Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Brasil

Cruz, Andrea O.; Santana, Amanda L. S.; Santos, Andréia C.; Athanazio, Daniel A.
Frequency of the morphological criteria of prostate adenocarcinoma in 387 consecutive prostate needle biopsies: emphasis on the location and number of nucleoli
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 52, núm. 2, marzo-abril, 2016, pp. 107-111
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

J Bras Patol Med Lab, v. 52, n. 2, p. 107-111, April 2016

10.5935/1676-2444.20160018

Frequency of the morphological criteria of prostate adenocarcinoma in 387 consecutive prostate needle biopsies: emphasis on the location and number of nucleoli

Frequência de critérios morfológicos de adenocarcinoma da próstata em 387 biópsias de agulha da próstata consecutivas: com ênfase na localização e no número de nucléolos

Andrea O. Cruz[1]; Amanda L. S. Santana[2]; Andréia C. Santos[2]; Daniel A. Athanazio[2]

1. Hospital Universitário Professor Edgard Santos, Bahia, Brasil. 2. Universidade Federal da Bahia, Bahia, Brasil.

ABSTRACT

Introduction and objectives: We evaluated the sensitivity and specificity of morphological criteria for the diagnosis of prostate adenocarcinoma in consecutive, prostate needle biopsies specimens with emphasis on the location and number of nucleoli. **Methods:** The morphological features of 387 consecutive prostate needle biopsies specimens, in 2013, were systematically examined and stratified according to the final diagnosis of benign, suspicious and malignant lesions. We also tested how well each criterion predicted the final diagnosis after the immunohistochemical evaluation for expression of the basal cell markers (p63 and high molecular weight cytokeratin) and racemase. **Results:** A prominent nucleoli is the most common feature of carcinoma; however it is also relatively common in benign cases. The frequencies of prominent central nucleoli in malignant, suspicious and benign cases were 99%, 89% and 27%, respectively. Marginated nucleoli (85%, 60% and 7%), double nucleoli (86%, 53% and 10%), and multiple nucleoli (47%, 14% and 2%) were less common in benign cases, with significant difference among the groups. From the 36 cases initially diagnosed as suspicious, the presence of marginated nucleoli and mitoses were associated with the final diagnosis of malignancy. Prominent central nucleoli were more associated with cases which the final diagnosis after immunohistochemistry was benign. **Conclusion:** The location and number of nucleoli may be valuable morphological markers to identify suspicious lesions, since these features are more specific for malignancy than nucleolar prominence. The presence of prominent nucleoli commonly leads to the initial diagnosis of suspicious lesion that, subsequently, will be resulted in benignity confirmed by immunohistochemistry.

Key words: prostate cancer; large-core needle biopsy; surgical Pathology.

INTRODUCTION

The widespread use of prostate cancer screening based on digital rectal examination and serum prostate specific antigen dosage in the last three decades increased the number of minimal volume prostate adenocarcinoma detected in needle biopsies. However, misdiagnosis of carcinoma in prostate needle biopsies is still a major problem in Uropathology[1]. There is an extensive list of well-known morphological criteria recognized for the diagnosis of prostate acinar adenocarcinoma[1-3]. Prominent nucleoli and infiltrative growth pattern of small acini are probably the most widely recognized and less specific of all criteria.

An old study by Helpap suggests that marginated nucleoli and multiple nucleoli are specific morphological markers of prostate acinar adenocarcinoma. In a series of 476 biopsies, the author found that the presence of multiple nucleoli was a unique feature of the malignant lesions; the frequency of double nucleoli ranged from 1.2% to 11.9%, whereas the frequency of more than two nucleoli per nucleus ranged from 0.4% to 0.7%, which depended on the histological grade. An eccentric (marginated) nucleoli was

First submission on 07/10/15; last submission on 25/12/15; accepted for publication on 26/12/15; published on 20/04/16

found in carcinomas (13%-48%) and suspicious lesions (26%), but it was not present in benign samples[4]. In a recent review, Epstein listed an extensive list of features that favor malignancy and benignity[2]. Previously, the same author commented on the potential use of the location and number of nucleoli as morphological markers of acinar adenocarcinoma, emphasizing the need of further validation[1].

