Partial Sampling of Radical Prostatectomy Specimens

Detection of Positive Margins and Extraprostatic Extension

Viacheslav Iremashvili, MD, PhD, Soum D. Lokeshwar,* Mark S. Soloway, MD,* Lisét Pelaez, MD,† Saleem A. Umar, MD,† Murugesan Manoharan, MD,* and Mercé Jordá, MD, PhD†*

Abstract: Currently there is no global agreement as to how extensively a radical prostatectomy specimen should be sectioned and histologically examined. We analyzed the ability of different methods of partial sampling in detecting positive margin (PM) and extraprostatic extension (EPE)—2 pathologic features of prostate cancer that are most easily missed by partial sampling of the prostate. Radical prostatectomy specimens from 617 patients treated with open radical prostatectomy between 1992 and 2011 were analyzed. Examination of the entirely submitted prostate detected only PM in 370 (60%), only EPE in 100 (16%), and both in 147 (24%) specimens. We determined whether these pathologic features would have been diagnosed had the examination of the specimen been limited only to alternate sections (method 1), alternate sections representing the posterior aspect of the gland in addition to one of the mid-anterior aspects (method 2), and every section representing the posterior aspect of the gland in addition to one of the mid-anterior aspects, supplemented by the remaining ipsilateral anterior sections if a sizeable tumor is seen (method 3). Methods 1 and 2 missed 13% and 21% of PMs and 28% and 47% of EPEs, respectively. Method 3 demonstrated better results missing only 5% of PMs and 7% of EPEs. Partial sampling techniques missed slightly more PMs and EPEs in patients with low-risk to intermediate-risk prostate cancer, although even in high-risk cases none of the methods detected all of the studied aggressive pathologic features.

Key Words: extraprostatic extension, partial embedding, positive margin, prostate cancer, radical prostatectomy

(*Am J Surg Pathol* 2013;37:219–225)

Radical prostatectomy is the most widely used treatment for prostate cancer, with approximately 40% to 50% of newly diagnosed men selecting this treatment modality.[1] The prognosis after a radical prostatectomy is strongly dependent on the tumor characteristics revealed by the pathologic examination. For instance, the risk of recurrence is significantly increased if the cancer is not confined to the gland and/or to the surgical specimen.[2] Depending on the presence of these and other aggressive features, such as grade and tumor volume, the clinician can assess the likelihood of a recurrence and consider additional therapy.[3] Therefore, the accuracy of the pathologic findings has important patient-care implications.

Most of the current patients with prostate cancer have a relatively small volume of disease, which is not macroscopically identifiable. For this reason a systematic technique for radical prostatectomy specimen sampling is required to derive appropriate prognostic parameters. Surprisingly there is no agreement as to how much of a radical prostatectomy specimen should be histologically examined.[4] Although analysis of the entire gland would allow for the most thorough analysis, processing a completely sampled specimen requires more resources compared with partial sampling. Several studies have suggested that a systematic partial sampling can provide almost as much information as total submission.[5–7] However, these studies included a limited number of patients, and it is not well established whether any of the recommended methods of partial sampling is superior to others. To address this we analyzed the ability of different methods to detect positive margins (PMs) and extraprostatic extensions (EPEs)—2 pathologic features of prostate cancer that are most easily missed by partial sampling of the surgical specimen.

MATERIALS AND METHODS

Patient Population

Between 1992 and 2011, 1981 patients underwent open retropubic radical prostatectomy by 1 surgeon (M.S.S.). All specimens were uniformly processed and entirely submitted (see below). Of these patients, 775 had either PM or EPE or both. Patients were excluded if they had received preoperative radiation or neoadjuvant hormonal therapy (n = 111), or if the information about the slides containing PM or EPE was not available (n = 47). The remaining 617 patients formed the study cohort. The study was approved by the institutional review board of University of Miami.

From the Departments of *Urology; and †Pathology, Miller School of Medicine, University of Miami, Miami, FL.

Conflicts of Interest and Source of Funding: The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Correspondence: Viacheslav Iremashvili, MD, PhD, Department of Urology, Miller School of Medicine, University of Miami, PO Box 016960 (M-14), Miami, FL 33101 (e-mail: iremashvili@hotmail.com).

