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Antibody-Cytokeratin Marker 34βE12 in Prostate Cancer Detection

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Abstract: Introduction: The histological diagnosis of prostate cancer is commonly based on morphological patterns. The presence of malignant tissue mixed with benign tissue, or the presence of carcinoma that mimics benignity may generate difficulty in the diagnostic elucidation. Therefore, the application of immunohistochemistry contributes its diagnostic value. Objectives: To evaluate the *34βE12* marker in the detection of adenocarcinoma (ADn), atypical small acinar proliferation (ASAp), regular prostatic tissue (RPT) and regular prostatic tissue alternated by atrophy spotlights (RPTa) in transrectal biopsy guided by ultrasonography of patients with suspected prostate cancer. Method: Analysis of 34 patients who underwent ultrasound-guided transrectal biopsy with subsequent analysis by *H&E* staining and *34βE12* labeling for elucidation of neoplasms or diseased tissues with doubtful diagnosis. Results: The marker *34βE12* showed negativity in 100% of the neoplasms ADn, positivity in 100% of the benign prostatic tissues (RPT and RPTa); the patients with ASAp presented positivity (20%) and negativity (80%). The chi-square test (χ^2) showed that there is an association (χ^2= 29.55 and $p < 0.0001$) between the groups, that is, the *34βE12* marker has a significant value ($p < 0.0001$) in the elucidation of patients with prostatic neoplasia and benign prostatic tissues. Discussion and Conclusion: With the early screening of prostate cancer in the modern era, pathologists have become increasingly challenged to diagnose small outbreaks of cancer when only a few atypical glands are present in transrectal biopsy-guided ultrasonography. The *34βE12* marker becomes an important tool in elucidating diagnoses such as ADn and ASAp.

Key words: Antibody-cytokeratin marker *34βE12*, adenocarcinoma (ADn), atypical small acinar proliferation (ASAp), regular prostatic tissue (RPT), regular prostatic tissue alternated by atrophy spotlights (RPTa).

1. Introduction

Prostate cancer is a highly prevalent disease and has been observed in about three million individuals in the US population in the year 2014 [1]. It ranks second among malignant neoplasms that affect men worldwide only behind of lung cancer [2]. In 2012, estimates revealed approximately 1.1 million new cases, constituting 15% of cancers in males [2-4]. In 2017, out of every 5 new cases of cancer diagnosed 1 was of prostate cancer [5].

Prostate specific antigen (PSA), digital rectal examination are the most commonly used tools for prostate cancer screening. However, transrectal biopsy guided by ultrasound remains the gold standard for the

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final diagnosis. The histological diagnosis of prostate cancer is commonly based on morphological patterns. However, prostate cancer is a heterogeneous disease that can exhibit varying degrees of aggressiveness and metastasis patterns; in addition, variable responses to treatments. There is a need to go beyond the morphological characteristics to stratify patients with prostate cancer into more homogeneous groups [6]. Histological diagnosis may be challenging because there may be malignant tissue mixed with benign tissue, or the presence of carcinoma mimicking benignity. Therefore, the application of immunohistochemistry contributes diagnostic value [7].

The differentiation between prostatic adenocarcinoma, benign prostatic lesions and the process of hyperplasia can be made based on the presence or absence of the basal cell layer, considering the fact that in the adenocarcinoma there is no cellular basal layer. Therefore, the use of immunomarkers such as *p63* and *34βE12* seems to be useful in differentiating these two types of lesions [8-12].

Although the immunoreaction may indicate a discontinuous or fragmented basal layer, this is not indicative of malignancy. Only a few studies indicate that there is a positive reaction for neoplastic cells with the *34βE12* marker. In contrast, healthy cells react continuously to *34βE12*, except when they are affected by inflammation [13-15].

Our objective was to evaluate the *34βE12* marker in the detection of adenocarcinoma (ADn), atypical small acinar proliferation (ASAp), regular prostatic tissue (RPT) and regular prostatic tissue alternated by atrophy spotlights (RPTa) in transrectal biopsy guided by ultrasonography of patients with suspected prostate cancer.

2. Materials and Methods

2.1 Ethical Conditions

The present study was approved by the Research Ethics Committee of the Base Hospital Institute of the Federal District, Bras Ília, CAAE: 90199518.0.0000.8153.

2.2 Data Collected

The information was collected through the electronic prognosis and anatomopathological reports retrospectively. The biopsy indication took into account alterations in the PSA level and alterations in digital rectal examination. The transrectal image of the prostate was obtained with a sectorial transducer for subsequent collection of the biopsies, the samples obtained by puncture were sextants using 18-gauge needle (18-Gauge). These fragments were sent for anatomopathological study, being stained by the Hematoxylin-Eosin (*H&E*) technique and diagnosed by the same group of pathologists. Subsequently, all fragments were stained with the *34βE12* anti-cytokeratin antibody marker. The immunohistochemistry analysis was performed by the same group of pathologists and the criteria for positivity and negativity were: immunohistochemistry with basal layer marking was considered as positive and immunohistochemistry without labeling the basal layer was considered negative.

