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Diagnostic Value of ERG in Prostate Needle Biopsies Containing Minute Cancer Foci

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Background: Prostate carcinoma (PC) is the second most diagnosed cancer in men population worldwide. The small amount of the tissue in prostate needle biopsy is often sufficient for the correct interpretation. Novel antibodies, as ERG, could add to the diagnostic value of IHC study in analysing difficult core biopsies.

Aim: The aim of the present study was to establish a diagnostic use of ERG in a work-up of prostate needle biopsies containing minute PC, individually and in combination with AMACR/34βE12.

Materials and methods: From total number of 1710 consecutive prostate needle biopsies based on HE stain 114 biopsies containing minute PC. Selected biopsies were incubated with anti-ERG, AMACR and 34βE12 antibodies using immunohistochemical technique.

Results: Among 98 selected biopsies, 57 showed positive and 41 negative ERG staining. AMACR staining was positively expressed in 86 of the cases and completely absent in remaining 12. In 9 of the AMACR-negative cases the final diagnosis was establish by manifestation of ERG expression in the tumour foci. 95 of the biopsies demonstrated lack of 34βE12 expression and only 3 cases showed weak patchy staining. Among these cases 2 were ERG-positive.

Conclusion: ERG antibody could be especially helpful in the cases with controversial expression of AMACR and 34βE12.

INTRODUCTION

Prostate carcinoma (PC) is the second most diagnosed cancer in men and accounts for the sixth most frequent cause of cancer-related deaths worldwide.[1] The small amount of tissue in prostate needle biopsy available for the analysis is often sufficient for correct interpretation. There is a wide range of benign mimickers such as adenosis and atrophy that could imitate architectural and cytological features of PC. Although the currently used immunohistochemical (IHC) markers such as alpha-methylacyl-CoA racemase (AMACR) and basal cell markers such as high molecular weight cytokeratin 34βE12 (34βE12) and p63 assist in making a definitive diagnosis of malignancy, various immunostains remain difficult for interpretation leading to a possible misdiagnosis.[2]

Erythroblastosis E26 Rearrangement Gene (ERG) was discovered in 2005 by Tomlins et al.[3] It is a member of the family of genes encoding erythroblast transforming specific transcription factors (ETS) with frequent expression in PC. The mechanism of ERG overexpression is a consequence of recurrent gene fusion involving ERG and androgen presenting gene Transmembrane Protease Serine 2 gene (TMPRSS2). In summary, TMPRSS2 fused with ERG after inter- or intra chromosomal rearrangements. This leads to overexpression of ETS-related gene as a consequence of androgen dependent stimulation following gene fusion.[3] Among all members of the ETS family genes, ERG has the highest rate of fusion with TMPRSS2 gene and located in over 90% of all cases with TMPRSS2: ETS rearrangements.[4]

Subsequent studies confirmed that about 50% of prostate specific antigen (PSA) monitored patients

with PC demonstrate ERG expression.[5,6] Furthermore, this gene fusion appears to be exclusive for the PC since it has not been identified in any other epithelial tumour.[7] Recent investigations reported a strong concordance between ERG expression detected by IHC method and a presence of TMPRSS2 gene rearrangements using fluorescence in-situ hybridization. Nuclear ERG staining was present in tumour cells in ~65% of the patients and absent in benign epithelial cells thus demonstrating extremely high specificity (~99%).[8,9] This high specificity of ERG to PC may be helpful for resolving difficult prostate biopsies.

AIM

The aim of the present study was to establish a diagnostic use of ERG in a work-up of prostate needle biopsies containing foci of minute PC, individually and in the combination with AMACR/34βE12 using IHC method.

