

## Pathologic Reassessment of Prostate Cancer Surgical Specimens Before Molecular Retrospective Studies

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### Abstract

**Purpose:** The retrospective use of prostate cancer tissue is crucial to design tumor marker prognostic studies. We hypothesize that comparison between recent and more historical cases could introduce biases due to stage and grade migration upon time.

**Design:** We reviewed 544 margin-free specimens from patients treated for clinically localized prostate cancer by radical prostatectomy between 2000 and 2005. One hundred and ninety-two patients that underwent biochemical recurrence after surgery were matched with 352 patients without progression, according to pretreatment prostate specific antigen, Gleason score, pathologic stage, and follow-up duration (at least 36 months).

**Results:** The reassessment led to histopathologic reclassification of standard prognostic variables in 15% of cases, including modifications in the Gleason score ( $n = 63$ ), pathologic stage ( $n = 12$ ), and margin status ( $n = 30$ ). Most discrepancies with the initial reports are explained either by differences in the scoring system upon time, or by the exam of additional tissues sections. The impact of reclassification led to increase adverse prognostic factors more frequently in the group of patients with progression ( $\text{Chi}^2$ ,  $P < 0.0001$ ).

**Conclusion:** Careful reassessment of prostate cancer samples should be mandatory before molecular prognostic studies to ensure a more uniform pathologic evaluation, and might be reported in the "recommendations for tumor markers prognostic studies" (REMARK). *Clin Cancer Res*; 17(4); 836–40. ©2010 AACR.

### Introduction

The prognosis of clinically localized prostate cancer treated by radical prostatectomy depends upon pretreatment prostate specific antigen (PSA), pathologic staging, Gleason score, and surgical margin status. Histopathologic examination of the surgical specimens allows to stratify patients into risk groups or to build postoperative models that can help to predict the risk of recurrence after treatment (1, 2). Although helpful, these prediction tools need however to be improved, since after curative treatment the disease recurs in 10% of low-risk patients (3, 4), and 30% of high-risk patients are disease progression free after 10 years follow-up (5, 6). There is therefore a need for molecular markers that could improve the prediction accuracy.

Because prostate cancer can recur several years after treatment, molecular studies on cancer tissues that aimed

to discover predictive biological markers have to be designed mainly on retrospective cases. The comparison between historical and more recent cases could however introduce biases mainly due to stage or grade migration and reclassification upon time, named the Will Rogers phenomenon (7). Few previous studies have focused on the review of prostatectomy specimens to evaluate histopathologic modifications (8–10). In 2 of them, the number of cases reviewed was small (7 and 38, respectively), and concerned only patients with disease recurrence (8, 9). In addition, all these previous studies focused only on gleason grade migration upon time, and not on both stage and margin status reassessment.

In this study, we reviewed the surgical specimens from 2 groups of matched patients, with and without recurrence after radical prostatectomy, to reassess all the histopathologic parameters before molecular studies.

### Materials and Methods

From the radical prostatectomy database of the Montsouris Institute between 2000 and 2005, we identified 1,187 cases with at least 36 months follow-up, coded by the urologists as margin negative, with PSA before surgery less than 20 ng/mL. Of these, 192 men (16%) developed biochemical recurrence, defined as 2 consecutive PSA levels 0.2 ng/mL or greater. The median time to biochemical relapse was 17 months (range 1–60 months). Each of these

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### Translational Relevance

Despite hundred of reports on prostate cancer prognostic markers, almost none have been applied in clinical practice. In order to improve the quality of tumor marker study conduct and reporting, guidelines for "reporting on tumor marker studies" (REMARK) have been published simultaneously in several journals. However, most of the studies that aimed to analyze prognostic markers on retrospective and often historical cancer samples do not reevaluate the pathologic data. We demonstrate herein that molecular studies on prostate cancer prognostic markers without pathologic reassessment could introduce biases mainly due to stage and grade migration upon time. Given its impact on the reclassification of standard prognostic variables, preliminary centralized review of archival cancer samples should be mandatory before molecular studies, in order to improve the clinical application of prostate cancer prognostic markers.