To the best of our knowledge, a single study of consecutive, routine prostate needle biopsies focused attention on this issue after the original suggestion by Helpap. Varma *et al*. (2002)[5] evaluated 250 biopsies and found the ensuing sensitivity and specificity to the following criteria: prominent nucleoli (98%/75%, respectively), marginated nucleoli (88%/93%) and multiple nucleoli (64%/100%). This work highlights the potential gain in specificity when evaluating the position and number of nucleoli.

METHODS

All consecutive prostate core needle specimens examined in the Laboratory of Pathology IMAGEPAT (Salvador, Brazil) and the Hospital Universitário Professor Edgard Santos (Salvador, Brazil) during the year of 2013 were included in the study. The project was approved by the Research Ethics Committee of the Hospital Universitário Professor Edgard Santos (368.350/2013).

Using hematoxylin and eosin (HE) stained sections, thirteen morphological criteria for acinar adenocarcinoma were assigned. The number of nucleoli per nucleus and eccentric (marginated) nucleoli were recorded as previously described[5]. In addition, we further distinguished the frequency of double nucleoli or more than two nucleoli per nucleus as different findings/variables. The nucleoli was counted as for prominence, eccentricity and multiplicity if readily detected at 200× magnification[5]. Malignant cases were restricted to acinar adenocarcinoma of the prostate, and other types of cancer were excluded. All cases with high grade prostatic intraepithelial neoplasia (HGPIN) foci were excluded. When available, data on new biopsies were recorded. Cases were compared based on the diagnostic conclusion stratified by benign, malignant or suspicious (atypical small acinar proliferation [ASAP]) lesion.

In biopsies diagnosed with cancer or ASAP, only malignant or suspicious foci were evaluated based on the presence of morphological criteria of malignancy. All samples of biopsies with benign final diagnosis were evaluated for the presence of each morphological feature. Benign cases were not yet classified for specific diagnoses. Illustrative images of blue mucin, mitoses and marginated, double and multiple nucleoli are shown in **Figure**.

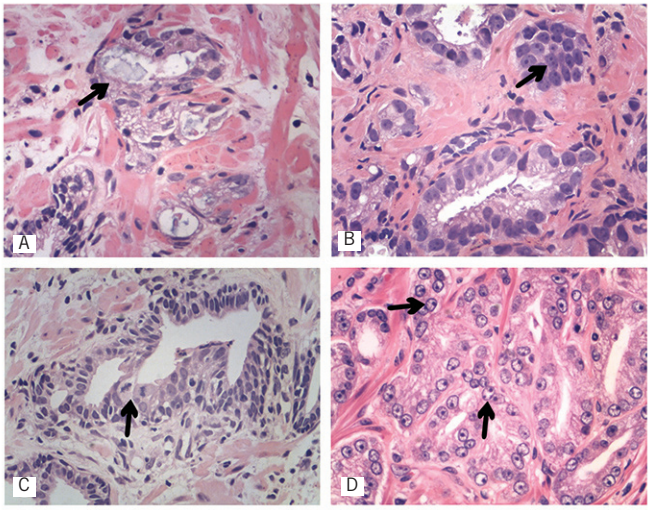

FIGURE – *Illustrative images of some morphological criteria for the diagnosis of prostate cancer*

A) blue mucin (arrow) in acinar adenocarcinoma (HE, 200×); B) multiple nucleoli (arrow) in acinar adenocarcinoma (HE, 400×); C) two mitoses (arrow) in reactive epithelium (HE, 200×); D) double and marginated nucleoli (arrows) in acinar adenocarcinoma (HE, 400×).

HE: hematoxylin and eosin.

We also investigated how each criterion would predict the final result after an initial diagnosis of ASAP and immunohistochemical evaluation of basal cell markers. In the laboratory of Pathology IMAGEPAT, basal cell markers were stained with the cocktail alpha-methylacyl-CoA-racemase (P504S) + p63 (DBS, Pleasanton, CA, USA) and anti-high molecular weight cytokeratin 34β12 (Dako, Carpinteria, USA). At the Hospital Universitário Professor Edgard Santos, the presence of basal cells was studied using 34βE12 antibody.