MATERIALS AND METHODS

Cohort

A total number of 1710 consecutive prostate needle biopsies diagnosed between January 2008 and December 2012 in our medical institution were reviewed. Based on haematoxylin eosin (HE) staining 98 biopsies containing minute PC foci (carcinomas 6 in Gleason score, < 1 mm in size or occupying <1 ×40 field in a single needle specimen) were selected for the purposes of the study.[10]

Evaluation of ERG, AMACR and 34bE12 expression by IHC method

Formalin-fixed paraffin-embedded prostate biopsies were sectioned at 4 μm. IHC study was performed manually using monoclonal antibodies against ERG, AMACR and 34βE12. Antigen retrieval was performed in EDTA buffer (pH 6.0) for 40 min at 95ºC and then 10 min at room temperature. The slides were then incubated with primary antibodies as follows: anti-ERG (ERP 3864, 1:200, monoclonal, Abcam), AMACR (P504S 13H4, RTU, monoclonal, BioSB) and 34βE12 (34βE12, RTU, monoclonal, BioSB) for 45 min at room temperature. The IHC reaction was visualized with a peroxidase-based brown detection (Mouse/Rabbit PolyDetector HRP/DAB System, BioSB). The slides were contra stained with haematoxylin. Known positive (skin and prostate cancer) and negative controls were run in parallel and gave appropriate results.

Only sections with positive endothelial ERG expression were included and only nuclear staining was considered valid. ERG expression was evaluated as absent (0), weak (+1), moderate (+2) and strong (+3). Any staining (weak, moderate and strong) was considered as positive. Similarly granular cytoplasmic AMACR staining was scored as negative (0), weak (+1), moderate (+2) and strong (+3). 34βE12 was recorded as negative (lack of any staining) or positive (diffuse or patchy cytoplasmic and membrane staining).[11] All cases were independently evaluated based on HE sections by 3 pathologists (BS, SD, BV) and only biopsies containing foci of PC GS6 were analysed.

Statistical analysis

All data were statistically analysed using descriptive and nonparametric tests. Statistical analysis of antibodies' sensitivity and specificity was performed by ROC-curve analysis. Statistical significance was accepted at $p<0.05$. The data were analysed with SPSS 19.0.

RESULTS

The mean age of the men was 71.1 years (range, 50 to 87 yrs) and mean pre-biopsy PSA serum level was 28.6 ng/mL (range 4.3 to 353 ng/mL). All patients underwent sextant needle biopsy.

Out of 98 PC biopsies, 57 (58%) showed positive (**Fig. 1**) and 41 (42%) negative ERG staining in the tumor glands. The mean age of ERG-positive and ERG-negative patients did not differ significantly. The mean PSA level of the ERG-negative patients was twice as high as that of ERG-positive patients. Summarized clinicopathological data are presented in **Table 1**.

ERG staining was strongly positive in vascular endothelial cells which were used as an internal control. The intensity of ERG staining in ERG-positive glands was moderate to strong (2+ or 3+) in 77% and weak in remaining 23% of the cases.

Eighty-five (86.7%) cases demonstrated a classic IHC pattern: positive AMACR and negative 34βE12 expression in tumour glands (**Fig. 2**). AMACR staining was positively expressed in 86 (87.7%) of the neoplastic glands and completely absent in remaining 12 (12.3%). In 8 of the AMACR-negative PC the final diagnosis was established by presence of ERG expression in the tumour glands (**Fig. 3**). Ninety six (97%) of the biopsies demonstrated lack of 34βE12 expression and only 3 (3%) biopsies showed weak patchy 34βE12 staining in a single glands as well as moderate to strong nuclear ERG

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staining (**Fig. 4**). Summarized data of IHC results are presented in **Table 2**.

In 10 biopsies including 2 cases with high-grade prostatic intraepithelial neoplasia (HGPIN), we observed positive nuclear staining in benign glands close to the PC areas (**Fig. 5**).

DISCUSSION

The present study is the first ever investigation of ERG expression in PC in a Bulgarian population. Moreover, to the best of these authors' knowledge, there is no other research on ERG expression in PC population of the Balkan Peninsula. Although TMPRSS2-ERG gene rearrangements were extensively studied in Northern American, Western European and Asian populations, there is still limited information about Central Eastern and South Eastern European regions.[5,12-14] PSA screening has been used only in the last few years in our country. As a result, the number of prostate needle biopsies has dramatically increased leading to the greater number of difficult cases encountered by clinicians containing minute PC and its benign mimickers. This force to the more wide usage of the IHC method in a daily pathology practices.