Pathologic Examination

The radical prostatectomy specimens were weighed, measured, marked with ink, and fixed in zinc formalin overnight. A 2 to 3 mm apical margin was obtained from the most distal part of the prostate. The bladder neck was also removed as a thin margin. Both were sliced sagittally (cone method). The remaining prostate was step-sectioned at 3 to 4 mm intervals in transverse planes into separate blocks according to the Stanford protocol.[8] The blocks were examined as quarter mounts from which histologic maps were constructed. Outlines of each cancer area were drawn on the slides under the microscope and copied to paper diagrams for prostate sectioning. PM was defined as tumor cells present at the inked margin, whereas EPE was defined as the extension of the tumor cells beyond the prostatic capsule into the periprostatic adipose tissue. For this study, all slides recorded as containing a PM or EPE were reexamined by a single pathologist (L.P.).

Statistical Analysis

We retrospectively analyzed the potential findings of 3 methods of partial sampling of prostatectomy specimens in the studied cohort of patients. To that end, for every patient we determined whether PM or EPE would have been diagnosed had the examination of the specimen been limited only to the slides included in each of the 3 studied methods of partial sampling (Fig. 1). This was initially done in the entire cohort. The following additional analyses were also performed:

1. Pathologic characteristics of prostate cancer have changed considerably over the last 2 decades,[9] and findings in a cohort of men who had surgery over this time period may not be directly applicable to contemporary radical prostatectomy patients. To investigate whether the year of surgery affected the performance of different methods of partial sampling we separately analyzed patients who were operated on before 2000 and in the period from 2000 to 2011.[10]
2. Larger PM and EPE, characteristic of patients with more aggressive disease, may be less likely to be missed by partial sampling. Therefore, we repeated all analyses in patients stratified into preoperative risk groups according to the classification proposed by D'Amico et al.[11]
3. Apical margins are included in each of the studied partial sampling protocols and therefore could not be missed. However, the prognostic significance of this PM location was suggested to be inferior to that of other locations. Some studies suggest that apical PMs do not increase the risk of biochemical recurrence as much as other locations.[12–14] Therefore, to study the effect of partial sampling on the detection of potentially more clinically important PMs, we calculated the number of missed nonapical PM cases.

The rates of PMs and EPEs missed by the studied partial sampling technique in different subgroups of patients were compared using the Pearson χ^2 test. Stata version 11.0 (College Station, TX) was used for all data analyses.

RESULTS

Of the 617 assessable patients, 370 (60%) had only PM, 100 (16%) had only EPE, and 147 (24%) had both PM and EPE. Table 1 lists the patient characteristics.

Figures 2A and B illustrate the prevalence of PMs and EPEs at different locations. The apex was the most common location of PMs. The frequency of PMs decreased along the urethral axis from almost 30% at the distal apex to $<10\%$ at the base. This decrease primarily resulted from the change in the prevalence of anterior PMs, which were much more common in the apical third of the gland, whereas the frequency of posterior PMs was similar at different levels. In contrast, the prevalence of EPE was much higher in the mid and basal thirds of the prostate than in the apical third. Most of the EPEs were located in the posterior gland. Both PMs and EPEs were rarely detected simultaneously in the anterior and posterior parts of the prostate at the same level.

The partial sampling methods would have required examination of considerably fewer prostate slides (Table 2), but this would have resulted in missing a substantial numbers of PMs and EPEs (Fig. 3). For instance, the number of patients from the entire cohort in whom neither PM nor EPE would have been found was 81 (13%), 144 (23%), and 25 (4%) for methods 1, 2, and 3, respectively. The number of PMs missed by the studied methods ranged from 107 (21%) for method 2 to 28 (5%) for method 3. EPEs were much more likely to be missed by partial sampling, particularly if it included examination of alternate posterior slides. For example, methods 1 and 2 would have missed almost 30% and 50% of cases of EPE, respectively.

The rates of the studied aggressive pathologic features missed by different methods of partial sampling were similar in patients operated on before 2000 and in the period from 2000 to 2011 (data not shown). The probability of missing PMs and EPEs in men with high preoperative risk tended to be lower than that in patients with low-risk to intermediate-risk prostate cancer, although the differences were not statistically significant for most pairs of comparison (Figs. 4A–C). Finally, in a subgroup of 353 patients with at least 1 nonapical PM, 100 (28%), 108 (31%), and 51 (14%) specimens would have been considered margin negative or would have had only apical PM if methods 1, 2, and 3, respectively, had been used.