Categorical data were compared with a Chi-square test or Fisher's exact test, and the numerical data were compared using the student's *t* test.

RESULTS

A total of 387 consecutive biopsies were studied; 344 were from the Laboratory of Pathology IMAGEPAT and 43 from the Hospital Universitário Professor Edgard Santos. As expected, patients with cancer diagnosis by large-core needle biopsy were older and had higher serum prostate specific antigen (PSA) levels (**Table 1**). Eleven of thirteen morphological criteria had significantly different frequencies when comparing benign and malignant tissues at the initial diagnosis. These same criteria remained at significantly different frequencies when cases with initial diagnosis of ASAP

were included. Exceptions were angiolymphatic invasion and extraprostatic extension due to very low frequency in this series (**Table 2**).

Six cases had intraluminal crystalloids in benign glands (without foci of carcinoma). Unfortunately, none of these cases were biopsied again.

Table 3 shows how each criterion could predict the final result of needle biopsy after immunohistochemistry. Prominent nucleoli and intraluminal eosinophilic secretions were more common in cases with final diagnosis of benign. Marginated and double nucleoli, blue mucin, intraluminal crystalloids and mitoses were more common in cases with final diagnosis of malignancy. Multiple nucleoli were more common in cases with persistent result of ASAP and cancer, and they were not detected in any of the cases with final diagnosis of benign.

TABLE 1 – Frequency, age and serum PSA levels stratified according to the diagnosis after the examination of hematoxylin and eosin stained slides

Diagnosis	Frequency *n*/N (%)	Age (years)* mean ± SD	Serum PSA (ng/dl)* mean ± SD
Malignant	156/387 (40)	66.3 ± 9.7	16.2 ± 39.5
ASAP	55/387 (14)	63.3 ± 8.6	7.4 ± 6.7
Benign	176/387 (45)	61.8 ± 9	7.1 ± 5.7

ANOVA p < *0.05*.*
PSA: prostate specific antigen; SD: standard deviation; ASAP: atypical small acinar proliferation; ANOVA: analysis of variance.

TABLE 2 – Frequency of morphological criteria for the diagnosis of acinar adenocarcinoma of the prostate in 387 consecutive core needle biopsies stratified according to the diagnosis after the examination of HE stained slides

Criterion	Initial diagnosis after needle biopsy *n* = 387		
	Benign *n* = 176	ASAP (suspicious) *n* = 55	Malignant *n* = 156
Prominent nucleoli*	47/176 (27)	49/55 (89)	154/156 (99)
Marginated nucleoli*	12/176 (7)	33/55 (60)	133/156 (85)
Double nucleoli*	17/176 (10)	29/55 (53)	135/156 (86)
Multiple (> 2) nucleoli**	3/176 (2)	8/55 (14)	73/156 (47)
Intraluminal eosinophilic secretion*	32/176 (18)	20/55 (36)	94/156 (60)
Blue mucin**	1/176 (1)	2/55 (4)	26/156 (17)
Intraluminal crystalloids*	6/176 (3)	13/55 (24)	65/156 (42)
Collagenous micronodules**	0/176 (0)	0/55 (0)	10/156 (6)
Glomerulation**	0/176 (0)	0/55 (0)	17/156 (11)
Mitosis**	4/176 (2)	3/55 (5)	28/156 (18)
Perineural invasion**	0/176 (0)	0/55 (0)	18/156 (12)
Angiolymphatic invasion	0/176 (0)	0/55 (0)	2/156 (1)
Extraprostatic extension	0/176 (0)	0/55 (0)	2/156 (1)

Data are presented in n/N (%). *Chi-square test* p < *0.05*; Fisher's exact test* p < *0.05**.*
HE: hematoxylin and eosin; ASAP: atypical small acinar proliferation.

