Utility of seriated sections in prostate biopsies

J. Arista-Nasr, J. de Anda-González, L. Bornstein-Quevedo, F. Chablé-Montero*

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México DF, Mexico

Received 26 March 2012; accepted 5 April 2012

KEYWORDS
Prostate sampling; Needle biopsy; Additional sections; Focal carcinoma; Atypical small acinar proliferation

Abstract
Introduction: With the routine use of prostate specific antigen, focal carcinomas and atypical small acini proliferation (ASAP) are currently detected more frequently. The number of sections per cylinder needed to detect most of them is still unknown.

Methods: We reviewed 250 sextant prostate biopsies in the 2008–2011 period. The average number of cylinders per biopsy was 14. In each case, in addition to the original sections with three histological levels, three more sections were performed with three levels (total: 12 levels). Biopsies with focal lesion were analyzed immunohistochemically. The frequency of focal lesions was compared to a previous study of 1000 biopsies in which a single section was made with three histological levels. The main clinical and laboratory data were recorded.

Results: There were 16 focal lesions (6.4%). Seven (2.8%) corresponded to focal carcinomas and nine (3.6%) to atypical proliferation. In the previous study, thirteen (1.3%) focal carcinomas and 29 (2.9%) cases with atypical proliferation were found.

Conclusions: There was an increase of 4.2–6.4% of focal lesions carcinomas increased from 1.3% to 2.8%. Making additional sections in all biopsies may have practical drawbacks. However, they could be performed in patients with high clinical suspicion of carcinoma (especially in young patients), or when there is a history of atypical glandular proliferations consistent with carcinoma in previous biopsies.

© 2012 AEU. Published by Elsevier España, S.L. All rights reserved.

Utilidad de cortes seriados en biopsias prostáticas

Resumen
Introducción: Con el empleo rutinario del antígeno prostático específico, se detectan con mayor frecuencia carcinomas focales y proliferaciones atípicas de acinos pequeños (ASAP, siglas en inglés). El número de cortes por cilindro que debe practicarse para detectar la mayoría de ellos se desconoce.

Métodos: Revisamos 250 biopsias prostáticas por sextantes en el periodo 2008–2011. El promedio de cilindros por biopsia fue de 14. En cada caso se practicaron además del corte original con tres niveles histológicos, otros tres cortes con tres niveles (total: 12 niveles). En las biopsias con lesiones focales se practicó estudio inmunohistoquímico. La frecuencia de lesiones focales...
se comparó con un estudio previo de mil biopsias donde se realizó un solo corte con tres niveles histológicos. Se anotaron los datos clínicos y de laboratorio.

**Resultados:** Hubo 16 lesiones focales (6.4%); siete (2.8%) correspondieron a carcinomas focales y nueve a (3.6%) proliferaciones atípicas. En el estudio previo se encontraron trece (1.3%) carcinomas focales y 29 (2.9%) casos con proliferaciones atípicas.

**Conclusiones:** Hubo un aumento de 4.2% a 6.4% de lesiones focales y un incremento de carcinomas del 1.3% a 2.8%. Aunque realizar cortes adicionales rutinariamente tiene inconvenientes prácticos, podría realizarse en pacientes con alta sospecha clínica de carcinoma (en particular jóvenes) o en los que existan antecedentes de proliferaciones glandulares atípicas compatibles con carcinoma.

© 2012 AEU. Publicado por Elsevier España, S.L. Todos los derechos reservados.

**Introduction**

With the widespread use of the determination of serum of the prostate specific antigen (PSA) levels and increased sextant prostate biopsies, we detected focal lesions of the prostate more frequently. These lesions included focal carcinomas (minimal or limited)\(^1\)-\(^3\) and atypical small acinar proliferations (ASAP).\(^4\)-\(^9\) Many ASAPs correspond to areas of adenosis, atrophy, atypical hyperplasia of basal cells, seminiferous ducts, etc. that are difficult to identify as benign by the moderate atypia and/or the small number of glands. Others corresponded to carcinoma showing insufficient criteria to establish a categorical diagnosis of malignancy.\(^5\),\(^6\),\(^8\) Recognizing focal carcinomas is important, as they usually have a good prognosis. Additionally, many of the ASAP biopsies correspond to benign lesions that may be confused and treated as carcinomas.

In this study the frequency of focal carcinomas and ASAP is reviewed in 250 prostate biopsies in which several cuts per cylinder were performed, and it is compared to the lesions found in a previous study with 1000 biopsies in which a single cut was made.\(^10\)

**Material and methods**

We studied 250 needle biopsies (period: 2008–2011) for clinical suspicion of prostate carcinoma. On an average, 14 cylinders were obtained per biopsy. All underwent a routine cut with three histological levels, and additionally three cuts with three histological levels (total: 4 cuts with 12 levels). We selected the focal carcinomas (FCA), also called limited or minimum that measured less than a millimeter, and ASAP. In the biopsies with focal lesions, immunohistochemical studies were performed with high molecular weight keratins (Cytokeratin 34 BetaE12 and p63, Dako, CA). The age, the PSA levels, the findings in the DRE, the additional biopsies, and the extent of the neoplasia in the cases in which prostatectomy was carried out were noted.

