Significance of Prostate Cancer Missed on Needle Biopsy Tools for Retrieving Missed Cancer

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BACKGROUND. Prostate needle biopsy (PNB) is required for the diagnosis of prostate cancer (PCa), but little is known about the frequency and clinical implication of false-negative results.

OBJECTIVE. To investigate the incidence and clinical impact of minute PCa missed on routine haematoxylin and eosin (H&E) slides, but retrieved by α-methylacyl-CoA-racemase (AMACR) immunohistochemistry.

METHODS. AMACR immunohistochemistry was used to detect PCa missed on H&E slides in a series of consecutive 1,672 PNB including 1,003 patients without evidence of PCa, and 669 patients with PCa meeting pathological criteria for active surveillance (PCAS) under current clinical investigation, including Gleason score ≤ 7 $(3+4)$, $<33\%$ of biopsies involved by cancer, $<50\%$ of any core involved by cancer. Using improved multicore (pre-) embedding techniques a single AMACR immunostain/patient was sufficient to detect missed lesions.

RESULTS. In patients without histological evidence of PCa, AMACR immunohistochemistry retrieved minute PCa in 33 of 1,003 patients (3.29%) and atypical small acinar proliferations (ASAP) in 17 of 1,003 patients (1.69%). Among 116 of 669 (17.34%) PCa patients meeting PCAS, detection of additional core(s) involved by cancer was found responsible for disease reclassification in 63 of 116 of patients (54.31%). Limitations include the single-institutional design of the study.

CONCLUSIONS. PCa missed on routine H&E histology was retrieved by AMACR in 8.91% of PNB, including 17.34% of PCa patients meeting PCAS. 54.31% of them have finally lost their eligibility for active surveillance after detecting additional cores involved by cancer. Underdiagnosis of limited adenocarcinoma on PNB is a matter of concern, but can be prevented by a single AMACR immunostain/patient if improved multicore (pre-) embedding techniques are used. *Prostate 76:369–375, 2016.*

KEY WORDS: **false negative prostate biopsy; AMACR; active surveillance**

INTRODUCTION

The underdiagnosis of limited adenocarcinoma on prostate needle biopsy (PNB) is a significant problem in prostate pathology, but little is known about the frequency and clinical implications of false-negative biopsy results. It is hard to obtain related data, as most pathologists do not want for medicolegal or other reasons to go back and review old cases for potential missed cancer, or even publish their own false-negative rate. Most current information derives from studies of PNB sent in for consultation or prostate cancer screening trails [1,2]. Among 3.251 PNB cases seen in consultation, Epstein and coworkers have reported a false-negative rate of 2.7%, although these data underestimate the magnitude of the problem, as the entire specimen was not submitted for review in 41% of cases in this study [1]. In a series of the European randomized study of screening for prostate cancer (ERSPC), 196 biopsies which had been reported as "benign" at initial diagnosis, followed by a diagnosis

Conflicts of interest: none.

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of adenocarcinoma in a subsequent screening round, missed adenocarcinoma or atypical small acinar proliferations (ASAP) was identified in 8.2%, respectively, 9.7% of patients [2]. Few studies have used the prostate cancer (PCa) marker alpha-methylacyl CoA racemase (AMACR) to detect minute PCa missed on routine haematoxylin and eosin (H&E)-stained PNB [3,4]. In a series of 793 PNB specimens classified benign on H&E slides from 239 patients with PCa diagnosed in other biopsy cores taken at the same biopsy session, the authors have reported a false-negative rate of 1.1% [3]. A more recent study using dual-color immunostaining with a 3-antibody cocktail (AMACR, 34βE12, and p63) on PNB from 113 patients has retrieved 14 missed lesions (12.39%) on which consensus was reached in eight cases of limited adenocarcinoma (7.08%) and one ASAP (0.88%) [4]. Nevertheless, the risk of missing minute cancer on PNB from larger series of unselected patients and its clinical implication is currently unknown.