TABLE 3 – Frequency of morphological criteria for the diagnosis of acinar adenocarcinoma in 36 consecutive core needle biopsies with ASAP on initial diagnosis, stratified according to the final diagnosis after immunohistochemical evaluation of basal cell markers

Criterion	ASAP on initial diagnosis in needle biopsy *n* = 36		
	Benign *n* = 11	ASAP *n* = 4	Malignant *n* = 21
Prominent nucleoli*	11/11 (100)	2/4 (50)	18/21 (85)
Marginated nucleoli *	5/11 (45)	2/4 (50)	13/21 (62)
Double nucleoli*	3/11 (27)	2/4 (50)	13/21 (62)
Multiple (> 2) nucleoli*	0/11 (0)	1/4 (25)	2/21 (9)
Intraluminal eosinophilic secretion*	6/11 (55)	2/4 (50)	6/21 (29)
Blue mucin*	0/11 (0)	0/4 (0)	1/21 (5)
Intraluminal crystalloids*	0/11 (0)	1/4 (25)	6/21 (27)
Collagenous micronodules	0/11 (0)	0/4 (0)	0/21 (0)
Glomerulation	0/11 (0)	0/4 (0)	0/21 (0)
Mitosis*	0/11 (0)	0/4 (0)	2/21 (9)
Perineural invasion	0/11 (0)	0/4 (0)	0/21 (0)
Angiolymphatic invasion	0/11 (0)	0/4 (0)	0/21 (0)
Extraprostatic extension	0/11 (0)	0/4 (0)	0/21 (0)

Data are presented in n/N (%). *Chi-square test* p < *0.05*.*
ASAP: atypical small acinar proliferation.

DISCUSSION

In routine practice, it is not uncommon for the pathologist to find a marginated nucleoli or multiple nucleoli per nucleus in benign and malignant prostatic acini. Since these findings have been suggested as useful to differentiate cancer from its mimickers, we decided to test their frequency in our routine practice.

In a study design, different from those previously mentioned, Aydin *et al*. (2005)[6] evaluated a hundred prostate needle biopsies sent for consultation because of diagnosis difficulty. In their experience, they found more than one nucleoli per nucleus in 38% of cancer foci, 54% of HGPIN foci, 37% in atrophy and 30% in inflammatory foci. Multiple nucleoli were statistically more common in HGPIN than in cancer, and they were more common in cancer than in benign lesions. Eccentric nucleoli were found in 89% of all cancer foci, 99% of HGPIN, 80% of atrophy and 74% of inflammation. This feature was statistically more common in HGPIN than in cancer, and it was more common in cancer than in benign lesions, except for fully developed atrophy[6]. The authors concluded that the location and number of nucleoli per nucleus were not useful for distinguishing between malignant lesions and their benign mimickers.

Despite variations in the association of marginated and multiple nucleoli with malignancy, the wide variation of these

features in different studies is of concern. When compared to the series by Varma *et al*. (2002)[5], our series has similar rates of prominent nucleoli in malignant (99% in the present study vs 94%) and benign cases (27% vs 25%), as well as in marginated nucleoli (85% vs 88% in malignant and 7% vs 7% in benign)[5]. Data from Aydin *et al*. (2005)[6] are somewhat different, because central prominent nucleoli (85%) is less frequent than marginated nucleoli (89%) in malignant cases. A marginated nucleoli was detected in up to 8% of total benign glands in that study, 74% in inflammatory and 80% in atrophic foci[6]. The frequency of multiple nucleoli is probably similar when comparing our work and Varma's study, but it is not comparable because in the present study, we subdivided this finding into double or multiple (> 2 nucleoli per nucleus), with frequencies of 86% and 47% in malignant cases, respectively. Therefore, the frequency of multiple nucleoli in Varma's study (64%) is intermediate, and it was unexpectedly lower in Aydin's series (38%)[5, 6]. At least part of this variation may be explained by the different criteria used to assign these features. Varma *et al*. (2002)[5] recorded nucleoli that were readily visible at 200× magnification, whereas Aydin *et al*. (2005)[6] recorded nucleoli that were "not pinpoint"[5, 6]. Our findings reflect the frequencies observed by Varma *et al*. (2002)[5] because we used the same criteria for grading nucleolar prominence. However, it is not possible to overlook that the series of biopsies sent by Aydin *et al*. (2005)[6] for consultation accounts for the most difficult cases, because they have these benign mimickers of carcinoma with cytological atypia, such as marginated nucleoli and multiple nucleoli. However, the high frequency of marginated nucleoli in benign glands (normal) (8%) compared with the rate of 7% among the benign biopsies from the present study (which embraces all nonmalignant lesions) supports that this difference can be attributed to the observer criteria.