2.3 Patients

A total of 100 patients underwent ultrasound-guided transrectal biopsy with subsequent labeling of the *34βE12* anti-cytokeratin antibody and were inserted into a maintained retrospective database. The variables for inclusion of the patients in the study were: (1) transrectal biopsy guided by ultrasonography with primary analysis of the fragments by the *H&E* technique; (2) subsequent staining of the *34βE12* anti-cytokeratin antibody; (3) patients with complete data in the medical record, such as age, number of fragments, sum of Gleason scores. Exclusion criteria were: (1) patients who did not undergo transrectal biopsy guided by sextant ultrasonography as described by Hodge et al. [16] and with less than 12 fragments collected for analysis; (2) patients with incomplete data to formulate the research. Thus, based on these criteria,

34 patients were included for analysis.

2.4 Statistical Analysis

The analyzed variables were computed using SPSS version 20.0. Clinical and pathological data including age, PSA, number of fragments collected, localization of diagnosed adenocarcinoma in relation to sites collected from ultrasound-guided transrectal biopsy by the method of Hodge et al. [16], sum of Gleason score on biopsy, percentage of biopsy involvement, marking by *34βE12* were analyzed and correlated among the 34 patients. The analysis of frequencies, mean, standard deviation (SD) and range of variation in relation to clinicopathological data were described. The chi-square test (χ^2) with the respective groups and the *34βE12* marker was based on the 95% confidence interval and $p < 0.05$ as significant.

3. Results

Our study analyzed 34 patients who underwent ultrasound-guided transrectal biopsy with subsequent anatomopathological evaluation by *H&E* staining. These patients presented suspicion of prostate cancer or morphological changes characteristic of malignancy and, thus, diagnostic complementation by immunohistochemistry with the *34βE12* marker was required (Table 1). The patients analyzed had a mean age of 67.41 years, a standard deviation of 7.44 years, and the ages ranged from 55 to 82 years, patients aged < 65 years were 10 (29.41%) and ≥ 65 were 24 (70.58%). In the analysis of the biopsied fragments, we showed that the fragments varied from 12 to 23, obtained an average of 17.09 fragments, standard deviation of 3.08 fragments, patients with 12 fragments added 8 (23.52%), 13-19 added 21 (61.76%) and ≥ 20 added 5 (14.70%). The 34 patients analyzed included 134 fragments collected for analysis in *H&E* staining that showed 86 (64.17%) fragments with adenocarcinoma, 17 (12.68%) with atypical small acinar proliferation ranging from 0-5% of biopsy involvement, 6 (4.47%) with regular prostatic tissue, 25 (18.65%) with regular prostatic tissue alternated by atrophy spotlights. After the 134 fragments were stained with the *34βE12* marker, we noticed some

Table 1 Clinical-pathological characteristics of patients

Characteristic	N (%)
Age (Mean ± SD*)	67.41 ± 7.44
< 65/≥ 65 years	10 (29.41)/24 (70.58)
	Variation: 55-82 years
Biopsied Fragments (Mean ± SD)	17.09 ± 3.08
12/13-19/≥ 20	8 (23.52)/21 (61.76)/5 (14.70)
	Variation: 12-23 fragments
Diagnosis by the technique H&E**	134 Analyzed Fragments
ADn/ASAp/RPT/RPTa***	86 (64.17)/17 (12.68)/6 (4.47)/25 (18.65)
Diagnosis by technique IHQ****	134 Analyzed Fragments
ADn/ASAp/RPT/RPTa	97 (72.38)/3 (2.23)/9 (6.71)/25 (18.65)
PSA (ng/mL)	
<10/10-20/>20	27 (79.41)/4 (11.76)/3 (8.82)
IHQ *34βE12*	
Positive/negative	37 (27.61)/97 (72.38)
Gleason Scores on ADn Biopsy	
≤ 6/7/> 7	71 (82.55)/12 (13.95)/3 (3.48)

*****SD—Standard Deviation.

**H&E—Hematoxylin-Eosin staining.

***ADn—Adenocarcinoma; ASAp—Atypical Small Acinar Proliferation ranging from 0-5% of biopsy involvement; RPT—Regular Prostatic Tissue; RPTa—Regular Prostatic Tissue Alternated by Atrophy Spotlights.

****Immuno-Histochemistry Anti-Cytokeratin Antibody *34βE12*.

changes in the pathological report, and 97 (72.38%) fragments were confirmed with adenocarcinoma (Fig. 1), 3 (2.23%) with atypical small acinar proliferation (Fig. 4), 9 (6.71%) with regular prostatic tissue (Fig. 2) and 25 (18.65%) with regular prostatic tissue alternated by atrophy spotlights (Fig. 3).