The present study evaluates the incidence of minute PCa missed on routine H&E histology, but retrieved by AMACR immunohistochemistry in a series of 1672 consecutive and unselected patients, including 669 PCa patients meeting pathological inclusion criteria for active surveillance (PCAS), currently under investigation in large active surveillance cohorts [6,7]. AMACR was used, because it is considered currently the best tissue marker that highlights the malignant prostatic epithelium [5].

MATERIALS AND METHODS

The vast majority of PNB enrolled in the current study was submitted not conventionally, floating free in formalin-filled containers, but in two tissue cassettes (right and left), in which the core biopsies are straightened and orientated between two meshes before formalin fixation by the urologist (Fig. 1A). This sandwich pre-embedding method is known to increase the frequency of cancer diagnosis by decreasing loss of tissue during the cutting process [8,9]. In addition, a metal tamper was used to further enhance the flattening of the cores in the same plane during their embedding in paraffin. In this way, a maximum of 6–8 cores per cassette could be embedded safely without compromising their quality. The tissue blocks from right and left were cut at least four levels. Additional paraffin wax ribbons from the right and left block between the two levels are sampled on two slides suitable for immunohistochemical analysis (AMACR, and CK5/6). Using this multicore embedding technique, we were able to provide an immunohistochemical evaluation of the entire biopsy specimen with only two immunostains (Fig. 1B).

From a series of 2,499 consecutive patients with PNB during a period between January 2013 and April 2015, 1,672 patients with available immunohistochemistry were considered in the current study. Excluded from immunohistochemical evaluation were 95 benign PNB containing >50% non-glandular prostate tissue (stromal, capsular, and extraprostatic tissue). Submitted for study were 1,003 PNB without evidence of cancer on routine H&E slides, and 669 patients with diagnosed PCa meeting current pathological inclusion criteria for active surveillance (PCAS), that is, Gleason score ≤ 7 $(3+4)$, <33% of biopsies involved by cancer, <50% of any core involved by cancer [6,7]. It is noteworthy that the Gleason 7 $(3+4)$ is not considered in the inclusion criteria of all active surveillance cohorts currently under investigation [6,7].

Tissue blocks were cut and mounted on adhesive slides with a surface electric charge density. Endogenous peroxidase activity was blocked for 6 min with 3% hydrogen peroxidase in water. Subsequently, heat-induced epitope retrieval was performed according to manufacturers' instruction. Immunohistochemistry was performed by using a monoclonal rabbit Anti Human AMACR antibody, clone 13H4 (Biologo; Kronshagen, Germany) and a monoclonal mouse Anti Human CK5/6 antibody, clone D5/16 B4 (Dako, Hamburg, Germany). Internal positive control was benign prostate glandular tissue for CK5/6, and rectum mucosa, frequently included in PNB specimens, for AMACR. All cases including the initial (H&E stained) biopsies and the immunohistochemical stains (AMACR, basal cell keratins) were reviewed and reported by the same pathologist.

RESULTS

In PNB classified as benign on routine H&E slides from 1,003 patients, minute PCa was detected by AMACR immunohistochemistry in 33 cases (3.29%). Additional ASAP's were identified in 17 of 1,003 cases (1.69%) (Fig. 2A–H). In patients with diagnosed PCa meeting current pathological inclusion criteria for active surveillance (PCAS) [6,7], additional core(s) were detected by AMACR immunohistochemistry in 116 of 669 cases (17.34%). Among these patients, detection of additional core(s) involved by cancer did not affect their PCAS status in at least 54 of 116 cases (46.55%). In 63 of 116 (54.31%) patients, however, the retrieval of missed cancer in additional core(s) by AMACR was associated with a failure of PCAS, including Gleason score >7 $(3+4)$, ≥33% of biopsies involved by cancer, ≥50% of any core involved by cancer. Referring to all 669 PCa patients meeting PCAS, the