192 patients was matched with 1 or 2 patients without biochemical recurrence after at least the same follow-up, with identical Gleason score, pTNM (tumor node metastasis, system for staging cancer) stage and preoperative PSA. At the end, the 192 patients with recurrence were matched with 352 patients without recurrence. Patient's characteristics are summarized in Table 1.

The initial slides from the 544 radical prostatectomy specimens were reviewed in a blinded fashion by an experienced uropathologist (GF). All specimens have been serially sectioned and completely embedded. In case of doubtful margin or doubtful extra-prostatic status, new sections were cut from the paraffin blocks. In case of differences with the initial pathology report, slides were rescored by another uropathologist (PV), and reviewed by both pathologists until a consensus was reached. A positive surgical margin was characterized by carcinoma at the inked margin of the specimen. Extra-prostatic extension (EPE) was defined either as the presence of cancer cells in close contact to adipose tissue, or, in case of desmoplastic

reaction, as tumor located beyond the condensed smooth muscle of the gland.

### Statistics

Differences between groups were analyzed using the Chi-square test or the Fisher exact test.

### Results

Review of the initial slides led to reassessment in 85 on 544 cases (15.6%). Changes in pathologic characteristics were more frequent in the group of patients with biochemical recurrence when compared to patients without recurrence (Table 2). Ten on the 40 recurrent tumors initially classified pT2 Gleason 6 showed more adverse pathologic features after review.

The types of modifications are summarized in Table 2. Pathologic review showed higher Gleason score than initially coded in 25 cases. Of these, 21 tumors had Gleason pattern 4 not initially reported (poorly formed glands), leading to Gleason migration from 6 to 7 (3+4; Fig. 1A), and 14 tumors had a dominant nodule Gleason 8 (4+4) and tiny secondary foci Gleason 7, leading to Gleason migration from 7 to 8. In 18 patients, the pathologic review showed lower Gleason score than initially reported. In 8 cases, the review led to Gleason migration from 7 to 6, because initial upgrading that was due to tangential sectioning of few glands mimicking pattern 4. In 10 patients, some foci of small yet well-formed glands had not been previously reported, that led to Gleason migration from 8 to 7 (4+3). Of these 18 initially overgraded cases, 12 were patients without recurrence, and only 2 were patients with biochemical relapse. The pathologic review showed modifications in the pTNM stage in 12 cases. Of these, 6 were tumors with focal EPE (Fig. 1B). In 6 other patients, all of them without recurrence, tumors were reclassified from pT3 to pT2 because despite capsular invasion, cancer cells were not adjacent to adipose tissue, without evidence of extra-prostatic desmoplastic reaction. In 30 of the 192 patients with biochemical relapse after surgery, the review showed positive surgical margins on new tissue

**Table 1.** Tumor's characteristics before review

		Patients with recurrence	Patients without recurrence
Gleason	5-6	<i>n</i> = 47	<i>n</i> = 96
	7 (3+4)	<i>n</i> = 50	<i>n</i> = 99
	7 (4+3)	<i>n</i> = 73	<i>n</i> = 130
	8-9	<i>n</i> = 22	<i>n</i> = 27
pTNM	pT2	<i>n</i> = 122	<i>n</i> = 231
	pT3a	<i>n</i> = 70	<i>n</i> = 121
PSA	<10 ng/mL	<i>n</i> = 122	<i>n</i> = 221
	10-20 ng/mL	<i>n</i> = 70	<i>n</i> = 131

**Table 2.** Frequency, types, and impact of modifications following review of surgical specimen in patients with and without recurrence after radical prostatectomy