When we analyzed the PSA values, we obtained 27 (79.41%) patients with PSA < 10 (ng/mL), 4 (11.76%) patients with PSA between 10-20 (ng/mL) and 3 (8.82%) patients with PSA > 20 (ng/mL). When analyzing the 134 fragments submitted to staining with the *34βE12* marker, we showed that 37 (27.61%) fragments showed positivity and 97 (72.38%) of the fragments showed negativity for this marker. When fragments were diagnosed with adenocarcinoma previously by *H&E* staining, 71 (82.55%) fragments obtained ≤ 6 in the sum of Gleason scores, 12 (13.95%) fragments obtained 7 in the sum of Gleason scores and 3 (3.48%) fragments obtained > 7 in the sum of Gleason scores.

After the general stratification of the patients by type of diagnosis by *H&E* staining and subsequent staining by the *34βE12* marker, we showed that the marker showed negativity in all fragments containing adenocarcinoma, showed positivity in all fragments that appeared regular prostatic tissues with or without being alternated by atrophy spotlights (RPT and RPTa), however, when we analyzed patients with atypical small acinar proliferation ranging from 0-5% of biopsy (ASAp) we found that there were positivity (80%) and negativity (20%) (Fig. 5), showing the importance of the marker in the diagnostic elucidation in ASAp fragments (Table 2).

When stratifying the fragments diagnosed with adenocarcinoma by *H&E* staining and subsequent staining by the *34βE12* marker, we showed that the fragments with adenocarcinoma presented in 100% of the cases negativity for the *34βE12* marker in different