Table 1. Clinicopathologic features of ERG-positive and ERG-negative patients

	ERG+	ERG-
Mean age (yrs)	70.17	72.5
Mean PSA level (ng/ml)	21.2	37.39
Sextant biopsy	yes	yes

Table 2. Immunohistochemical profiles of the cases

	ERG+	ERG-	Total
AMACR+/CK-	47	37	84
AMACR+/CK+	2	0	2
AMACR-/CK-	8	3	11
AMACR-/CK+	1	0	1
Total	58	40	98

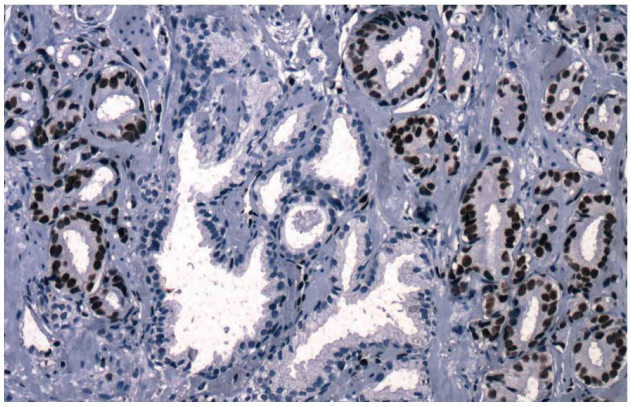

Figure 1. Prostate cancer Gleason scored 6 showing diffuse strong nuclear ERG staining in tumour glands arranged on both sides of benign acini (x100).

We evaluated the diagnostic utility of ERG-antibody separately and in combination with AMACR/34βE12 markers in a group of biopsies containing minute foci of PC. In this study, ERG was expressed in 57 cases (58%) with cancer glands with 98.3% specificity and 57.9% sensitivity. In the ERG-positive cases, the nuclear staining was moderate to strong (2+ to 3+) in 45 of 57 cases (77%) and was diffusely expressed in the tumour glands in 48 of 57 cases (84%). This type of expression make an evaluation process extremely easy, especially when only couple glands suspicious for PC are presented. Our results were highly concordant with the previous studies, reporting between 45 and 61% of ERG expression in PC diagnosed in needle biopsies. Similar to other authors we detected high levels of ERG staining intensity and a low level of the heterogeneity of the ERG expression.[11,15] In our series, we also found 10 biopsies with positive ERG staining in HGPIN (2 out of 57, 3.5%) and benign glands (8 out of 57, 14%). We should note that the ERG-positive HGPIN and benign areas were restricted only to the biopsies with concomitant ERG-positive PC and were located in close proximity to the neoplastic glands. These observations suggested that the presence of ERG-positive HGPIN separately on the biopsy could be a possible indicator of an undersampled PC within few millimetres in the same biopsy core.[16]

Routinely used in pathology practice, IHC markers (AMACR and 34βE12) were able to resolve 85 (84.7%) of the cases in this study. Of the remaining 13 cases, 12 biopsies demonstrated complete lack of staining of both markers (AMACR and 34βE12) in the suspicious glands. Three cases showed positive expression of both markers in the malignant glands and one biopsy exhibited benign IHC profile. In the group of double-negative biopsies we found 8 (72.7%) cases with positive ERG expression in suspected areas, which helped us make a definitive