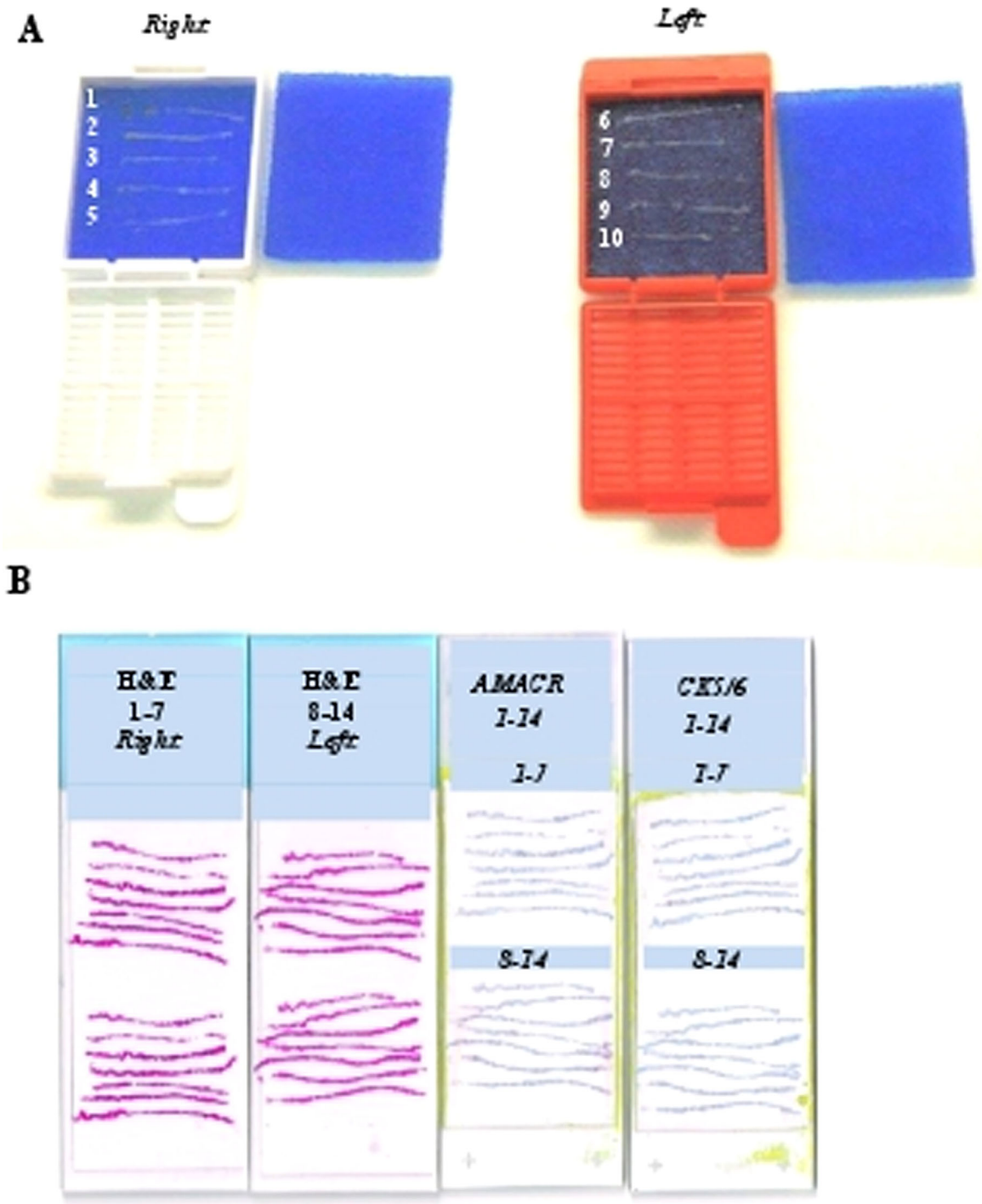

Fig. 1. Improved sandwich pre-embedding method (**A**), histology and immunohistochemistry (**B**). Urologists submit core biopsies not conventionally, floating free in formalin-filled containers, but in two tissue cassettes (right and left), in which core biopsies are straightened and orientated between two meshes before formalin fixation. The position of each core in the tissue cassettes indicates its clinical localization (e.g., 1–10). Histological and immunohistochemical slides of a 14-core biopsy. Please note that the cores on the H&E slides are perfectly straight without any fragmentation, deformation or tissue loss. The cores of the right and left side are mounted on two additional slides, which are suitable for immunohistochemical analysis of AMACR and a basal cell marker (CK5/6) on the entire biopsy specimen (B).

