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Algorithm optimization of pathoanatomical diagnosis of prostate cancer with application of an immunohistochemical method

ABSTRACT

Pathological differential diagnosis of primary high-grade prostatic adenocarcinoma (Gleason sum 6) and benign prostatic hyperplasia in transrectal prostate biopsy has been conducted with using an optimized panel of antibodies to cytokeratin 34βE12, cytokeratins 5 and 6, protein P63, AMACR, PSA and/or PSAP, which in 100% of cases conduced to establish the appropriate diagnosis. This immunohistochemical analysis can be recommended for carrying out life-time pathotogical diagnostics of prostate cancer besides the traditional research of a material from 12-24 points of biopsy with coloring of micropreparations with hematoxylin-eozin and determining a degree grade by Gleeson's scale.

Keywords: pathologic anatomy diagnosis, biopsy, prostate cancer, immunohistochemical study.

INTRODUCTION

Prostate cancer (PCa) is the most common solid tumors in men in the United States and in the European Union [1]. In Russia and other countries of UIS in men older than 60 years, prostate cancer is the most common malignant neoplasm (MN) [2]. In the period from 1999 to 2009 in our country, the incidence of prostate cancer has increased by 2.8 times, showing the highest values among all MN [3]. Due to the widespread use of prostate-specific antigen (PSA) screening number of men with prostate cancer detection at early stage increases in developed countries, including Russia [4, 5]. Under the new conditions the share of the biopsy increases, in which the differential diagnosis between high-grade adenocarcinoma of prostate, tumor-like "mimic" atrophic process, prostatic intraepithelial neoplasia (PIN), atypical small acinar proliferation (ASAP), sclerosing adenosis, which necessitates not only the use common differential diagnostic morphological criteria, but also to include in the currently under development standards of prostate biopsy reliable immunohistochemical methods in certain quantities. Among manuals, differential diagnostic immunohistochemical criteria of prostate well-differentiated adenocarcinoma occupy an important place [6]. If in one diagnostic case even 12 specimens (the amount of biopsies depends on the volume of prostate and can be increased up to 24) present, it is necessary to select a sufficient diagnostic panel, and question of the economic

feasibility sharply raises to use all possible number of biomarkers that increases the cost of biopsy.

The aim of our study was the optimization of prostate biopsy algorithm with using immunohistochemical biomarkers.

MATERIALS AND METHODS

We carried pathologic diagnosis of prostatic adenocarcinoma (n = 32) and benign prostatic hyperplasia (BPH) (n = 32) in combination with a PIN, chronic prostatitis, focal atrophy, atypical small acinar proliferation (ASAP) on biopsies of patients GBUZ "Volgograd Regional uronefrologichesky center".

Prostate biopsies were fixed in 10% buffered formalin solution for 24 hours at room temperature and worked up in the standard manner. Paraffin sections 3-5 μm were prepared, stained with hematoxylin and eosin. After micropreparations investigation pathological anatomical diagnosis (conclusion) was established.

For immunohistochemical investigation (IHC) next biomarkers (mouse and rabbit monoclonal antibody) were used for basal epithelial cells: cytokeratin 34βE12 (1:100, Thermo Scintific), cytokeratins 5 and 6 (1:100, Thermo Scintific), protein P63 (1:50, Santa Cruz); oncomarkers: alpha-methylacyl coenzyme A racemase, AMACR (1:100, Thermo Scintific), ERG 1,2,3 (1:50, Santa Cruz); and marker of low molecular weight calcium-binding protein - protein S-100 (1:00, Thermo Scintific) and prostate biomarkers: prostate specific antigen, PSA (1:200, Thermo Scintific) and prostatic acid phosphatase, PSAP (1:3000, Thermo Scintific). Procedures of dewaxing, unmasking antigens, hematoxylin staining were performed in accordance with the recommended protocol with further immunophenotype analysis. Pathological anatomical diagnosis (conclusion) based on results of immunohistochemical investigation of micropreparations.

RESULTS AND DISCUSSION

In each case of transrectal prostate biopsy at first we have examined micropreparations from 12 points of biopsies (768 micropreparations) stained by hematoxylin and eosin. After that, we have selected suspected to adenocarcinoma 2-4 paraffin blocks from each patient and made sections for IHC examination (571 slides). Thus, 16,7-33,3% of biopsy material often from the most questionable tissue samples examined by IHC method.

Immunophenotype of prostatic adenocarcinoma (Gleason 6) in 100% of cases was characterized by cytokeratin 34βE12 (-), cytokeratins 5 and 6 (-), protein P63 (-), AMACR (+),

PSA (+), PSAP (+). Biomarker ERG 1,2,3 demonstrated less informative diagnostic significance and was positive in 6 cases (18.8%). Virtually in all cases, prostate cancer occurred on a background of BPH. In 18 cases (43.8%) adenocarcinoma of the prostate was accompanied by PIN 2-3 degrees.

In contrast, 100% of BPH immunophenotype characterized by cytokeratin 34βE12 (+), cytokeratins 5 and 6 (+), protein P63 (+), AMACR (-), ERG 1,2,3 (-) at the PSA (+), PSAP (+). It should be noted, that the cytoplasmic expression of cytokeratins 5 and 6 in 9.4% of cases has been hailed as "questionable." In 6 cases (18.8%) BPH was accompanied by PIN. In addition, in 25 cases (78.1%) of BPH focal atrophy of prostatic glands was detected, and in 29 cases (90.6%) BPH accompanied by varying severity of lymphoid infiltration.

In 2 cases (6.3%) of BPH foci of atypical small acinar proliferation were detected and characterized by the presence of small glands with moderate cytological atypia and immunophenotype: cytokeratin 34βE12 (+), cytokeratins 5 and 6 (+), P63 protein (+), AMACR (-).

In one case (3.1%) of BPH thickened hyalinized periacinar basement membrane detected with atrophy of some prostatic glands and sclerosing adenosis suspected, however, expression of protein S-100 was negative.

Transrectal biopsy of the prostate is considered as "gold standard" for prostate cancer diagnosis [7], the implementation of IHC for differential diagnosis of prostate adenocarcinoma, PIN, focal atrophy, atypical small acinar proliferation is an essential element of diagnostic search and included in various national manuals [5, 6, 8].

To reduce the cost of the biopsy examination PIN-cocktail can be used for IHC, composed of antibodies against AMACR - tumor markers (cytoplasmic expression) and anti-p63 - a marker of basal cells (nuclear expression) that contributes to the proper establishment of diagnosis in 92-97% of cases [9,10]. Furthermore, it is noted that the use of the multiblock system reduces the antibody expense per specimen of tissue without loss of information about the object labeling [6, 7].

Thus, in the differential diagnosis of primary high-grade prostatic adenocarcinoma with Gleason score = 6 and BPH during transrectal prostate biopsy an optimized panel of antibodies to cytokeratin 34βE12, cytokeratin 5 and 6, protein P63, AMACR, PSA, PSAP presented, which allowed 100% of cases to establish correct diagnosis.

The results of this study allow to recommend to include immunohistochemical examination with using the following biomarkers: 34βE12, cytokeratins 5 and 6, protein P63,

AMACR, PSA and/or PSAP in standard method of biopsy to diagnose prostate cancer in addition to traditional analysis of material from 12-24 points of biopsy stained by hematoxylin and eosin with Gleason grades estimation.

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