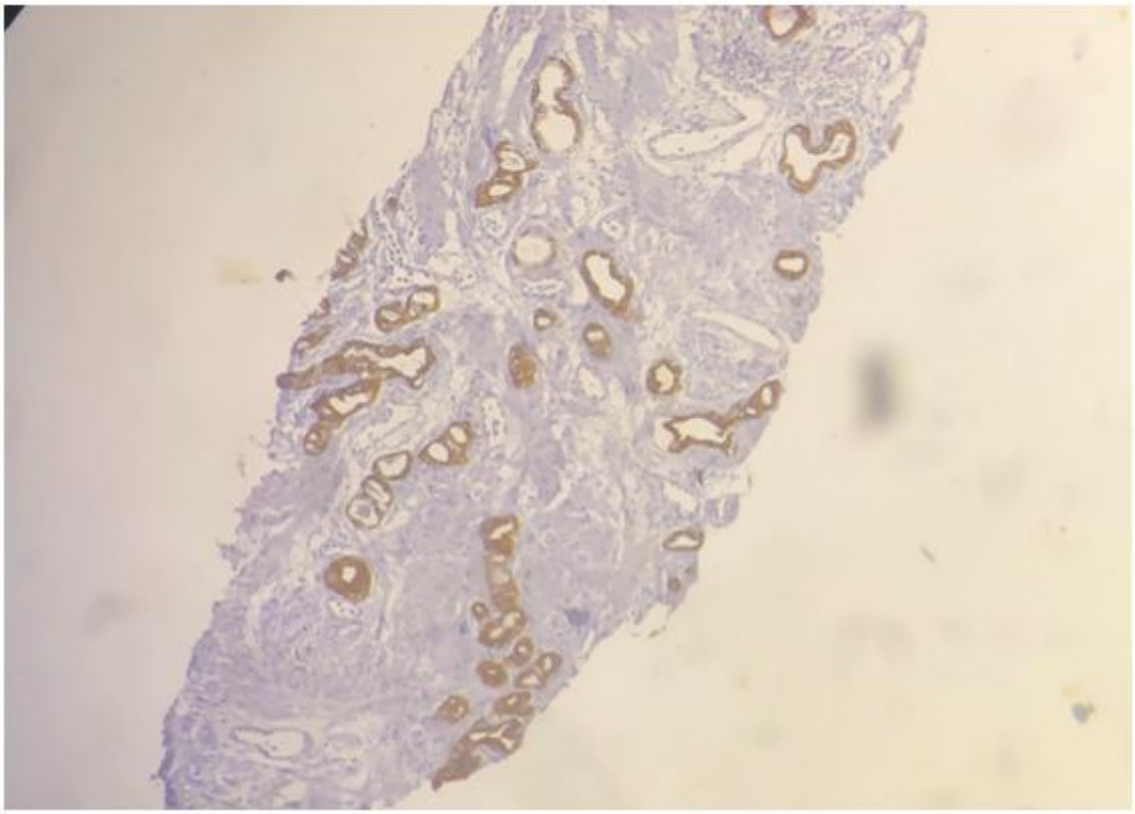
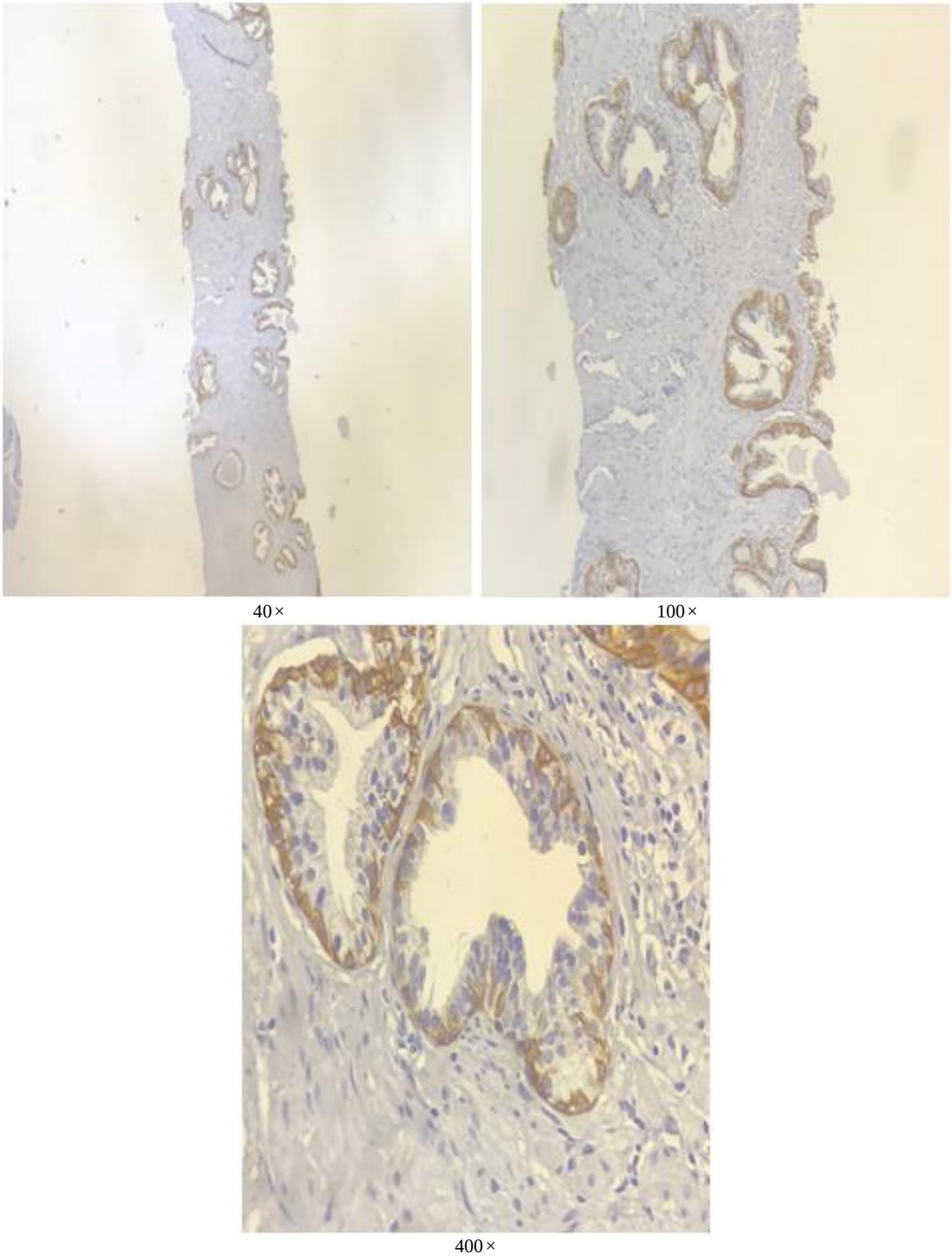