In the same series of consecutive needle biopsies by Aydin *et al*. (2005)[6], mitosis were observed in 13% of cancer, 12% of HGPIN, 6% of inflammation, 1% of atrophy cases, and was not observed in partial atrophy cases. The presence of mitoses was significantly different between cancer and partial atrophy cases[6].

We were also interested in how the presence of crystalloids in benign glands could predict the diagnosis of carcinoma in subsequent biopsies. The previous data suggested that the presence of intraluminal crystalloids in benign glands are more associated with concurrent in prostate needle biopsies than HGPIN[7]. However, the risk of carcinoma diagnosis in a subsequent biopsy, after observing intraluminal crystalloids in benign glands (23%), was not different from that in patients with previous negative biopsies without crystalloids (16%)[8]. Unfortunately, the presence of intraluminal crystalloids in benign glands (without concurrent cancer) is a rare phenomenon ($n = 6$ in this series), and none of the patients performed new biopsy.

Among other features of carcinoma, our study showed a lower frequency of intraluminal eosinophilic secretion (60% vs 87%) and intraluminal blue mucin (17% vs 52%) in the malignant cases when compared to Varma's series. The frequency of intraluminal crystalloids was similar (42% vs 40% in malignant, 3% vs 1% in benign). Our observations are consistent with observations of others authors, that collagenous micronodules, glomerulations, perineural invasion, angiolymphatic invasion and extraprostatic extension are specific for cancer, but they occur very rarely in core needle biopsies.

From Table 1, we may infer that the frequency of some morphological features could alternatively be influenced by age, because a definitive diagnosis of carcinoma is more common in older patients. To exclude the influence of age on morphological findings, we repeated all analyses presented in Table 2 after excluding all patients younger than 70 years. All associations were maintained (data not shown).

Furthermore, we investigated how each criterion could predict the final result of the needle biopsy after immunohistochemistry. In current medical practice in Brazil, immunohistochemical evaluation of suspicious lesions is not automatic and requires the approval of health insurance corporations. Therefore, a second complementary report usually follows the initial diagnosis of ASAP. Prominent nucleoli and intraluminal eosinophilic secretions are well known morphological features of carcinoma with low specificity. Therefore, small benign lesions with these features may be labeled as suspicious, and the diagnosis of malignancy can be discarded according to the immunohistochemical confirmation of the presence of basal cells. Marginated and double nucleoli, blue mucin, intraluminal crystalloids and mitoses were more common in cases with a final diagnosis of malignancy. These features are probably the most useful for decision-making in initial diagnosis of ASAP or the definitive diagnosis of malignancy in difficult cases.

CONCLUSION

In conclusion, the position and number of nucleoli are important tools for identifying acinar adenocarcinoma of the prostate. These features are also common in cases of malignancy, whereas they are more specific than prominent nucleoli alone. The presence of low specificity criteria, such as prominent nucleoli and intraluminal eosinophilic secretions, probably result in an

initial diagnosis of suspicious, which in many cases will finally be recognized as benign after immunohistochemical analysis.

COMPLIANCE WITH ETHICAL STANDARDS

This work was supported by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 474596/2012-5.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its subsequent amendments or comparable ethical standards. For this type of study, informed consent is not required. The research project was approved by the Ethics Comitee of the Hospital Universitário Professor Edgard Santos (368.350 08/22/2013).

