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# Histopathologic Diagnosis and Classification of Prostate Adenocarcinoma: Biologic Significance

Jill M. Peters, MD,\* and John D. Crissman, MD†

*Early diagnosis and accurate, biologically meaningful classification of prostate neoplasia remain important goals. The relation of evolving clinicopathologic concepts of histologic appearances to potential tumor progression is a major advance in classification of prostatic neoplasia. The criteria for recognizing incidental or "occult" stage A-1 adenocarcinomas remain problematic in diagnosis, and focal neoplasms with little or no propensity to progression must be differentiated from cancers with a high likelihood of aggressive behavior. Current histologic grading systems in classifying prostate adenocarcinoma accurately identify two cancer subsets: 1) focal well differentiated tumors which rarely progress, and 2) diffuse poorly differentiated tumors which invariably develop metastatic disease. Unfortunately, the majority of prostatic cancers are classified in the intermediate group in which the prognosis is variable and difficult to differentiate purely by histology. Our laboratory recently adapted image analysis of cellular DNA quantitation—a major improvement in accurately predicting tumor behavior, especially in the intermediate histologic grades. We and others have found that tumors with abnormal (aneuploid) DNA content are more likely to progress than neoplasms with normal (diploid) DNA content. (Henry Ford Hosp Med J 1989;37:8-13)*

Confirmation of prostate carcinoma requires tissue or cellular biopsy. When prostatic adenocarcinoma is suspected either by clinical symptoms, palpation, or ultrasound examination, a needle biopsy or aspirate is the most common method of tissue sampling. When a nodule is palpated, the biopsy is directed at the nodule. Successful tissue sampling depends on the location and size of the nodule as well as the skill of the urologist. When the suspected neoplasm is identified by ultrasound examination, a guided biopsy is required. Either removal of a core of the tissue (traditional needle biopsy) or aspiration of cellular material (aspiration biopsy) can be done. The "automatic gun" approach, which samples multiple small tissue fragments, has become popular recently, but tissue samples are smaller and provide pathologic information in-between that of needle core and aspiration biopsies. Accurate diagnosis for aspiration biopsies depends on the skill of the aspirator, the quality of the aspirate, and the experience of the cytopathologist. Aspiration biopsies can be interpreted accurately, but only after considerable practice and detailed clinical pathologic correlation by urologist and pathologist. Needle core tissue biopsies are interpreted by surgical pathologists and remain the standard method of diagnosing prostatic adenocarcinoma.

Prostate adenocarcinoma is the most common cancer in elderly men. Asymptomatic occult neoplasms or prostatic nodules detectable by physical examination are found in approximately one third of men in their 70s (1). The frequency of asymptomatic occult neoplasms increases appreciably when histologic step sections are examined from prostates removed at autopsy. Clearly, histologic demonstration of adenocarcinoma is

common in males in the eighth decade or greater. These observations raise questions about the relationship of asymptomatic occult cancers and their propensity to progress to a clinically significant invasive neoplasm with metastatic potential. This spectrum of neoplastic disease behavior also raises important clinical questions as to which prostate cancers are truly "occult," not likely to progress, and can therefore be treated in a conservative manner, and which prostate cancers are potentially life-threatening and require therapy, often radical in extent.

## Histologic Grading

Numerous schemes describing grading systems for the classification of prostatic adenocarcinoma have been reported. The three major groups of observations incorporated to varying degrees in these grading schemes include:

1. Cytologic or nuclear grade: This set of observations includes nuclear size (shape), chromatin content and staining pattern, and presence of nucleoli and amount of cytoplasm (nuclear/cytoplasmic ratio). These observations provide the major criteria for diagnosis of needle aspiration biopsies and are also

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integrated into many of the proposed histologic grading schemes.

2. Neoplastic cell organization or formation of tubules/acini: These observations reflect the extent to which the neoplasm recapitulates normal prostate tissue organization. Generally the greater the proportion of neoplasm forming tubules (with identifiable lumens), the better the differentiation.

3. Neoplasm growth pattern: While less commonly incorporated into grading schemes, this observation is an integral part of the Gleason classification (2,3). The pattern in which the neoplastic cells (or glands) infiltrate the adjacent host stroma (ie, pushing borders versus single cell invasion) is significant. Small differentiated foci of tumor usually have well demarcated tumor-host borders. In contrast, poorly differentiated tumors commonly infiltrate as single cells or cords of cells.