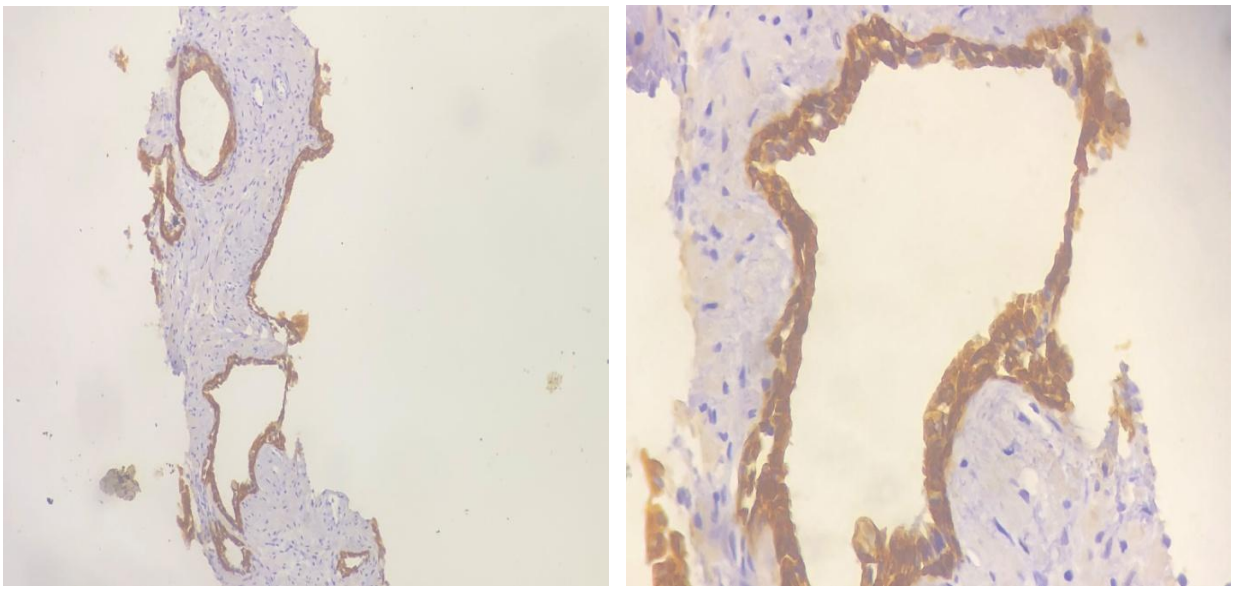
Fig. 1 Some habitual glands, positivity for labeling with *34βE12* (brownish staining), that is, they are delimited by basal cells. Other glands were negative for the marker, indicating absence of basal cell delimitation (IHQ-*34βE12*, 40×).



40× 100×

400×

Fig. 2 Usual glands showing positivity for labeling with *34βE12* (brownish staining), that is, they are delimited by basal cells. Increasing images of, respectively, 40×, 100× and 400× (IHQ-*34βE12*).



100× 400×

Fig. 3 Atrophic glands with scarce cytoplasm that could be confused with adenocarcinoma in routine staining (*H&E*). On labeling with *34βE12*, these glands showed to be positive (brownish staining) or they are delimited by basal cells. Increasing images of, respectively, 100× and 400× (IHQ-*34βE12*).

