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# Electronic expert consultation using digital still images for evaluation of atypical small acinar proliferations of the prostate ☆ A comparison with immunohistochemistry

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ABSTRACT

This study was performed on a series of prostate needle biopsies with diagnosis of atypical small acinar proliferation (ASAP) to verify to what extent the application of immunohistochemistry (IHC) for p504s and p63 markers as well as expert consultation by still images could affect the diagnosis. The results of these 2 methods were compared. Immunohistochemistry staining for p504s and p63 was performed on sections from 42 patients with a primary diagnosis of ASAP. Meanwhile, digital still images were taken from hematoxylin and eosin–stained slides of cases and were sent to an expert uropathologist, blind to IHC staining interpretations. The results of IHC staining were compared with diagnostic interpretations of the consultant pathologist. In 13 cases, the focus of concern was not detectable on IHC slides. In the remaining 29 cases, IHC showed a benign and malignant expression pattern in 17 and 9 patients, respectively. In 3 cases, IHC findings were inconclusive and retained the diagnosis of ASAP. The consultant pathologist diagnosed 11 cases of benign and 7 cases of malignant processes. He retained the diagnosis of ASAP in 11 cases. There was high concordance between the results of IHC and electronic consultation in the group of benign cases. All 11 cases with the diagnosis of benignancy by electronic consultation showed a benign IHC pattern. Among 7 cases with the diagnosis of malignancy by the consultant pathologist, 5 were classified as malignant, 1 as benign, and 1 as inconclusive IHC groups. Considering problems with IHC staining of prostate needle biopsy, including loss of focus of interest, expert consultation using still images can provide very useful diagnostic information. This approach can be used as an adjunct to other diagnostic activities like IHC or even as an independent source of information to reach more accurate diagnoses in ASAP cases, particularly in institutions with limited resources.



## 1. Introduction

After the beginning of screening programs for prostatic carcinoma and widespread application of transrectal ultrasound–guided prostatic needle biopsy with thin needles, pathologists more frequently encounter lesions, which cannot be interpreted as either benign or malignant with certainty. Originally described by Bostwick et al [1] in 1993, the phrase “atypical small acinar proliferation (ASAP) of uncertain significance” was used to define some of these changes. Iczkowski et al [2] further outlined the clinical importance and microscopic findings, resulting in the terminology being modified to “ASAP suspicious for but not diagnostic of malignancy” or simply “ASAP.”

Atypical small acinar proliferation is a diagnostic term and not a disease entity. It is usually used to define the lesions that are suspected to be malignant but for which the microscopic findings are not satisfactory to fulfill the criteria of malignancy [3]. The uncertainty with these lesions can be due to several confounding factors but in many cases is related to the small size of the lesion or the low number of acini showing pathologic changes.

Usually, the patient with a diagnosis of ASAP is put under surveillance, which includes periodic clinical examinations, serum prostate–specific antigen (PSA) measurement, and repeated needle

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biopsies at appropriate intervals [4-6]. These evaluations have both economic and emotional implications for patients, physicians, and health insurance systems. Accordingly, pathologists attempt to clearly define the nature of the prostatic pathologic changes as much as possible before rendering a diagnosis of ASAP. Two basic approaches to the cases with ASAP are preparation of additional microscopic cuts for hematoxylin and eosin (H&E) staining [7] and immunohistochemical examinations [8].

This study attempts to determine to what extent IHC can be helpful in the recategorization of ASAP cases into benign and malignant groups. The study also attempts to determine to what extent consultation with an expert uropathologist by electronic still images can be useful for the same purpose.

## 2. Materials and methods

### 2.1. Case selection

During the period between January 2004 and December 2008, the prostate needle biopsies from 1750 cases were examined in the pathology laboratory of Hasheminejad Kidney Center in Tehran, Iran. A total of 52 samples from 45 patients had the histopathologic diagnosis of ASAP. For each patient, the diagnosis was made independently by 1 of 3 pathologists. At the time of diagnosis, the pathologists each had between 8 to 12 years experience in general pathology and 1 to 5 years experience in urologic pathology. At the time of diagnosis, immunohistochemistry (IHC) was not carried out for any of the cases. Three patients were excluded from study as they had foci of adenocarcinoma in the samples from other areas of the prostate. Hematoxylin and eosin slides of all 49 cases were reviewed by one of the authors (MM), and 7 additional cases with apparently benign or malignant lesions were excluded from this study.

The remaining 42 samples were those that had ASAP diagnoses in the original pathology reports, had remained areas of interest in additional cuts (when it was performed at the time of original diagnosis), and had available paraffin blocks for IHC examination. The demographic data and serum levels of total and free PSA were obtained from pathology reports and hospital information systems.