DISCUSSION

Although currently there is no consensus on the optimal method of prostatectomy specimen processing, more centers seem to rely on total sampling. For instance, in 1994, only 12% of the surveyed members of American Society of Clinical Pathologists processed the entire radical prostatectomy specimen[15]; in 2009, 48% of North American responders to an International Society of Urological Pathology survey reported using total sampling routinely.[16] Although the latter society consists primarily of urological pathologists who are more likely to submit the entire prostate for research purposes, this difference may reflect a change in practice over time. Interestingly, total embedding

seems to be much more common in other parts of the world. Of the responders to the aforementioned International Society of Urological Pathology survey who were from outside North America, 78% reported using this method. Although the results of a survey may indicate what the pathologists thought was the "correct" method rather than the reality in their institution, the increase in the use of total sampling may have resulted from an appreciation of the significant changes in the characteristics of prostate cancer that took place after the introduction of prostate-specific antigen screening and systematic needle biopsies. Most contemporary patients have relatively small-volume prostate cancer, which consists of several foci, and accurate description of all tumor characteristics requires examination of different areas of the prostate. However, there is a lack of evidence for or against the use of particular sampling methods obtained from well-constructed, sufficiently powered studies.

In the current study we validated and compared the performance of several methods of partial sampling in detecting PMs and EPEs in a relatively large cohort of patients all of whom had at least 1 of these adverse pathologic features. None of the studied methods of partial sampling detected all PMs and EPEs, but the proportion of cases missed by different techniques varied considerably. Methods 1 and 2, which were based on examination of alternate slides, missed from 13% to 21% of PMs and from 28% to 47% of EPEs. In contrast, method 3, which included examination of all posterior slides along with 1 mid-anterior section, and, in case a sizeable tumor was found in the latter, the rest of the contralateral anterior slides, missed only 5% of PMs and 7% of EPEs. The reasons for the poor performance of methods 1 and 2 and relatively good performance of method 3 become clear when one considers the distribution of the PMs and EPEs at different prostate sections (Figs. 2A, B). Most of the cases of both studied pathologic features were found on the posterior surface of the prostate, with PMs being more common in the apical part of the gland and EPE in the basal part. Thus, exclusion of any of the posterior slides inevitably results in missing