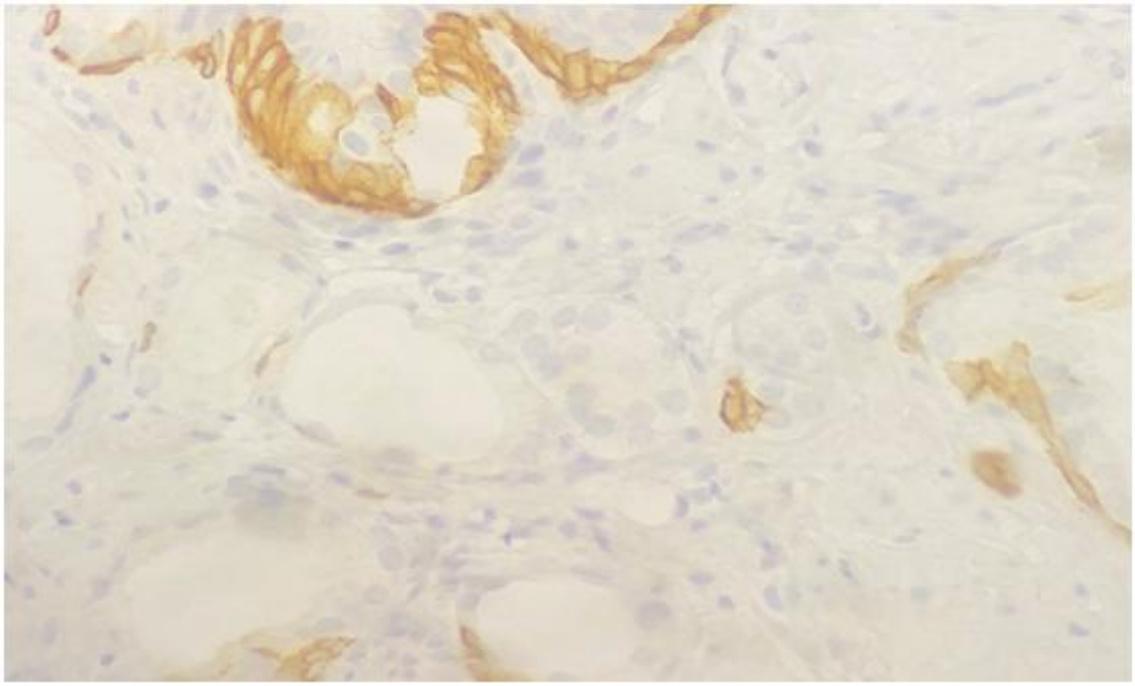
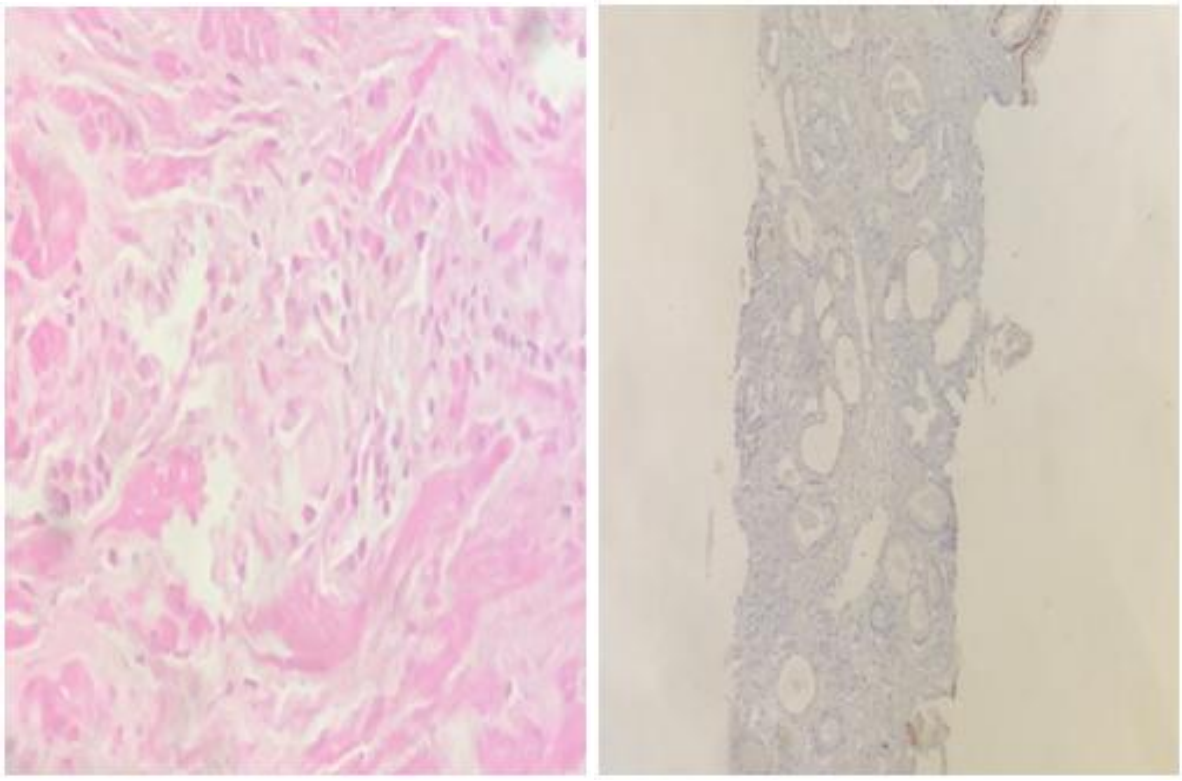


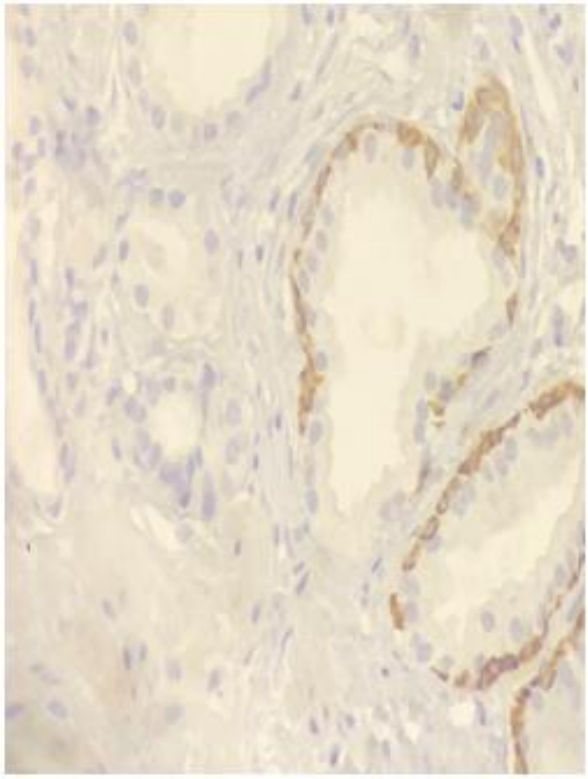
Fig. 4 Multiple glands not evident, negative for labeling with *34βE12*. This demonstrates that these glands are not delimited by basal cells, something characteristic of the neoplastic alterations in prostatic glands. In the upper part of the image, a normal gland, positive for *34βE12* (brownish color) in the basal cells is observed.

fragments with the sum of the Gleason scores and different percentages of involvement of the biopsy by adenocarcinoma (Table 3). Therefore, it shows that the marker has wide utility regardless of the biopsied location, Gleason score and percentage of involvement of the biopsied fragment.