The focal lesions were classified as follows:

1. Focal carcinomas (FCAs): biopsies that showed architectural and cytological criteria sufficient to establish the undoubted diagnosis of malignancy and that measured less than one millimeter (Fig. 1).\(^1\),\(^2\)
2. Atypical small acinar proliferations (ASAP): biopsies with architectural and cytological changes, suggestive but not diagnostic of carcinoma (Figs. 2–4).\(^5\),\(^6\),\(^8\)

We noted the following histological changes: (a) irregular arrangement of the glands or infiltrative appearance; (b) number of glands; (c) nucleomegaly; (d) nuclear hyperchromasia; (e) prominent nucleoli; (f) mitosis; (g) amphophilic cytoplasm; (h) acute and chronic inflammation; (i) eosinophilic and basophilic intraluminal secretions; (j) crystalloids; (k) atrophic glands; (l) cytoplasmic lipofuscin; (m) neural infiltration; and (n) amylaceous bodies.\(^5\),\(^8\)

**Statistical analysis**

We used the Chi-square test to evaluate differences in proportions using the SPSS 15 statistical package.

**Results**

The frequency of focal lesions in the biopsies from the previous study and those found in the present study are shown in **Table 1**. The frequencies of lesions, overall and divided by type of lesion, were all higher in the current study than in the previous one, particularly in focal carcinomas, in which the current study had a frequency of more than double the previous study, although it only reached marginal statistical significance \((p < 0.10)\). **Table 2** shows clinical and laboratory data. As a group, there were no significant differences between the two of them.

![Figure 1](image-url)  
**Figure 1** Focal carcinoma of the prostate. Nest of neoplastic glands with nucleomegaly and prominent nucleoli. The infiltrative pattern is inconspicuous (hematoxylin and eosin staining, 350×).
hyperchromatic arrangement.

Figure 2 Scattered atypical glands with apparent nucleomegaly and nucleoli surrounded by a fibrous stroma. The diagnosis is of atypical glands of uncertain significance (ASAP) (hematoxylin and eosin staining, 350×).

Figure 3 Same histological field as Fig. 2 in the second additional level. Although the number of glands did not increase significantly, the glands are now easily recognizable as atrophic (hematoxylin and eosin staining, 250×).

Focal carcinomas (7 biopsies)

The number of glands ranged from 5 to 20 (average 12 glands), which showed infiltrative aspect or irregular arrangement. The constant findings included nucleomegaly, hyperchromatic nuclei, prominent nucleoli, and intraluminal secretions; the latter were basophilic in a biopsy

Table 1 Comparison of the prevalences of lesions in needle prostate biopsies with a cut (previous study) vs. 4 cuts (current study).

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Previous study</th>
<th>Current study</th>
<th>Chi square p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCA</td>
<td>13/1000 = 1.3%</td>
<td>7/250 = 2.8%</td>
<td>0.091 marginal</td>
</tr>
<tr>
<td>ASAP</td>
<td>29/1000 = 2.9%</td>
<td>9/250 = 3.6%</td>
<td>0.56 not significant</td>
</tr>
<tr>
<td>Overall</td>
<td>42/1000 = 4.2%</td>
<td>16/250 = 6.4%</td>
<td>0.14 not significant</td>
</tr>
</tbody>
</table>

Table 2 Age, PSA, and digital rectal examination in focal glandular lesions.

<table>
<thead>
<tr>
<th>Age</th>
<th>PSA</th>
<th>S-DRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal carcinoma (7 cases)</td>
<td>54–78 years (P: 69 years)</td>
<td>4.2–11.9 ng/ml (P: 8.0 ng/ml)</td>
</tr>
<tr>
<td>ASAP (9 cases)</td>
<td>56–72 years (P: 63 years)</td>
<td>4.1–13.9 ng/ml (P: 7.4 ng/ml)</td>
</tr>
</tbody>
</table>

PSA, prostate-specific antigen; ASAP, atypical small acinar proliferation; S-DRE, suspicious digital rectal examination.

(Figs. 1 and 2). Less frequent changes included crystalloids (one case) and mitosis (one case). One of the carcinomas showed xanthomatous appearance. The immunohistochemical study confirmed the absence of basal cells. The Gleason score was 6 in 6 cases and 7 in another.

Atypical glandular proliferation (9 biopsies)

The number of glands ranged from 4 to 22 (average 13). In several biopsies, irregular glandular arrangement and little apparent infiltrative pattern were observed. The nucleomegaly was focal, and in 3 the nuclei were hyperchromatic and small. The nucleoli were less apparent than in the cases with focal carcinoma, and they were present in less than 20% of the glands. Eosinophilic intraluminal secretions were found in 4 cases. Two biopsies showed few lymphocytes between the atypical glands. Three ASAPs showed
doubtful basal cells in the immunohistochemical study suggesting their benign nature.