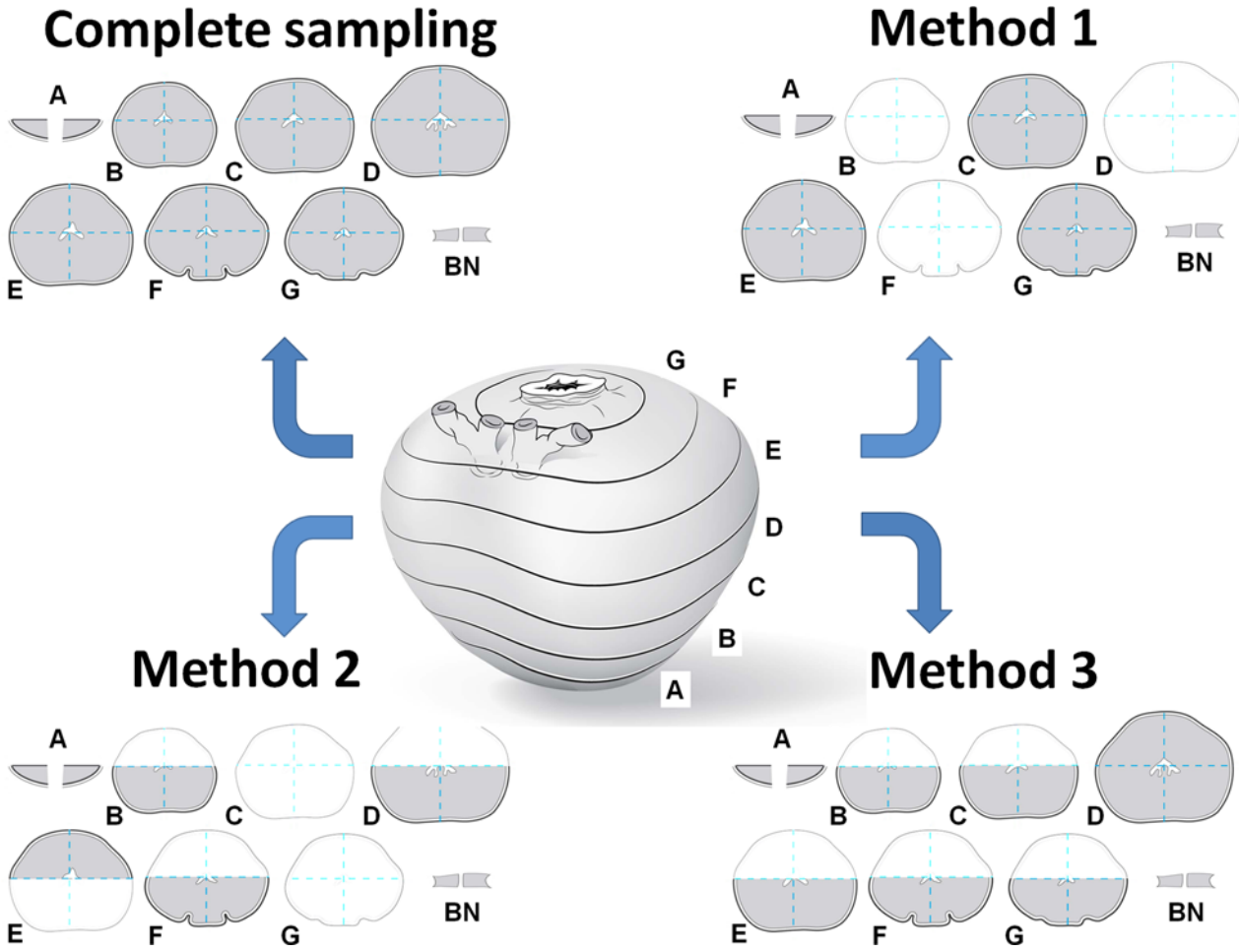

FIGURE 1. Schematic representation of the studied methods of partial sampling as compared with complete sampling of the prostatectomy specimen. Method 1—alternate sections; method 2—alternate sections representing the posterior aspect of the gland in addition to one of the mid-anterior aspects; method 3—every section representing the posterior aspect of the gland in addition to one of the mid-anterior aspects, supplemented by remaining ipsilateral anterior sections if a sizeable tumor is seen.

TABLE 1. Patient Characteristics

Variable	
No. patients	617
Median age at surgery, y (IQR)	61.7 (55.8-67.2)
Median PSA, ng/mL (IQR)	6.6 (4.8-9.3)
Median no. biopsy cores (IQR)*	8 (6-12)
Median no. positive cores (IQR)†	3 (2-4)
Median average percent core involvement (IQR)‡	25 (10-40)
No. biopsy Gleason score (%)	
$\leq 3+3$	292 (47.3)
7 (3+4)	163 (26.4)
7 (4+3)	76 (12.3)
$\geq 4+3$	86 (13.9)
No. clinical stage (%)	
T1	389 (63.0)
T2	213 (34.5)
T3	15 (2.4)
No. preoperative risk (%)§	
Low	224 (36.3)
Intermediate	261 (42.3)
High	132 (21.4)
Year of surgery (%)	
1992-1999	182 (29.5)
2000-2011	435 (70.5)
No. pathologic Gleason score (%)	
$\leq 3+3$	162 (26.3)
7 (3+4)	231 (37.4)
7 (4+3)	108 (17.5)
$\geq 4+3$	116 (18.8)
Median prostate weight, g (IQR)	42 (34-52)
Median visually estimated percent of carcinoma (IQR)	12 (7-20)
No. SVI (%)	89 (14.4)
No. BNI (%)	46 (7.5)
No. LNI (%)	19 (3.1)

*Not available for 22 patients.
†Not available for 30 patients.
‡Not available for 71 patients.
§Risk group stratification system described by D'Amico et al[11]
BNI indicates bladder neck involvement; IQR, interquartile range; LNI, lymph node involvement; PSA, prostate-specific antigen; SVI, seminal vesicle invasion.