RESUMO

Introdução e objetivos: *Avaliamos a sensibilidade e a especificidade de critérios morfológicos para diagnóstico de adenocarcinoma da próstata em biópsias de agulha da próstata, consecutivas, com ênfase na localização e no número de nucléolos.* ***Métodos:*** *A morfologia de 387 biópsias de agulha consecutivas do ano de 2013 foi sistematicamente examinada e estratificada como diagnóstico de benigno, lesão suspeita ou maligno. Também testamos como cada critério previu o diagnóstico final após avaliação imuno-histoquímica para expressão de marcadores de células basais (p63 e citoqueratina de alto peso) e racemase.* ***Resultados:*** *Um nucléolo proeminente foi o achado mais comum do carcinoma, mas também foi relativamente comum em casos benignos. As frequências de um nucléolo proeminente central em lesões malignas, suspeitas e benignas foi de 99%, 89% e 27%, respectivamente. Nucléolo marginado (85%, 60% e 7%), nucléolos duplos (86%, 53% e 10%) e nucléolos múltiplos (47%, 14% e 2%) foram menos comuns em casos benignos, com diferenças significativas entre os grupos. Dos 36 casos com diagnóstico inicial de suspeito, a presença de nucléolo marginado e mitoses foi associada ao diagnóstico final de malignidade. Um nucléolo proeminente central foi mais associado a casos cujo diagnóstico final após imuno-histoquímica foi benigno.* ***Conclusão:*** *A localização e o número de nucléolos pode ser um marcador morfológico valioso para identificar lesões suspeitas, uma vez que esses achados são mais específicos para malignidade do que a proeminência nucleolar. A presença de nucléolo proeminente comumente leva ao diagnóstico inicial de lesão suspeita que, posteriormente, terá a conclusão de benignidade confirmada pela imuno-histoquímica.*

Unitermos: *doenças prostáticas; biópsia com agulha de grande calibre; patologia cirúrgica.*

REFERENCES

1. Epstein JI. Diagnosis and reporting of limited adenocarcinoma of the prostate on needle biopsy. Mod Pathol. 2004; 17(3): 307-15.

2. Epstein JI. Diagnosis of limited adenocarcinoma of the prostate. Histopathology. 2012; 60(1): 28-40.

3. Iczkowski KA, Bostwick DG. Criteria for biopsy diagnosis of minimal volume prostatic adenocarcinoma: analytic comparison with nondiagnostic but suspicious atypical small acinar proliferation. Arch Pathol Lab Med. 2000; 124(1): 98-107.

4. Helpap B. Observations on the number, size and localization of nucleoli in hyperplastic and neoplastic prostatic disease. Histopathology. 1988; 13(2): 203-11.

5. Varma M, Lee MW, Tamboli P, et al. Morphologic criteria for the diagnosis of prostatic adenocarcinoma in needle biopsy specimens. A study of 250 consecutive cases in a routine surgical pathology practice. Arch Pathol Lab Med. 2002; 126(5): 554-61.

6. Aydin H, Zhou M, Herawi M, Epstein JI. Number and location of nucleoli and presence of apoptotic bodies in diagnostically challenging cases of prostate adenocarcinoma on needle biopsy. Hum Pathol. 2005; 36(11): 1172-7.

7. Svatek RS, Karam JA, Rogers TE, Shulman MJ, Margulis V, Benaim EA. Intraluminal crystalloids are highly associated with prostatic adenocarcinoma on concurrent biopsy specimens. Prostate Cancer Prostatic Dis. 2007; 10(3): 279-82.

8. Henneberry JM, Kahane H, Humphrey PA, Keetch DW, Epstein JI. The significance of intraluminal crystalloids in benign prostatic glands on needle biopsy. Am J Surg Pathol. 1997; 21(6): 725-8.

CORRESPONDING AUTHOR

Daniel Abensur Athanazio

Universidade Federal da Bahia, Faculdade de Medicina da Bahia, Departamento de Patologia e Medicina Legal; Praça XV de novembro, s/n; Largo do Terreiro de Jesus; CEP: 40025-010; Salvador-BA, Brasil; Phone: +55 (71) 3283-5563, Extension: 41; Fax: +55 (71) 3240-8406; e-mail: daa@ufba.br.