When we classified the patients into 3 groups for analysis of the chi-square test: neoplastic patients (sum of ADn diagnoses) ($\chi^2 = 29.55$ and $p < 0.0001$) between the groups, i.e., the marker *34βE12* has a significant



(a) (b)



(c)

Fig. 5 Discrete area containing suspicious, ill-defined, fused glands. Initial diagnosis of atypical small acinar proliferation (ASAp) (*H&E*: 400×). Figs. 5b and 5c. Extensive area containing glands negative for this marker, not delimited by basal cells. A partially positive area is shown in Fig. 5c (IHQ-*34βE12*) (Fig. 5b: 100×; Fig. 5c: 400×).

Table 2 Analysis of patients by type of diagnosis with *H&E* staining and *34βE12* marker.

		Immunohistochemistry		
Types of Diagnostics*	*H&E* (%)	*34βE12*		N (%)
		Negative	Positive	
ADn	22 (64.70)	22	0	26 (76.47)
ASAp	5 (14.70)	4	1	1 (2.94)
RPTa	6 (17.64)	0	6	5 (14.70)
RPT	1 (2.94)	0	1	2 (5.88)

*ADn—Adenocarcinoma; ASAp—Atypical Small Acinar Proliferation ranging from 0-5% of biopsy involvement; RPT—Regular Prostatic Tissue; RPTa—Regular Prostatic Tissue Alternated by Atrophy Spotlights.

Table 3 Analysis of the fragments diagnosed with adenocarcinoma and the labeling pattern of *34βE12*.

Biopsy site of the prostate	Sum of the Gleason scores in the biopsy			Percentage of biopsy impairment (%)				*34βE12*	
	≤ 6	7	> 7	0-20	> 20-40	> 40-60	> 60-80	Negative	Positive
Base right	16	0	1	11	2	0	4	17	0
Third middle right	6	0	0	4	0	2	0	6	0
Apex right	10	0	0	10	0	0	0	10	0
Base left	16	3	0	16	3	0	0	19	0
Third middle left	11	3	0	12	2	0	0	14	0
Apex left	14	6	0	17	3	0	0	20	0

Table 4 Analysis of patients by type of diagnosis and staining by the *34βE12* marker.

Type of diagnosis by *H&E* staining	All N (%)	*34βE12*	
		Negative	Positive
Malignant	22 (64.70)	22	0
ASAp	5 (14.70)	4	1
Benign Prostatic Tissue*	7 (20.58)	0	7

(χ^2 = 29,55 p < 0,0001), *Benign Prostatic Tissue (sum of the regular prostatic tissue and regular prostatic tissue alternated by atrophy spotlights).

Table 5 Analysis of the validity parameters of the diagnosis by H&E having the marker *34βE12* as reference standard.

				Validity parameters of marker (%)			
	Diagnosis by immunohistochemistry						
Diagnosis by *H&E* staining	ADn	ASAp	BPT*				
	34βE12						
	Negative	Positive		Sensibility	Specificity	**PVPR	***PVNR
ADn	22	0	0	85	100	100	67
ASAp	4	1	0	100	88	20	100
*BPT	0	0	7	100	100	100	100

*BPT—Benign Prostatic Tissue (sum of the regular prostatic tissue and regular prostatic tissue alternated by atrophy spotlights); ** PVPR—Predictive Value of a Positive Result; *** PVNR—Predictive Value of a Vegative Result.

value ($p < 0.0001$) to aid the distinction of patients previously diagnosed with prostatic neoplasia and benign prostatic tissue using the *H&E* technique. However, the *34βE12* marker has 80% of the negativity and 20% of positivity for patients previously diagnosed with ASAp by the *H&E* technique (Table 4) (Fig. 5). Therefore, it is evident the use of this marker in the aid in patients diagnosed with ASAp by *H&E* to elucidate cases undefined by morphology and to avoid underdiagnosis of adenocarcinoma.

When analyzing the validity parameters of the diagnosis by *H&E* staining with the *34βE12* marker, we showed that the sensitivity for ADn was 85%, 100% for ASAp and 100% for BPT (Benign Prostatic Tissue—sum of the regular prostatic tissue and usual prostatic tissue alternated by atrophy spotlights) (Table 5). The specificity for ADn was 100%, 88% for ASAp and 100% for BPT. The PVPR for the ADn was 100%, 20% for the ASAp and 100% for the BPT; the PVNR for ADn was 67%, 100% for ASAp and 100% for BPT. We evidenced the need for immunohistochemistry in the elucidation of doubtful diagnoses or not specific for morphology, since patients previously classified with ASAp contained malignancy characteristics.