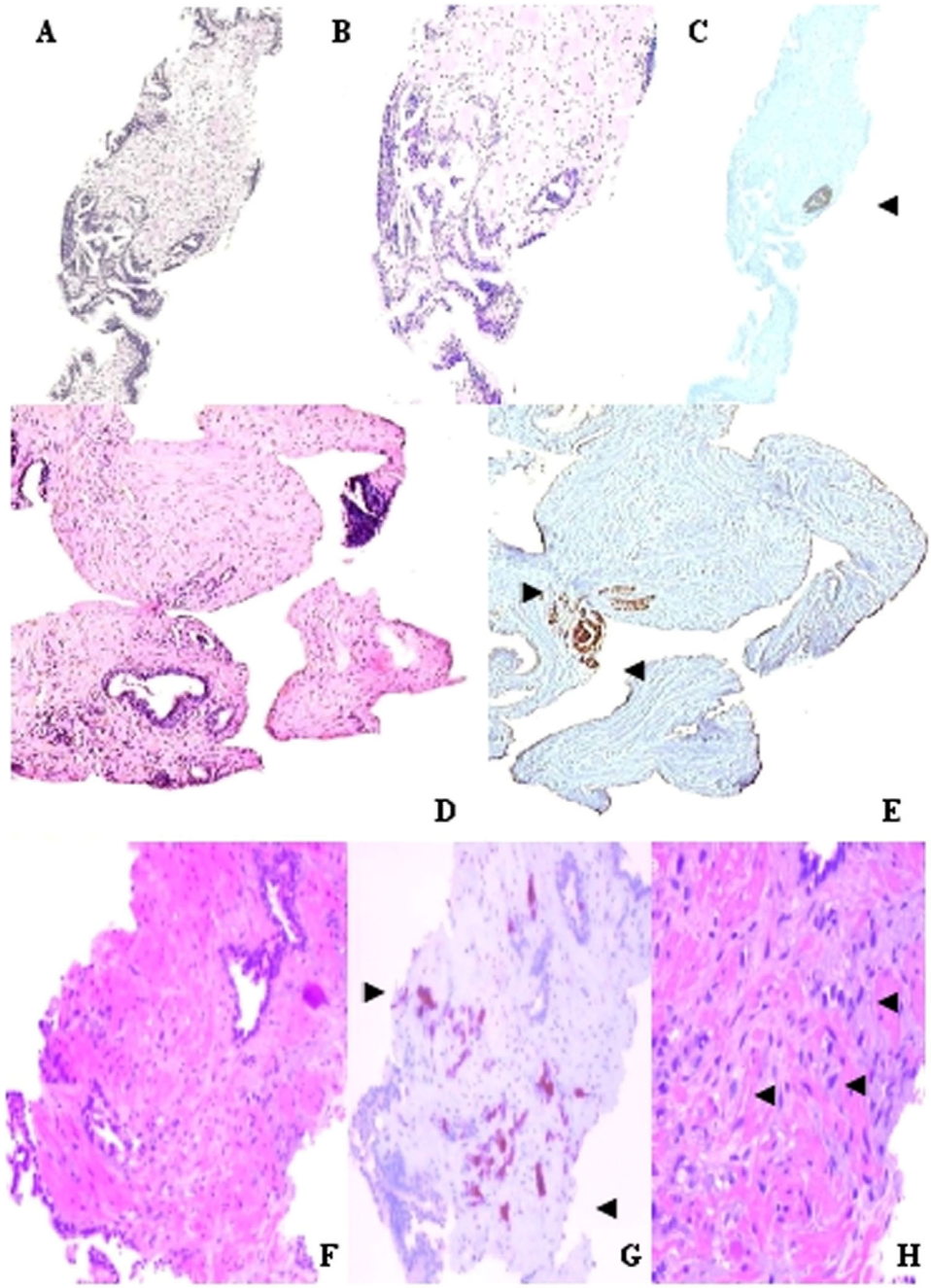

Fig. 2. Atypical small acinar proliferation (ASAP) consisting of one atypical glandular structure (<0.3 mm) missed on H&E slide at low (**A**) and medium power (**B**), but retrieved by AMACR immunohistochemistry (arrow head) (**C**). Prostate cancer (0.4 mm), Gleason 3 + 3 = 6 missed on H&E slide (**D**), but retrieved by AMACR immunohistochemistry (arrow heads) (**E**). Note that the atrophic lesion at the edge of the core with inflammatory stromal changes is easily overlooked on H&E slide. Prostate cancer (0.8 mm), Gleason 5 + 5 = 10 missed on H&E slide (**F**) and retrieved by AMACR immunohistochemistry (arrow heads) (**G**). Note that the diffuse solid growth pattern (arrow heads) recognizable at high power (**H**) is easily overlooked at medium power (F). It is noteworthy that the lesions shown in A–H lack immunoreactive basal cells (CK5/6). Magnification: A (×5), B (×10), C (×5), D (×10), E (×5), F (×10) G (×10), and H (×20).

detection of additional core(s) had no impact on the pathological inclusion criteria for active surveillance in 54 of 669 (8.07%) of patients, whereas 63 of 669 patients (9.42%) have lost their eligibility for active surveillance (Tables I and II).

Considering that two cases of ASAP and 14 cases of PCa identified on H&E slides were AMACR negative, the two-tailed χ^2 test could be applied to examine the statistical significance of retrieving PCa by AMACR immunohistochemistry. The difference in

TABLE I. Frequency and Characteristics of Missed Lesions on Prostate Needle Biopsy Retrieved by AMACR Immunohistochemistry

PCaTot	149:1,672 (8.91%)
PCaN	33:1,003 (3.29%)
ASAP	17:1,003 (1.69%)
PCAS	116:669 (17.34%)
AS no change	54:669 (8.07%)
AS failure	63:669 (9.42%)
CRL	113:1,672 (6.76%)
Gleason 6 (3 + 3)	140:149 (94%)
Gleason 7 (3 + 4)	5:149 (3.35%)
Gleason 8 (4 + 4)	3:149 (2.01%)
Gleason ≥9 (4 + 5)	1:149 (0.7%)
Mean tumor extent	0.43 mm (0.2–1.5 mm)