Results after review	Patients with recurrence	Patients without recurrence	P
No modification	<i>n</i> = 145	<i>n</i> = 314	<0.0001
Modification	<i>n</i> = 47 (24%)	<i>n</i> = 38 (11%)	
Gleason score modifications	Increased <i>n</i> = 12 Decreased <i>n</i> = 2	Increased <i>n</i> = 13 Decreased <i>n</i> = 16	0.02
TNM stage modifications	Increased <i>n</i> = 3 Decreased <i>n</i> = 0	Increased <i>n</i> = 3 Decreased <i>n</i> = 6	
Positive margins	<i>n</i> = 30	<i>n</i> = 0	
Adverse prognostic factors increased	<i>n</i> = 45	<i>n</i> = 16	<0.0001
Adverse prognostic factors decreased	<i>n</i> = 2	<i>n</i> = 22	

sections (that were cut because of doubtful margins on initial slides), with intraprostatic incision into the tumor.

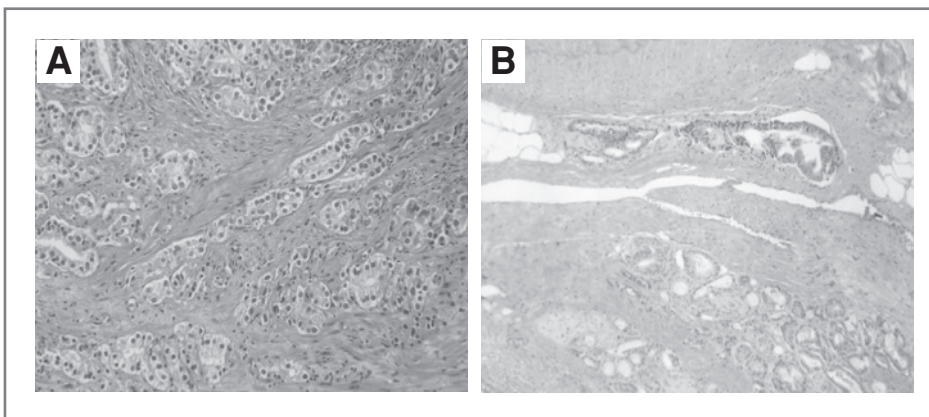
The impact of the pathologic review is summarized in Table 2. Reevaluation led to increase adverse prognostic factors (either margin, higher Gleason score or higher stage) more often in the group of patients with recurrence (95.7% of the modifications), than in patients without recurrence (42.1% of modifications). The difference is also significant when excluding margin status modifications and considering only either the reclassification of Gleason score and stage ( $P = 0.002$ ), or the Gleason score alone ( $P = 0.02$ ).

As examples, 2 tumors with initially a low risk of recurrence (pT2, Gleason 6 and negative margins), but that recurred 3 months and 3 years after surgery, were reclassified after review with, respectively, a positive margin and a Gleason score 7. On the contrary, in the group of patients with initially a very high risk of progression (pT3 and Gleason score 8), 5 of the 11 tumors that did not recur after at least 4 years follow-up showed after review an updated Gleason score of 7 instead of 8.

## Discussion

This review of 544 radical prostatectomy specimens from a single institution led to histopathologic reclassification in about 15% of total cases, and in 25% of the 40 patients initially considered low risk (pT2, Gleason 6, margin negative) that underwent biochemical recurrence. The previous studies on the same topic focused mainly on Gleason grade migration upon time, and not on stage and margin status reassessment (8–10). Two of them reported on a total of, respectively, 7 and 38 cases of low risk tumors with recurrence, a rate of 85 and 71% of modifications after review (8, 9). This difference in the reclassification rates could be due to the fact that these studies have reviewed cases more historical than ours (from the years 1980). In fact, another recent study reported that the concordance between original reading and standardized review of the Gleason score increased in the most recent cases when compared to historical cases (10).

In our study, the Gleason score was reclassified in 43 cases, increased when compared to the initial score in 25 tumors and decreased in 18 tumors. The 2005



**Figure 1.** Gleason score 3 + 4 = 7 carcinoma, where the pattern 4 is composed of poorly formed glands (A). Focal extraprostatic extension with tumor glands adjacent to periprostatic adipose tissue (B).