Grading schemes generally utilize two of the three sets of observations described in Table 1. For example, grading schemes described by Bocking et al (4) and by Gaeta et al (5) quantify both the cytologic and histologic patterns deriving a tumor score. Mostofi (6) integrates both cell features and histologic pattern into a tumor grade, and the scheme by Brawn et al (7) derives a grade based on the proportion of the tumor forming identifiable glandular structures. This system is similar to the grading scheme that has been used for many years at the Mayo Clinic (8). The histologic grading system proposed by Gleason et al (3) varies from the other grading schemes in two ways: 1) tumor histologic heterogeneity is recognized, and two distinct patterns are routinely factored into the final tumor grade or score; and 2) the pattern of tumor growth or invasion into the host stroma is also incorporated into the five distinct patterns or grades recognized by these authors. Well differentiated tumors tend to have well formed glands and "pushing" borders or well defined tumor host-stroma interfaces. Conversely, poorly differentiated neoplasms grow as single cells or irregular infiltrating cords with little or no evidence of gland formation.

The Gleason grading system has been embraced by the urology community, although there is little objective evidence that its predictive value is greater than other systems. Studies of reproducibility in grading have suggested that the simpler systems, such as the MD Anderson scheme (7), are more reproducible (9). In a comparative study of reproducibility and predictive value, the Mostofi (6) and Bocking (4) proposals had the best correlation with tumor stage (9). Both grading schemes incorporate cytologic factors in deriving tumor grade.

Lack of agreement in adapting a uniform grading scheme underlines the absence of an optimum system for predicting neoplasm behavior. In general, all proposed grading systems identify the relatively rare (5% to 15%) poorly differentiated adenocarcinomas that invariably progress, as well as the well differentiated tumors that are unlikely to progress. Well differentiated carcinomas have well demarcated tumor borders in addition to differentiated cytology and tubular formation. The former feature requires adequate tissue to determine the volume of tumor present and its growth pattern, parameters not always available from needle core biopsies and invariably absent in needle aspiration biopsies. Most prostate adenocarcinomas fall into the middle range of differentiation, some of which progress and some which do not. Most urologists and pathologists agree that:

**Table 1**  
**Histopathologic Grading Systems for Adenocarcinoma of the Prostate**

Grading System	Histologic Observations
Gleason (2)	<ol style="list-style-type: none"> <li>Growth pattern including:               <ol style="list-style-type: none"> <li>Formation of tubules and acini</li> <li>Tumor-host-stroma interface</li> </ol> </li> <li>Selected cytologic changes (eg, hypernephroid appearance, central comedo-like necrosis)</li> <li>Primary and secondary pattern scores combined for total of nine grades</li> </ol>
Mostofi (6)	Three grades dependent on: <ol style="list-style-type: none"> <li>Proportion of glandular differentiation</li> <li>Nuclear anaplasia</li> </ol>
Bocking (4)	<ol style="list-style-type: none"> <li>Four histologic growth patterns</li> <li>Three nuclear (anaplasia) grades</li> <li>Summation of two scores</li> </ol>
Gaeta (5)	<ol style="list-style-type: none"> <li>Four histologic growth patterns</li> <li>Four nuclear grades</li> <li>Grade both features and assign highest score (either growth pattern or nuclear grade)</li> </ol>
MD Anderson (7)	Four grades depending on proportion of tumor forming glandular structure (somewhat analogous to Broder's approach to grading)

1. All grading systems identify a minority subset of poorly differentiated or high grade tumors with a high likelihood of progression.

2. While the majority of prostate cancers fall in the middle or intermediate group of histologic grades, current histologic grading systems are not reliable or accurate in differentiating tumors likely to progress from the more indolent or slow proliferating neoplasms.

3. Well differentiated adenocarcinomas can also be segregated by histologic appearance. This small subset generally replicates slowly, and progression, if it occurs, is only after extended intervals. When well differentiated neoplasms are focal, confirmation of stage A-1 "incidental" neoplasms is appropriate. Since an adequate tissue sample is required to insure that the neoplasm is truly focal, needle tissue cores or aspirates can be excluded as methods of diagnosing stage A-1 cancers.