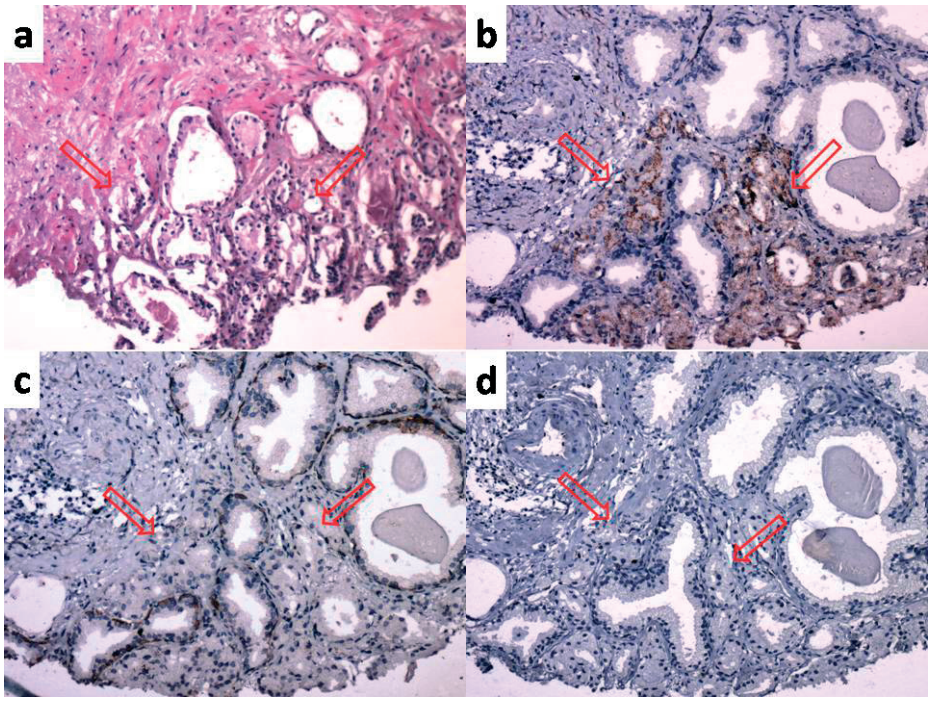

Figure 2. A. The small foci of glands showed architectural and cytological features of malignancy (red arrows) (HE, x100). **B.** The same glands demonstrating strong granular cytoplasmic AMACR expression (red arrows) (x100). **C.** The same glands presenting lack of 34βE12 expression (red arrows) (x100). **D.** Cancer glands with diffuse strong nuclear ERG staining (red arrows) (x100).