4. Discussion

Several studies have attempted to find methods of elucidating histological diagnosis in prostate cancer which in undetermined cases account for approximately 1.5-9% of prostate biopsies, therefore, the immunohistochemistry application contributes to its diagnostic value [17].

34βE12 keratin (also referred to as keratin *903-K903*) is a high molecular weight keratin and was first proposed as a basal cell marker. The *34βE12* antibody reacts strongly with the total thickness of all stratified squamous epithelium (prostate, skin, larynx, esophagus, ectocervix and others). In general, positively-labeled cells tend to have a more basal location within the gland, best represented in the prostate gland. *34βE12* antibody has not observed reactivity with mesenchymal or nerve tissue [18].

Wien et al. [19] in a study with 796 biopsies with *34βE12* staining demonstrated a reduction in the rate of doubtful cases from 5.1% to 1.0% and additionally offered a means of quality assurance when confirming the diagnosis of 61 prostate carcinomas made on the basis of samples of biopsy. Abrahams et al. [20] in a study with 30 cases, the staining for the *34βE12* antibody was positive in 26 of the cases with involvement classification between < 50% (5 cases), 50-75% (9 cases), > 75% (10 cases) and > 95% (2 cases) of the benign glands and in 4 cases (13%) the *34βE12* failed to stain any tissue, even after repeated staining, the *34βE12* antibody in no case stained the malignant glands and using the cut of > 75% of staining in the benign glands, the sensitivity of *34βE12* was 40% for benign glands. Several studies have found that benign prostatic glands stain positively for the *34βE12* marker [21, 22]. According to the staining pattern, extent and intensity of basal cell markers in the benign glands, the *34βE12* marker presented the best results, the *34βE12* marker presented the best sensitivity and specificity values (95% and 98%, respectively). Our study showed that all patients (100%) with fragments of benign prostatic glands (regular prostatic tissue, regular prostatic tissue alternated by atrophy spotlights) presented positivity for the *34βE12* marker, revealing its high significance ($p < 0.0001$) in the elucidation of benign glands (Tables 2 and 4). When analyzing the validity parameters of the *H&E* staining using the *34βE12* marker, we showed a sensitivity of 85% for the diagnosis of ADn by *H&E* staining in doubtful cases or not representative by biopsy, therefore showing the need for immunohistochemistry in cases that do not present a totally representative sample by biopsy (Table 5).

One study evidenced that the *34βE12* marker stained negative in all neoplastic areas and stained most (85%) of non-neoplastic epithelium [23]. In our study 86 fragments with diagnosis of ADn by *H&E* staining

were stained with the *34βE12* marker and all showed negativity to *34βE12* (Table 1). When stratifying the 86 (64.17%) fragments of biopsies diagnosed with ADn by the sum of the Gleason score (≤ 6, 7, > 7), percentage of biopsy involvement (0-20, > 20-40, > 40-60, > 60-80) and positivity for the *34βE12* marker, we showed that regardless of the sum of the Gleason scores and the amount of biopsy affected, the *34βE12* marker was negative in all ADn samples analyzed (Table 3).

Wojno et al. [24] studied 228 cases with *34βE12* immunostaining and found that the marker was useful in establishing, confirming or changing the diagnosis in 74% of the cases and 64% of the biopsies per needle. From these data, they concluded that staining with *34βE12* is a useful tool in confirming, establishing or changing the diagnosis in questionable focus seen in the daily practice of pathology [24]. When analyzing the patients diagnosed with atypical small acini proliferation as an abnormality in the collected fragments, we showed that the *34βE12* marker in these patients becomes very significant, because of the 5 (14.70%) patients analyzed, 80% of the cases were negative and 20% positive (Fig. 5). Therefore, patients with negative staining (80%) were reclassified with ADn and the *34βE12* marker was shown to aid in the elucidation and differentiation of patients with ASAp or ADn (Tables 2 and 4).

Despite our findings, there are some points that could be improved in future studies, including a relatively larger population sample. Due to the fact that the study was retrospective, it was not possible to add other markers for diagnostic comparison and to further enrich the statistical analysis. More studies are needed to confirm if this would affect the search results.

5. Conclusions

Due to the early detection of prostate cancer in the modern era, pathologists have become increasingly challenged to diagnose small outbreaks of cancer when only a few atypical glands are present in needle biopsies. The advent of immunohistochemistry has become an essential tool in the evaluation of such focus to confirm the absence of basal cells.

Therefore, we conclude that the *34βE12* marker becomes an important tool for pathologists in the attempt to elucidate diagnoses such as prostatic ADn and ASAp. In our study, the *34βE12* marker was very significant ($p < 0.0001$) in the elucidation between benign and malignant prostatic tissues, showing a strong correlation between *34βE12* marker negativity and ADn diagnosis, positivity and diagnosis of benign glands.

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Conflict of Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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