Considered are all patients enrolled in the current study with prostate cancer (PCaTot) missed on routine H&E slides; patients with benign prostate tissue on H&E slides, and detection of newly diagnosed prostate cancer (PCaN) or atypical small acinar proliferation (ASAP) by AMACR; patients with prostate cancer meeting pathological inclusion criteria for active surveillance (PCAS) with detection of missed cancer involving additional core(s) by AMACR, including patients still meeting inclusion criteria (AS no change) and those failing inclusion criteria (AS failure) after detection of additional positive core(s); and finally all patients with clinically relevant lesions (CRL), including PCaN, ASAP, and AS failure. Gleason scores and mean extent of minute prostate cancer missed on routine H&E slides.

Abbreviations: PCaTot, total number of patients with prostate cancer missed on routine H&E slides and retrieved with AMACR; PCaN, newly diagnosed prostate cancer missed on H&E slides and retrieved by AMACR; ASAP, atypical small acinar proliferation; AS, active surveillance; PCAS, patients with prostate cancer meeting pathological inclusion criteria for active surveillance with detection of additional cancer by AMACR; AS no change, patients still meeting inclusion criteria after detection of missed cancer; AS failure, patients failing inclusion criteria after detection of missed cancer; CRL, patients with clinically relevant lesions missed on H&E slides.

TABLE II. Frequency and Characteristics of Prostate Cancer That Fail Pathological Criteria of Active Surveillance After Detection of Missed Cancer by AMACR

≥33% of cores involved by cancer	59:116 (50.86%)
≥50% of any core involved by cancer	0:116 (0%)
>Gleason score 7 (3 + 4)	4:116 (3.45%)
Gleason score 8 (4 + 4)	3:116 (2.59%)
Gleason score 10	1:116 (0.86%)
Total	63:116 (54.31%)

detection rates between routine H&E histology and AMACR immunohistochemistry was found to be highly statistically significant ($P < 0.0001$).