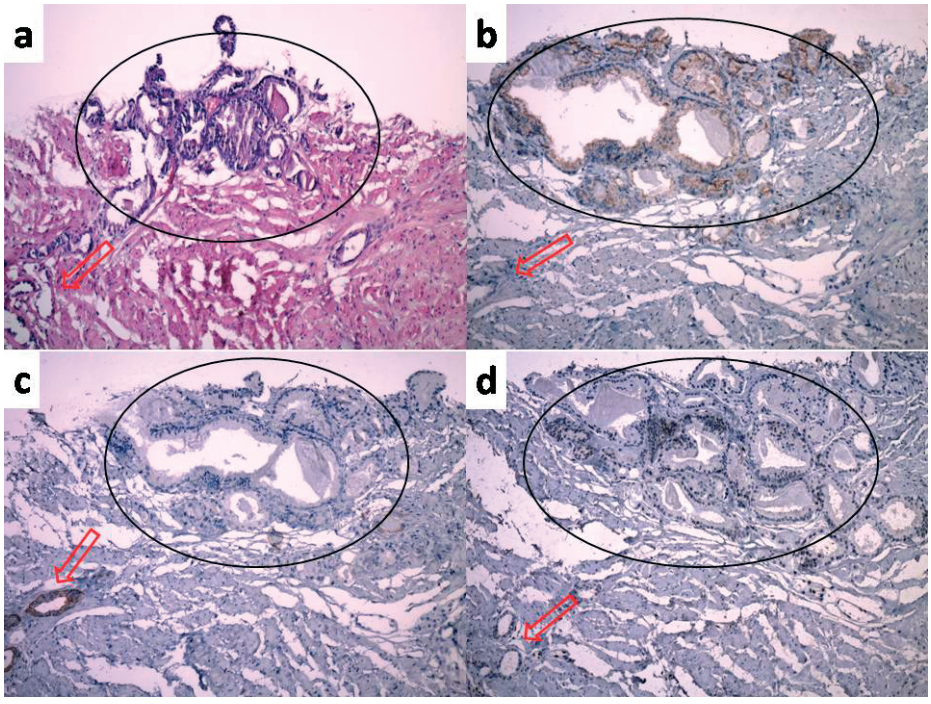

Figure 3. A. Small focus of neoplastic acini with amphiphilic cytoplasm and crystalloids (black circle) and small, atrophic benign glands (red arrow) (HE, x100). **B.** Tumour glands showing moderate cytoplasmic granular staining of AMACR (black circle) and benign glands showing lack of AMACR expression (red arrow) (x100). **C.** The same area demonstrating lack of 34βE12 expression (black circle) except single benign gland (red arrow) (x100). **D.** Cancer glands demonstrating focal weak nuclear ERG stain (black circle) and negative nuclear ERG expression in benign gland (red arrow) (x100).

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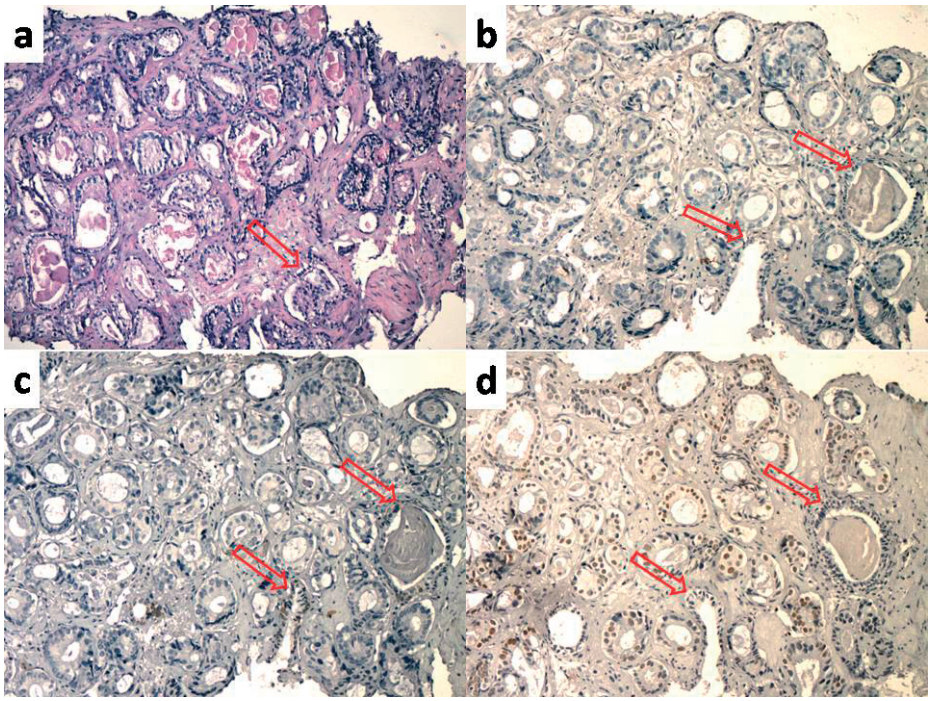

Figure 4. A. Prostate cancer Gleason scored 6 showing architectural and cytological features of a malignancy and single benign gland (red arrow) (HE, x100). **B.** Prostate cancer Gleason scored 6 presenting completely lack of AMACR expression in tumour focus and few benign glands (red arrows) (x100). **C.** Prostate cancer Gleason scored 6 presenting complete lack of 34βE12 expression in tumour glands and positive expression in few benign glands (red arrows). (x100). **D.** Cancer glands demonstrating diffuse strong nuclear ERG staining and benign glands with negative staining (red arrows) (x100).