### 2.2. Telepathology consultation

The foci of ASAP on H&E slides were photographed at different magnifications by an Olympus DP12 camera, which was mounted on an Olympus BX50 microscope. The images were taken from the areas, which were marked during the first microscopic evaluation. In the absence of marking, the suspected foci were selected by one of authors (MM). Sets of images from each case were sent via e-mail to an expert uropathologist (JAB). For each case, 3 images were taken at ×4, ×10, ×20, or ×40 objective magnifications. Additional images were attached according to the request of the consultant pathologist. In adjunct with images, a questionnaire about the quality and adequacy of images, definite diagnosis, and potential problems in rendering a definite diagnosis was attached.

### 2.3. Immunostaining for p63 and p504s

Immunohistochemistry stainings for p504s (Monoclonal Rabbit Anti-Human α-methylacyl-CoA-racemase [AMACR], clone 13H4) and p63 (Monoclonal Anti-Human p63-protein, clone 4A4) were performed manually using antibodies from Dako, Denmark according to manufacturer guidelines. Normal prostatic tissue and adenocarcinoma of the prostate were used as external positive controls for p63 and p504s staining, respectively. Heat-induced antigen retrieval was performed before staining with the use of citrate buffer, pH 6. Endogenous peroxidase was blocked using 1% hydrogen peroxide.

### 2.4. Interpretation of immunostaining

All IHC slides were examined by 2 observers (AB and MM) at ×10, ×40, and if necessary, at ×100 magnifications using a multihead microscope.

p63 staining interpretation was semiquantitative, and the results were expressed as +2 (diffuse basal cell nuclear staining pattern), +1 (focal and patchy basal cell nuclear staining pattern), or 0 (absent) as defined by previous studies [9,10].

p504s reactivity was scored as −2 (strong continuous dark cytoplasmic or luminal staining), −1 (discontinuous dark glandular or weak diffuse cytoplasmic staining), or 0 (absent at ×100 oil immersion objective) as described in previous studies [9]. Total IHC score was calculated by the sum of the results of 2 immunostainings and yielded a range of −2 to +2.

According to IHC findings, the patients were categorized into 4 groups. The grouping was based on the results of IHC for p504s as a marker for the presence of prostate cancer cells and p63 as a marker for presence of basal cells.

- Benign pattern (total score +2 and +1 in the absence of reactivity for p504s): cases with positive reactivity for p63 (score +2, score +1) and negative reaction for p504s (total score 0).
- Malignant pattern: cases with a strong and continuous (score −2) or discontinuous/weak (score −1) p504s staining patterns in the absence of reactivity for p63 (total score 0).
- Noninvasive pattern (total score −1 to +1 in the presence of definite positive reaction against p63): cases with strongly p63 reactive basal cells (score +2) and weak or discontinuous reaction against p504s (score −1) as well as cases that showed strong continuous staining pattern for p504s and patchy p63-positive basal cells. Three possible differential diagnoses in these cases are atypical adenomatous hyperplasia (AAH), typical benign gland with aberrant p504s reaction, and high-grade PIN (HGPIN). For statistical evaluations, this pattern was considered as a benign IHC expression pattern.
- Unclear pattern (total score 0 in the absence of reaction against p63): cases with simultaneous absence of p63 and p504s reactivity or noninterpretable immunohistochemical staining, which could not be allocated definitely into one of the aforementioned groups.

### 2.5. Data collection and processing

All demographic and clinical data of patients, IHC studies, and electronic consultation were analyzed by SPSS (Chicago, Illinois/USA) 16 software. The sensitivity and specificity of telepathology assessments in comparison with IHC results were calculated using $\chi^2$ statistical analysis.

## 3. Results

### 3.1. Demographic data

The patients had a mean age of 65.67 years (54-79) and mean serum PSA serum level of 11.07 ng/mL (0.8-40 ng/mL). Free PSA serum levels were measured in 14 cases, with a mean of 1.56, and prostate volume was calculated in 24 cases with mean of 58.43 $cm^3$.

### 3.2. Additional cuts of paraffin blocks

In 20 cases (47.6%), additional cuts were done for H&E staining at the time of original diagnosis, and the recuts contained the suspicious lesions.

### 3.3. Consultation with uropathologist

The expert uropathologist reclassified 19 (45.2%) cases as benign and 9 (21.4%) cases as malignant. In 14 (33.3%) cases, the consultant kept the diagnosis of ASAP. Three major groups of problems were