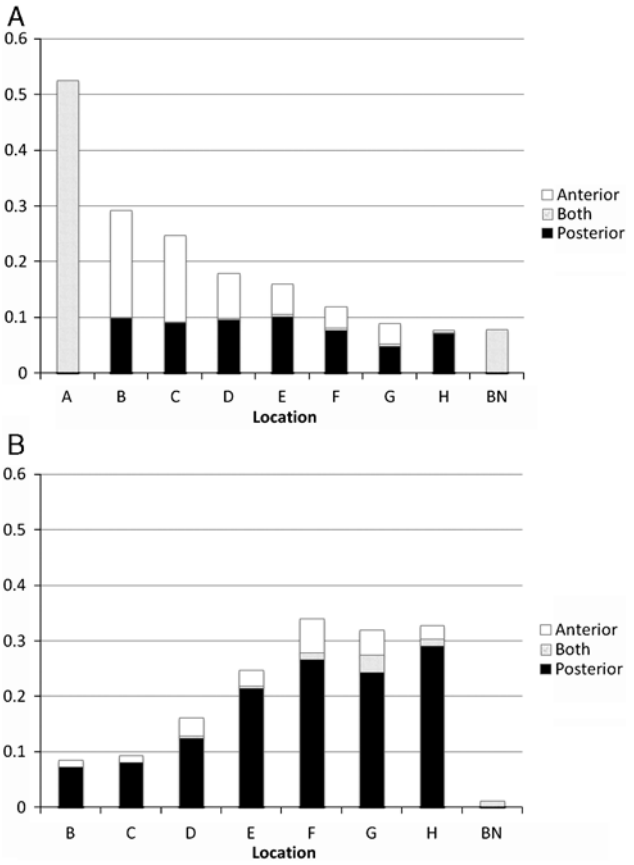

FIGURE 2. Rates of PMs (A) and EPEs (B) at different locations. The letters correspond to the prostate sections as shown in Figure 1.

some of these features. In contrast, with the exception of the relatively high rate of PMs in the apical third of the prostate, only a few PMs and EPEs were detected in the anterior slides. In many patients anterior PMs and EPEs were successfully detected by method 3, as these tumors often involved mid-anterior slides.

The partial sampling technique, which we called method 3, was originally described by Sehdev et al[6] in a study that included 78 entirely submitted prostatectomy specimens. The authors compared the ability of 9 partial sampling methods to detect Gleason score ≥ 7, PM, and EPE, and this technique demonstrated the best results and detected almost all of the cases of PMs and EPEs. The current study essentially validated the high sensitivity of this technique in a much larger cohort of prostatectomy patients.

As there was a profound change in the characteristics of prostate cancer over the period of time our cohort was recruited, we analyzed patients in 2 groups, on the basis of the date of surgery, to determine whether our findings in the entire cohort were applicable to a contemporary radical prostatectomy patient. Overall, the results of all studied methods of partial sampling in more recent patients were similar to those in the entire cohort, suggesting that the limitations or partial sampling are relevant for current patients. We also tried to identify a group in which partial sampling may be more effective by separately analyzing patients with different preoperative risks. In particular, we hypothesized that the negative consequences of partial sampling may be less pronounced in patients with high-risk disease, because the PMs and EPEs in these patients are more likely to extend over larger areas of the prostate or specimen surface and therefore are less likely to be missed when fewer slides are examined. However, although the proportion of missed PMs and EPEs was slightly lower in patients with high-risk

TABLE 2. The Numbers of Prostate Slides That Were Studied by Total Sampling and Would Have Been Required for Different Methods of Partial Sampling

	Mean	Median	Range	% Decrease Compared with Total Sampling
Total sampling	28.6	28	22-48	—
Method 1	16.3	16	14-28	42.9
Method 2	12.2	12	8-24	57.5
Method 3	21.1	18	16-48	26.3

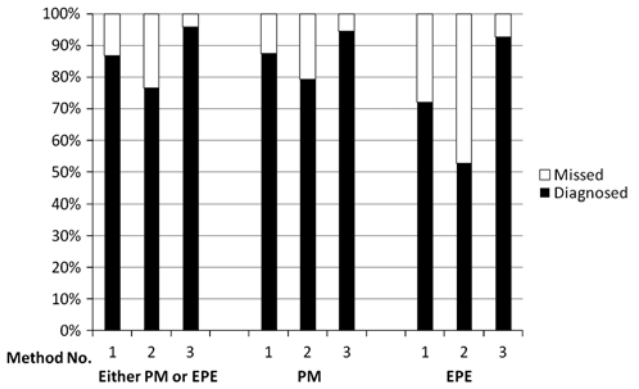

FIGURE 3. Rates of PMs and EPEs missed by the studied methods of partial sampling.

prostate cancer, findings of none of the studied methods demonstrated full conformity with those of total sampling.