The vast majority (140 of 149 cases [94%]) of missed cancer had a Gleason score 6 (3 + 3) and measured at the average 0.43 mm (range 0.2–1.5 mm). Only five cases with Gleason 7 (3 + 4) (3.35%), three case with Gleason 8 (4 + 4) (2.01%), and one case with Gleason score 10 (0.7%) were missed on H&E, but retrieved by immunohistochemistry (Fig. 2F–H).

DISCUSSION

The present study is the first reporting on the frequency and clinical impact of false-negative PNB in a large series of 1,672 consecutive and unselected patients, including 669 PCa patients meeting pathological inclusion criteria for active surveillance (PCAS), currently under investigation in large active surveillance cohorts [6,7]. Minute PCa missed on routine H&E slides, but retrieved by AMACR immunohistochemistry was observed in 8.91% of all patients indicating that underdiagnosis of limited adenocarcinoma is not an infrequent event in routine (H&E based) evaluation of PNB. The vast majority of missed cancer (94%) had a Gleason score 6 (3 + 3), and measured at the average 0.43 mm. Nevertheless, five cases of Gleason 7 (3 + 4), and four cases of Gleason ≥8 were missed on H&E slides, although upgrading cancer on missed biopsies affecting patient eligibility was relevant in only 3.45% of cases.

Giving the multifocal nature of PCa, it is perhaps not surprising that the risk of missing minute cancer in patients with clear evidence of PCa in at least one core is much higher than in patients, in which a minute focus of cancer missed on H&E slide is the first manifestation of the disease (17.34% vs. 3.23%). In the current study, 78% (116 of 149 cases) of missed minute cancer were retrieved in additional core(s) from patients with established low-intermediate risk PCa diagnosed at the same biopsy session. The most prominent risk factors for a false-negative diagnosis encountered in the current study were the lack of histoarchitectural features of cancer at low and medium power (in particularly at the edge of the core, and in cases of intense intermingling with preexistent glands), together with low number of atypical glands (<10 glands), followed by inflammatory stromal changes, crushing artefacts, and finally the presence of some rare PCa variants that may mimic benign glandular tissue, such as adenocarcinoma of atrophic type (n = 18) and pseudohyperplastic type (n = 5). It is noteworthy that AMACR is a marker with high sensitivity not only for PCa but also for its precursor [5]. High grade prostatic intraepithelial neoplasia (HGPIN) missed on H&E slides, but retrieved by AMACR may have potential implications in the risk evaluation of patients with negative biopsy, but this issue is beyond the scope of the current study. It is

clear that not all prostate lesions missed on H&E slides and retrieved by AMACR are HGPIN or cancer. False positive AMACR staining may occur, for example, small acinar proliferation and partial atrophy may be positive for AMACR and negative for basal cell markers. A definitive diagnosis of prostate cancer requires sufficient histoarchitectural and cytological criteria as assessed in H&E slides.