The development of histologic grading schemes has contributed to the clinical care of patients with prostate cancer (10). Nevertheless, the problems outlined above are major deficiencies in determining the biologic potential of each cancer and deciding appropriate therapy.

### Histologic Definitions of Localized Prostate Adenocarcinoma

Focal or incidental (stage A-1) prostate cancer varies greatly with the patient's age and the type of surgical procedure (transurethral resection of the prostate [TURP] versus prostatectomy) (11). The incidence of focal prostate cancer in autopsy studies varies from 4% in the third decade to 80% in the ninth decade

**Table 2**  
**Staging Designations for Carcinoma of the Prostate**

Description	Clinical Stage	
	Modified Jewett (25)	TNM (26)
Clinically unsuspected		
incidental histologic finding	A	T-1
Focal, well differentiated	A-1	T-1a
Diffuse, high grade	A-2	T-1b
Risk recognized clinically	B	T-2
Tumor confined to one lobe	B-1	T-2a
Tumor in both lobes	B-2	T-2b
Periprostic spread	C	T-3 to T-4
Base of seminal vesicle		T-3
Base of seminal vesicle and/or other structure		T-4
Distant metastases	D	T-1-4, N-1-3, M-0-1
Pelvic lymph node	D-1	T-1-4, N-1, M-1
Bones, lung, etc	D-2	T-1-4, N-0-1, M-1
Elevated acid phosphatase	D-0	T-1-4, N-0-3, M-0

(12). Evaluation of prostatectomies for benign prostatic hypertrophy (BPH) reveals focal cancers in 3.5% to 24% of prostates examined (13,14). Because of the small size of most stage A-1 carcinomas, step sections of all tissue are required (not routinely performed in most pathology laboratories) to diagnose all small latent neoplasms (15,16).

The separation of focal (stage A-1) and diffuse (stage A-2) incidental adenocarcinomas discovered in transurethral prostate resections is defined variably (17). Unfortunately, criteria for separation of stage A tumors into A-1 and A-2 are not completely agreed upon:

1. Three (18-20) to five (21) isolated foci of cancer has been adopted by some investigators as the maximum allowable foci (usually in TURP specimens) for stage A-1 cancers.

2. Of the specimen involved by the malignant tumor, 5% of area or less (as measured on the tissue slides) is used by other investigators to define stage A-1 (22,23).

3. One cc of tumor volume is used by yet another group of investigators to separate stage A-1 from more extensive stage A-2 cancers (24).

All of these definitions attempt to apply a quantitative approach to differentiating the "incidental," presumably latent cancer from diffuse adenocarcinomas thought to have a high likelihood of progression. In addition, all authors factored the histologic grade into the definition of occult cancer foci (stage A-1). Since urologists and pathologists generally agree that small or focal well differentiated tumors are unlikely to progress, they are appropriately classified as stage A-1 (11). Conversely, since poorly differentiated focal cancers are likely to progress, they are excluded from stage A-1 regardless of tumor extent. Staging designations commonly used for prostatic carcinoma include the modified Jewett (25) and the American Joint Committee or tumor, node, metastasis (TNM) classifications (26) (Table 2). A poorly differentiated focal incidental tumor is considered to be stage A-2 according to this staging system. Un-

fortunately, little is known about the intermediate histologic grade neoplasm which constitutes a sizable proportion of these "early" cancers. This is a major deficiency in current histologic grading systems and is not addressed in most studies.

Golimbu et al (21) found that most unsuspected prostate carcinomas are diffuse (stage A-2). They also observed that patients with stage A-2 neoplasms had a higher frequency of lymph node metastases than patients with either stage A-1 or B-1 cancers. Stage A-1 was defined in their study as five or fewer isolated foci (chips in TUR specimens) with a well-differentiated histologic pattern. Any tumor with a poorly differentiated tumor grade was classified as stage A-2. Blute et al (24) studied 23 untreated patients who were less than 60 years of age with stage A cancer. Two of the eight classified as stage A-2 (greater than 1 cc or high grade histology) progressed. Four of the 15 classified as stage A-1 also progressed after an average interval of 10.2 years. Cantrell et al (23) followed 117 patients with stage A cancer and determined that the cancer seldom progressed in patients with less than 5% of surface area examined containing foci of well differentiated tumor histologies (Gleason score 2-4). Conversely, the cancer progressed in 32% of patients with greater than 5% surface area and in 17% with a histologic grade greater than Gleason score 4 (23). A follow-up study of the same patient population restricted to stage A-1 tumors (less than 5% of surface area and Gleason score 2-4 histology) showed that eight of 50 (16%) patients at risk for eight years or longer developed disease progression (27). The authors concluded that stage A-1 disease carried substantial risk, but only after prolonged periods of follow-up, an important consideration in younger patients. The Mayo Clinic study also demonstrated that in the long term patients with stage A-1 neoplasms developed a substantial proportion of clinically significant cancers (24).