Our findings may have important implications for research on the outcomes in the area of surgical treatment of prostate cancer. The demonstrated differences in the detection of PMs and EPEs between total and partial sampling techniques indicate that information about the methods of specimen handling is essential to the understanding of comparative characteristics of disease in different patient groups. For example, the method of prostate sampling should be taken into account when a prognostic tool (eg, a nomogram) is tested in different patient populations. The difference in the methods of prostate processing between the original and validation cohorts potentially results in variations in the detection of PMs and EPEs and may lead to a deterioration of the calculated predictive performance of the tool. However, this remains speculative and needs further empirical validation in studies examining the prognostic implications of the PMs and EPEs missed by partial sampling techniques.

Although the results of earlier studies may seem to differ from ours, a careful examination of their findings demonstrates that in most cases they are quite similar (Table 3).[18] For example, the study of Hall et al[17] was conducted on patients who were diagnosed in the pre–prostate-specific antigen era and included only cases with grossly identifiable tumors. This group most likely included patients with what would have been called high-risk disease, and indeed the rates of missed PMs and EPEs in this study are similar to those in our patients with high-risk prostate cancer. The only earlier study with results that were remarkably different from ours is that of Vainer et al.[5] In this analysis the alternate sections method detected >95% of both PMs and EPEs. This discrepancy may have resulted from some differences in processing technique. For instance, whereas we obtained