The question arises whether the retrieval of missed glandular lesions by AMACR immunohistochemistry is feasible and cost-effective in routine evaluation of PNB. The most likely way to retrieve virtually all minute cancer missed on H&E slides is to perform immunohistochemistry (AMACR and a basal cell marker) on every "benign" core biopsy. If prostate biopsies are embedded separately (one core/block), this approach would impose a huge burden, for example, for a 12-core biopsy without evidence of cancer, 24 additional slides have to be cut and 24 immunostains have to be performed in each case, which, in turn, is too time consuming and expensive for routine diagnostic application. Using the multicore (pre-) embedding procedure described here, pathologists are able to provide two additional slides per patient, which can be used for immunohistochemical evaluation of the entire biopsy specimen with only one or two immunostains (i.e., AMACR, and a basal cell marker, if missed lesions are retrieved by AMACR). Although this approach is much more cost-effective, the burden of immunohistochemistry and the benefit of retrieving missed lesions by AMACR has to be considered carefully. Approximately 67% of PNB included in current study (1,672 of 2,499 cases) underwent AMACR immunohistochemistry before a definitive diagnosis was established. At first sight, this seems to be much higher than the previously reported rate of 40% for immunohistochemistry used in the routine workup of PNB performed in a Canadian tertiary academic institution [10]. A crucial factor, however, is the number of blocks/patient submitted for immunohistochemistry. In the Canadian institution two immunostains (AMACR and a basal cell marker) were performed on an average of 1.8 blocks/case, which correspond to 3.6 immunostains/patient. Considering that only 2.0 immunostains/patient were performed in the current study, the average number of immunostains/patient in both studies is very similar (1.34 vs. 1.44). The diagnostic benefit of retrieving missed cancer by AMACR on the entire specimen, however, is provided only by the multicore sampling technique used in the current study. This obviously raises the question of whether the multicore embedding is suitable and safe for routine evaluation of PNB.

In a recent survey on the variation in the handling of PNB in 241 Laboratories across Europe revealed that about 40% of European genitourinary pathologists processes one core/block, whereas the others prefer a multicore embedding [11]. The multicore processing has been criticized in a consensus statement of the College of American Pathologists stating that multiple cores embedded in a paraffin block often result in uneven levels among cores and result in the loss of tissue when cutting for histology [7]. In order to overcome the inherent shortcomings of multicore embedding, we have implemented the improved sandwich preembedding technique to submit PNB (Fig. 1A), and the routine use of a metal tamper to further enhance the flattening of the cores during their embedding in paraffin. In the hand of experienced laboratory technicians, the combination of both preembedding and embedding techniques ensures that the form and shape of the cores present on the histological slides are equal to the cores submitted by the urologist. In a recent Finish study comparing the quality of 12-core biopsies separately embedded (one core/block) versus multicore embedded biopsies, no significant differences in lengths of the biopsies or detection rates were obtained by both methods. There was no evidence regarding the superior quality of separately submitted and embedded biopsies as advocated by current guidelines [12]. Hence, the suitability of multicore embedding for routine application is basically a matter of technique and diagnostic outcome. Beside the diagnostic benefit of retrieving missed cancer by AMACR on the entire specimen, a further argument that may convince urologists to use the multicore processing is that this sandwich preembedding method is known to increase the frequency of cancer diagnosis by decreasing loss of tissue during the cutting process [8,9].

Detection of missed lesions on PNB by AMACR immunohistochemistry has clinical implications in all patients without evidence of cancer on routine H&E slides (3.29%), all cases with missed ASAP (1.69%) and all PCa patients meeting PCAS, in which detection of additional core(s) involved by minute cancer affects patient eligibility for active surveillance (9.42%). Accordingly, missed lesions on routine H&E slides have clinical significance in 6.76% of all patients enrolled in the current study. The most striking observation is that additional positive core(s) were detected by AMACR in 17.34% of patients meeting PCAS, and that 54.31% of these patients finally have lost their eligibility for active surveillance accordingly. The proportion of patients may vary depending on the inclusion criteria, for example, whether the Gleason score $3+4$ is included or not. Nevertheless, with increasing acceptation of conservative management of

low risk disease, the issue of missed minute cancer on PNB will become more and more important.

Despite the single-institutional design of the current study, the present data give at least some indication as to the magnitude of the problem. The issue of false-negative PNB has to be addressed not only by genitourinary pathologists, but also by general pathologists not specialized in prostate pathology. The use of the multicore embedding and sampling for immunohistochemistry as described here enables pathologists to address adequately this issue, whenever immunohistochemistry is required to establish a definite diagnosis in a selected case.

CONCLUSION

Underdiagnosis of limited adenocarcinoma on PNB is a matter of concern. Affected are patients without evidence of cancer on routine H&E slides (3.29%) and patients with established cancer meeting PCAS (17.34%), in which detection of additional core(s) may prompt curative treatment. In this study, about 54% of these patients have lost their eligibility for active surveillance after detecting additional cores involved by cancer. Using the multicore embedding and sampling procedure as described here, retrieval of missed lesions is feasible with a single AMACR immunostain/patient, whenever immunohistochemistry is required in a selected case. This approach provides a simple and cost-effective way to improve the diagnostic accuracy and reliability of PNB.

