 14(3), 365-368, 2006.

Zhou M, Aydin H, Kanane H, Epstein JI, How often does alpha-metylacyl-CoA-racemase contribute to resolving an atypical diagnosis on prostate needle biopsy beyond that provide by basal cell markers? Am J Surg Pathol, 28(2), 239-243, 2004.