International Society of Urologic pathology (ISUP) consensus conference on Gleason grading led to clear guidelines that aimed to apply the Gleason system in a more uniform manner (11). The effects of grade migration have been previously reported in prostate biopsies, leading to a significant upward shift of the Gleason score and risk classification (12, 13, 14). In this study, initial undergrading was mainly due to ill-defined glands with poorly formed glandular lamina, classified as Gleason pattern 4 only since 2005 (11). Another cause of undergrading was the presence of separate nodules with different differentiation that were initially graded together. The recommendation of the 2005 consensus conference is to assign a separate Gleason score to each dominant nodule (11). The reasons for overgrading were either the presence of a Gleason pattern 3 that occupy less than 10% of the area of the tumor and was not initially taken into account, or tangential sectioning of rare glands identified only at high magnification that have led to an overdiagnosis of pattern 4. The clinical significance of the Gleason score shift was previously reported, since standardized review of the score has been shown to improve the prediction of prostate cancer survival (10). In this study, the updated Gleason grading also better correlated with prognosis, since the score was significantly more often upgraded after review in patients with biochemical recurrence than in patients without progression.

The pTNM stage was modified in 12 cases after review. Understaging occurred in 6 cases, due to focal EPE that has not been initially reported. In these cases, we considered EPE as nonequivocal, because of the presence of tumor either in close contact with extra-prostatic fat or adjacent to adipose tissue beyond the rounded boundary of the gland. In 6 other cases initially reported as pT3 because of capsular invasion, we found in contrast no evidence of EPE, i.e., no contact with fat without disruption of the normal rounded contour of the prostate in this area, and without evidence of desmoplastic reaction of the extra-prostatic tissue. Problems with EPE definition have been recently well described, mainly due to the lack of a true histologic capsule (15). The updated TNM stage also tends to better correlated with prognosis, also the difference was not significant due to the low number of reclassifications. In fact, the reassessment led to upstaging in all patients with biochemical recurrence, and in only one-third of patients without progression.

After review and examination on new tissue sections performed because of initial equivocal margin status, we found in 30 cases evidence of positive margins (cancer cells reaching the ink) with intraprostatic incision into the tumor. This finding is of clinical importance, since it has been shown that patients with intraprostatic positive surgical margins have a recurrence rate significantly higher than patients with organ confined tumors and negative margins, and equivalent to cases with positive margins and

focal EPE (16, 17). In fact, all the 30 patients that we reclassified as margin positive underwent biochemical recurrence after surgery. In the situation of equivocal surgical margin, it could be therefore useful to cut additional levels from the block in question, in order to clarify the margin status.

In this study, patients with biochemical recurrence and patients without progression were matched according to the Gleason score, pathologic stage, and follow-up duration. The study is however limited by a relatively short follow-up that does not allow the use of prostate cancer mortality as a primary endpoint. When considering together the review of all histopathologic parameters, we observed that reassessment led to modifications more often in the group of patients with progression. In addition, the reclassification increased adverse prognostic factors more often in patients with recurrence when compared to matched patients without progression. The difference is more significant when considering all parameters together ( $P < 0.0001$ ), than when considering either the Gleason score alone ( $P = 0.02$ ) or the Gleason score and TNM stage ( $P = 0.002$ ). These results emphasize the clinical significance of all the histopathologic changes upon reassessment, including not only the Gleason score, but also the TNM stage and margin status. That could prompt to reevaluate the surgical specimens in case of biochemical recurrence, particularly in low risk patients. Thirty of our patients that underwent biochemical progression remained however classified as "low risk" after review, that emphasizes the need to improve the prediction accuracy by the identification of molecular markers. Given its impact on the reclassification of standard prognostic variables, preliminary reassessment of archival cancer samples might be reported in the "recommendations for tumor marker prognostic studies" (REMARK) guidelines (18).

## Conclusion

The present results demonstrated the importance to review all the pathologic characteristics of prostate cancer surgical specimens, before molecular studies performed on retrospective and often historical cases.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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