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REFERENCES

1. Kronz JD, Milord R, Wilentz R, Weir EG, Schreiner SR, Epstein JI. Lesions missed on prostate biopsies in cases sent in for consultation. Prostate 2003;54:310–314.
2. Wolters T, van der Kwast TH, Vissers CJ, Bangma CH, Roobol M, Schröder FH, van Leenders GJ. False-negative prostate needle biopsies: Frequency, histopathologic features, and follow-up. Am J Surg Pathol 2010;34:35–43.
3. Carswell BM, Woda BA, Wang X, Li C, Dresser K, Jiang Z. Detection of prostate cancer by alpha-methylacyl CoA racemase (P504S) in needle biopsy specimens previously reported as negative for malignancy. Histopathology 2006;48:668–673.
4. Tolonen TT, Kujala PM, Laurila M, Tirkkonen M, Ilvesaro J, Tuominen VJ, Tammela TL, Isola J. Routine dual-color immunostaining with a 3-antibody cocktail improves the detection of small cancers in prostate needle biopsies. Hum Pathol 2011;42:1635–1642.
5. Epstein JI, Egevad L, Humphrey PA, Montironi R, Members of the ISUP immunohistochemistry in Diagnostic Urologic Pathology Group. Best practices recommendations in the application of immunohistochemistry in the prostate: Report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol 2014;38:6–19.
6. Dall'Era MA, Albertsen PC, Bangma C, Carroll PR, Carter HB, Cooperberg MR, Freedland SJ, Klotz LH, Parker C, Soloway MS. Active surveillance for prostate cancer: A systematic review of the literature. Eur Urol 2012;62:976–983.
7. Amin MB, Lin DW, Gore JL, Srigley JR, Samaratunga H, Egevad L, Rubin M, Nacey J, Carter HB, Klotz L, Sandler H, Zietman AL, Holden S, Montironi R, Humphrey PA, Evans AJ, Epstein JI, Delahunt B, McKenney JK, Berney D, Wheeler TM, Chinnaiyan AM, True L, Knudsen B, Hammond ME. The critical role of the pathologist in determining eligibility for active surveillance as a management option in patients with prostate cancer: Consensus statement with recommendations supported by the College of American Pathologists, International Society of Urological Pathology, Association of Directors of Anatomic and Surgical Pathology, the New Zealand Society of Pathologists, and the Prostate Cancer Foundation. Arch Pathol Lab Med 2014;138:1387–1405.
8. Rogatsch H, Moser P, Volgger H, Horninger W, Bartsch G, Mikuz G, Mairinger T. Diagnostic effect of an improved preembedding method of prostate needle biopsy specimens. Hum Pathol 2000;31:1102–1107.
9. Rogatsch H, Mairinger T, Horninger W, Gschwendtner A, Bartsch G, Mikuz G. Optimized preembedding method improves the histologic yield of prostatic core needle biopsies. Prostate 2000;42:24–29.
10. Watson K, Wang C, Yilmaz A, Bismar TA, Trpkov K. Use of immunohistochemistry in routine workup of prostate needle biopsies: A tertiary academic institution experience. Arch Pathol Lab Med 2013;137:541–545.
11. Varma M, Berney DM, Algaba F, Camparo P, Compérat E, Griffiths DF, Kristiansen G, Lopez-Beltran A, Montironi R, Egevad L. Prostate needle biopsy processing: A survey of laboratory practice across Europe. J Clin Pathol 2013;66:120–123.
12. Tolonen TT, Isola J, Kaipia A, Riikonen J, Koivusalo L, Huovinen S, Laurila M, Porre S, Tirkkonen M, Kujala P. Length of prostate biopsies is not necessarily compromised by pooling multiple cores in one paraffin block: An observational study. BMC Clin Pathol 2015;15:4.