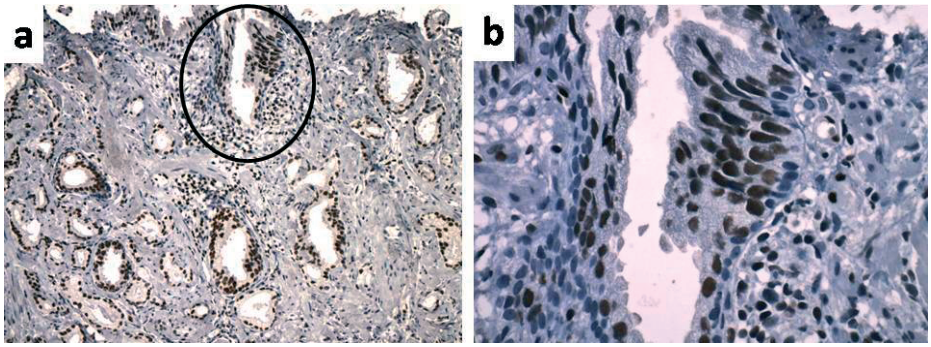

Figure 5. A. HGPIN (black circle) situated close to the cancer focus demonstrating strong nuclear ERG staining (x100). **B.** HGPIN exhibiting strong nuclear ERG expression (x400).

diagnosis. Despite controversial IHC results, the remaining 3 AMACR -/34βE12- cases demonstrated sufficient architectural and cytological criteria for PC and were diagnosed as ATYP. We identified lower mean staining intensity of ERG comparing to AMACR due to 42% ERG-negative cancer cases. Nevertheless, ERG showed less staining variability resulting in better level of observation and interpretation of its expression.

Focally positive ERG expression was found in 3 out of 3 34βE12-positive biopsies. Two cores were triple-positive presenting weak to moderate expression of ERG and AMACR in minute cancer foci and single glands with positive 34βE12 staining. One case exhibited IHC profile of benign tissue (AMACR-/34βE12+) but also demonstrated strong nuclear ERG staining. We speculate that 34βE12-positive glands in our series were actually small crashed benign glands amid malignant foci.

In conclusion, this is the first study that examined the frequency of ERG-positive PC in a Bulgarian population. ERG was expressed in 57% of GS6 PC in core needle biopsies. It is also expressed in HGPIN and a small percentage of benign glands adjacent

to PC. Although the IHC cocktail AMACR/34βE12 helps to resolve the majority of the difficult biopsies, ERG demonstrated higher specificity than does AMACR (p=0.38), supporting diagnosis of PC. Hence ERG could be added to the traditionally used markers (AMACR and 34βE12) as a component of the cocktail or in case these two failed.

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Диагностическое значение применения ERG в игольных биопсиях предстательной железы, содержащих миниатюрные раковые очаги

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Введение: Карцинома предстательной железы (КПЖ) является второй по частоте диагностирования формой рака среди мужской популяции в мире. Небольшое количество ткани при игольной биопсии предстательной железы часто является достаточным для правильного анализа данных. Новые антитела ERG (erythroblastosis E26 Rearragement Gene) могут способствовать диагностической точности данных иммуногистохимических исследований при анализе «трудных» биопсий.

Цель: Целью настоящего исследования является установление диагностической пользы применения ERG при обработке игольных биопсий предстательной железы, содержащих небольшие КПЖ, как самостоятельно, так и в комбинации с AMACR/34βE12.

Материал и Методы: Из общего количества 1710 последовательно проведенных игольных биопсий предстательной железы, окрашенных ХЕ, 114 содержат миниатюрные КПЖ. Отобранные биопсии были инкубированы анти-ERG, AMACR и 34βE12 антителами, с применением иммуногистохимической техники.

Результаты: Среди 98 отобранных биопсий, 57 демонстрировали положительное и 41 отрицательное ERG окрашивание. AMACR окрашивание экспрессируется положительно в 86 из случаев и полностью отсутствует в 12. В 9 из AMACR-отрицательных случаев заключительный диагноз был поставлен проявлением экспрессии ERG в раковых очагах. 95 биопсий демонстрируют отсутствие экспрессии 34βE12 и лишь в 3 из случаев проявляется слабое частичное окрашивание. Среди этих случаев 2 являются ERG-положительными.

Заключение: ERG-антитело может быть особенно полезным в случаях противоречивой экспрессии AMACR и 34βE12.