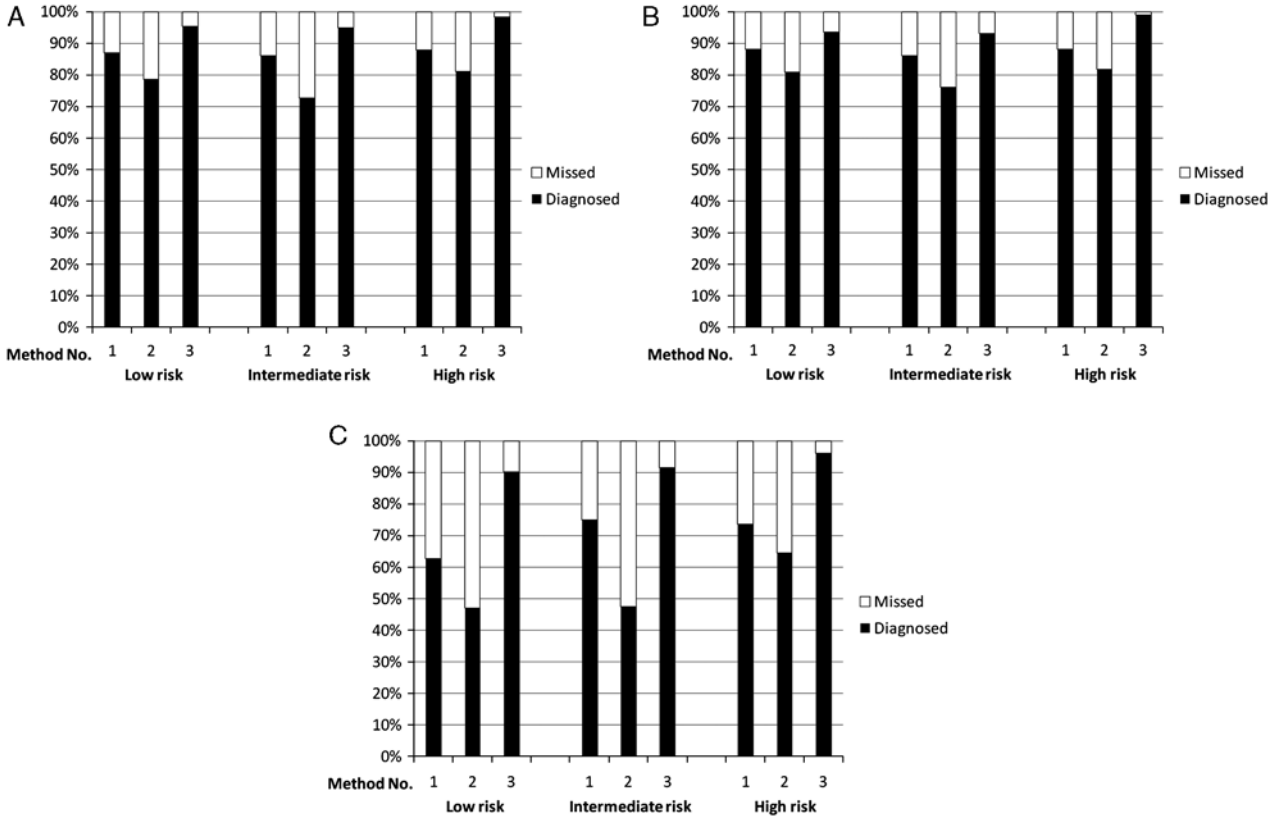

FIGURE 4. Rates of PMs and/or EPEs (A), only PMs (B), and only EPEs (C) missed by the studied methods of partial sampling in subgroups of patients with different preoperative risks (stratified by preoperative risk).

TABLE 3. Main Findings of the Earlier Studies for Partial Surveillance Protocols Included in Our Analysis

Reference	Method of Partial Sampling	No. Patients	No. PMs (% Missed)	No. EPEs (% Missed)
Hall et al[17]	Method 1*	90†	29 (10)	63 (6)
Cohen et al[18]	Method 1	100	10 (20)	8 (13)
Kim et al[7]	Method 1	148	46 (17)	50 (16)
Vainer et al[5]	Method 1	238	68 (2)	48 (4)
Sehdev et al[6]	Method 3	78	14 (0)	54 (4)

*Whole mount.
†With grossly identifiable tumors.

a 2 to 3 mm apical margin and thin bladder neck margin, the technique of Vainer and colleagues included apical and basal sections of 5 to 10 mm. These sections may have allowed the authors to better study corresponding areas of the gland that frequently contain PMs and EPEs, thus mitigating the effect of partial sampling of the rest of the prostate.

Salem et al[19] compared pathologic findings of a technique similar to method 2 in 525 radical prostatectomy patients with those of a whole mount, which was performed in 608 men with similar preoperative characteristics. The whole-mount technique used in this study included sectioning of the prostate at 4 to 5 mm intervals. The authors did not find statistically significant differences between the whole mount and partial sampling groups in detecting PMs, EPEs, and most other variables and concluded that both techniques yield similar pathologic information. It should be noted, however, that the whole-mount technique used by Salem and colleagues resulted in examination of somewhat less prostate tissue compared with our total sampling method. Thus, as the sampling techniques used were different, the results from Salem and colleagues are of limited relevance in the context of our analysis.

Our study is limited by its retrospective design and by its focus on a single surgeon's series. As our cohort included only patients who underwent open prostatectomy, our findings may not be directly applicable to men who were treated by robotic prostatectomy. We compared only partial sampling techniques, which could be simulated using the results of standardized total submission, although most of the practical methods of partial sampling are likely to be based on this technique. The study is also limited in that it did not analyze the effect of partial sampling on the detection of other predictors of prognosis. These include Gleason score, tertiary Gleason pattern, and tumor volume, as well as certain characteristics of the studied pathologic features, which could be associated with biochemical outcomes, such as focality of PM and EPE, their extent, location, and the Gleason score at PM.

It might be emphasized that many patients with either a PM or an EPE do not have a biochemical recurrence. The presence of a tumor at the inked margin suggests that there is a tumor remaining in the periprostatic tissue, but because of the contiguous structures this may not be the case. This is particularly true for the apex as the anterior capsule is often incised or exposed during the surgery and thus tumor cells may be exposed to the ink; however, this does not imply that any cells are remaining in the patient.