Discussion

The frequency of detection of focal lesions in prostate biopsies seems to be related to the number of levels performed. By comparing the incidence of lesions in isolated fields when performing a single histological section vs. 4 serial sections, the frequency increased from 4.2 to 6.4% (an increase of 2.2%). Of greatest interest was the detection of twice the focal carcinomas from 1.3 to 2.8%. This increase justifies making additional cuts in patients with a history of ASAP, or in those where there is a high suspicion of carcinoma, particularly if it is in young patients. In contrast, the frequency of ASAP was very similar (2.9 vs. 3.6%). In the biopsies with FCA in additional levels, they may show an increased number of glands which facilitate the interpretation of architectural and/or cytological criteria of malignancy, or reveal malignant glands that were not evident in the original cuts.  

Renshaw found that 3% of the carcinomas are not found in the first level of the routine cuts, and suggests making at least 3 additional cuts, particularly if there are areas with atypical glands. In their study, the additional levels in biopsies with prostatic intraepithelial neoplasia showed no associated carcinoma.

Reviewing the literature, we found that the additional levels are useful in approximately 20–25% of the biopsies with ASAP. We recently studied a group of 30 biopsies with ASAP in which 9 additional levels and immunohistochemical studies were performed. The additional cuts showed carcinoma in 4 cases in which the immunostains were not useful for the diagnosis of carcinoma. The combination of both procedures may be needed to detect a larger number of carcinomas limited to isolated histological fields. Routinely making several additional cuts in all prostate biopsies is not practical in terms of cost–benefit, and the time it takes for the viewer to review a considerable amount of lamellae.

The FCA diagnosis in needle biopsy does not imply that the neoplasia represents 100% of the carcinoma, and most of them will have to show other areas with adenocarcinoma. However, the focal carcinomas detected in needle biopsy are less spread and have a better prognosis than the carcinomas diagnosed in several fragments. In 20 patients with limited carcinoma, we found a patient in T0 stage, 18 in T2, and one in T3a; 62.5% were bilateral. In another series, Leroy et al. found that the neoplasia was located in the gland (T2) in 22 out of 24 patients with FCA, and two were classified in T3 stage.

The frequency of focal carcinoma in the general population is significant. Van der Kwast et al. found carcinoma in 5.1% of the 19,970 men screened, and 31.6% of them were classified as limited carcinomas. In cases of intraepithelial neoplasia or ‘suspected for malignancy’, adenocarcinoma was detected in 12.1 and 36.5% respectively in subsequent biopsies. FCA detection has resulted in an increase of small residual carcinomas, or apparent absence of neoplasia. The annual incidence of minimal residual carcinoma increased approximately from 0.5% in 1988 to 4% in 1993, and it has stabilized from 3 to 4% since then.

The main histological criteria to establish the diagnosis of FCA have been mentioned and referenced above. These changes are not unique to carcinoma and most can be found in ASAP. This overlapping of criteria explains the difficulties in interpreting these biopsies, as well as the discrepancy between observers. The presence of a higher amount of acini, infiltrative growth pattern, mitosis, prominent nucleolus in 10–20% or more of the cells, nuclear-omegaly, nuclear hyperchromasia, and basophilic secretions favor carcinoma. The immunohistochemical studies have improved diagnostic accuracy. The undoubted positivity to high-molecular weight keratins enables us to classify the proliferation as benign; however, a small nest of negative atypical glands to this antibody does not always mean that it is malignant, because in the cut-off level basal cells may not exist. The correlation with the conventional histological sections is required for the final interpretation. Additionally, the prostate-specific antigen levels and age can be very similar in ASAP and ASAP (Table 2) and of little use to favor one of these diagnoses.

Knowing the morphology and frequency of ASAP is important, because this is the injury with greatest predictive value for the diagnosis of carcinoma. The diagnosis of ASAP implies the possibility of finding prostate carcinoma in approximately 50% of the cases in a second biopsy, and on an average 3% of the biopsies will show atypical proliferations.

Conclusions

The increase in the number of serial sections in cylinders of sextant prostate biopsy increased the detection of focal lesions from 4.2 to 6.4%. The frequency of focal carcinomas doubled (1.3–2.8%) when 4 cuts per cylinder vs. only one cut were made. In contrast, the increase in atypical glandular proliferation (ASAP) was insignificant. Making serial sections in all prostate biopsies has limitations due to the cost–benefit and the time required to review a considerable number of cuts per biopsy. However, we think that making serial sections could be useful in patients with high suspicion of carcinoma (particularly if they are young patients) and patients with previous biopsies with ASAP suggestive of malignancy.

Conflict of interest

The authors declare that they have no conflict of interest.

References

4. Cheville JC, Resnick M, Bostwick DG. The focus of “atypical glands, suspicious for malignancy” in prostatic needle biopsy