In conclusion, all of the studied methods of partial prostatectomy specimen sampling missed some of the PMs and EPEs. The best results were demonstrated by method 3, originally described by Sehdev et al.[6] This can largely be attributed to the fact that this technique included examination of all of the posterior slides and also anterior slides in patients with anterior tumors of substantial size. This minimized, but not completely eliminated, the possibility of missing the studied adverse pathologic characteristics. Slightly more PMs and EPEs were missed in patients with low-risk to intermediate-risk prostate cancer, although even in high-risk patients none of the methods detected all of the studied features. However, the clinical importance of these differences between sampling techniques remains to be established.

ACKNOWLEDGMENTS

The authors thank Center for Urologic Research, Education, and Diseases (CURED) and Mr Vincent Rodriguez. We acknowledge Mr Michael Poltoratsky for his help with preparation of the illustrations.

REFERENCES

1. Cooperberg MR, Broering JM, Carroll PR. Time trends and local variation in primary treatment of localized prostate cancer. *J Clin Oncol*. 2010;28:1117–1123.
2. Stephenson AJ, Scardino PT, Eastham JA, et al. Postoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. *J Clin Oncol*. 2005;23:7005–7012.
3. McVey GP, Parker C. Adjuvant vs. salvage radiotherapy for pathologically advanced prostate cancer. *Curr Opin Urol*. 2010;20:229–233.
4. Samaratunga H, Montironi R, True L, et al. International Society of Urological Pathology (ISUP) Consensus Conference on Handling and Staging of Radical Prostatectomy Specimens. Working group 1: specimen handling. *Mod Pathol*. 2011;24:6–15.
5. Vainer B, Toft BG, Olsen KE, et al. Handling of radical prostatectomy specimens: total or partial embedding? *Histopathology*. 2011;58:211–216.
6. Sehdev AE, Pan CC, Epstein JI. Comparative analysis of sampling methods for grossing radical prostatectomy specimens performed for nonpalpable (stage T1c) prostatic adenocarcinoma. *Hum Pathol*. 2001;32:494–499.
7. Kim K, Pak PJ, Ro JY, et al. Limited sampling of radical prostatectomy specimens with excellent preservation of prognostic parameters of prostate cancer. *Arch Pathol Lab Med*. 2009;133:1278–1284.
8. McNeal JE, Haillot O. Patterns of spread of adenocarcinoma in the prostate as related to cancer volume. *Prostate*. 2001;49:48–57.
9. Dong F, Reuther AM, Magi-Galluzzi C, et al. Pathologic stage migration has slowed in the late PSA era. *Urology*. 2007;70:839–842.

10. Cooperberg MR, Broering JM, Kantoff PW, et al. Contemporary trends in low risk prostate cancer: risk assessment and treatment. *J Urol*. 2007;178:S14–S19.
11. D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA*. 1998;280:969–974.
12. Eastham JA, Kuroiwa K, Ohori M, et al. Prognostic significance of location of positive margins in radical prostatectomy specimens. *Urology*. 2007;70:965–969.
13. Blute ML, Bostwick DG, Bergstralh EJ, et al. Anatomic site-specific positive margins in organ-confined prostate cancer and its impact on outcome after radical prostatectomy. *Urology*. 1997; 50:733–739.
14. Kordan Y, Salem S, Chang SS, et al. Impact of positive apical surgical margins on likelihood of biochemical recurrence after radical prostatectomy. *J Urol*. 2009;182:2695–2701.
15. True LD. Surgical pathology examination of the prostate gland. Practice survey by American society of clinical pathologists. *Am J Clin Pathol*. 1994;102:572–579.
16. Egevad L, Srigley JR, Delahunt B. International Society of Urological Pathology (ISUP) consensus conference on handling and staging of radical prostatectomy specimens: rationale and organization. *Mod Pathol*. 2011;24:1–5.
17. Hall GS, Kramer CE, Epstein JI. Evaluation of radical prostatectomy specimens. A comparative analysis of sampling methods. *Am J Surg Pathol*. 1992;16:315–324.
18. Cohen MB, Soloway MS, Murphy WM. Sampling of radical prostatectomy specimens. How much is adequate? *Am J Clin Pathol*. 1994;101:250–252.
19. Salem S, Chang SS, Clark PE, et al. Comparative analysis of whole mount processing and systematic sampling of radical prostatectomy specimens: pathological outcomes and risk of biochemical recurrence. *J Urol*. 2010;184:1334–1340.