### Biopsy Techniques

Tissue or cellular sampling of prostatic adenocarcinoma is crucial for confirmation of diagnosis. Optimum tissue sampling must provide accurate diagnosis with minimal morbidity. Accuracy is critical, and the need for early diagnosis requires identifying smaller and smaller foci of cancer. The American College of Surgeons classified 22.9% of patients in clinical stage A in their 1978 survey and 27.2% in the 1983 survey. Pathologic confirmation of diffuse infiltrative neoplasms is usually not a significant problem, but biopsy of tumor nodules (stage B-1) and suspected tumor identified by ultrasound often proves to be difficult (28).

Needle core biopsy and needle aspirate cytology are the most common methods of sampling. Needle aspirate biopsy has achieved considerable popularity in the past decade because of decreased patient morbidity, although core needle biopsy techniques have the advantage of providing tissue for histologic examination. Interpretation of needle aspirate biopsy requires skilled personnel, but in experienced hands accuracy is comparable to that of core needle biopsies (29-32) (Table 3). Grading appears to be more reproducible in tissue sections from core biopsies.

**Table 3**  
**Accuracy of Needle Aspirate Biopsy in Diagnosis of Prostate Carcinoma**

Study	Total Patients	Malignant Cytology + FNA	Biopsy Confirmed Malignant +	Biopsy Confirmed Benign	Unsatisfactory or No Biopsy
Carter et al (32)	110	57	48 (86%)	8 (14%)	1
Epstein (29)	118	42	37 (90%)	4 (9.7%)	1
Kline et al (30)	540	170	142 (92%)	12 (7.8%)	16
Chodak et al (31)	75	19	12 (86%)	2 (14%)	5
Total		288*	239 (90%)	26 (10%)	23

\*Less patients with unsatisfactory or no biopsy.  
FNA = fine needle aspirate.

**Table 4**  
**Probability of Diagnosis of Carcinoma in TUR Prostate by Tissue Examined**

Study	Patients with Suspected Neoplasm	Number of Carcinomas	Proportion of Specimen Sampled	Result
Vollmer (33)	711	61 (8.6%)	5 blocks 10 blocks	90% Gleason 3,4,5 98% Gleason 3,4,5
Moore et al (34)*	151	39 (25.8%)	95% specimen 63% specimen 26% specimen	1 chip with Ca† 3 chips with Ca 10 chips with Ca
Murphy et al (35)	383	66 (17.2%)	6 g‡ 12-15 g	100% stage A-2 90% stage A-1
Rohr (36)	457	65 (14.2%)	8 blocks§	82% Ca 1 chip 95% Ca 3-5 chips 100% Ca > 5 chips

\*Consecutive cases.  
†95% probability.  
‡Average 1.5 g/block.  
§Average 1.6 g/block.  
Ca = cancer.

Transurethral resections of prostate occasionally reveal unsuspected adenocarcinoma. The incidence of stage A (especially A-1) tumors is dependent on patient age, tumor size, and completeness of the pathology examination. The latter two parameters are extremely important in identifying stage A-1 tumors. Urologists and pathologists require variable amounts of TUR tissue for histologic examination (Table 4). Several studies have evaluated the relationship of the amount of tissue examined to the sensitivity of detecting small or "early" A-1 adenocarcinomas (33-37). These reports confirm that six to eight blocks of approximately 1.5 g of tissue each is adequate to detect almost all high grade and/or diffuse stage A-2 cancers. To identify small foci of stage A-1 tumors, almost all of the specimen must be examined. Whether or not it is clinically relevant to diagnose each of the small A-1 nodules of neoplasm is a major issue. We think it is relevant to identify stage A-1 carcinomas in the younger age group. These small foci of neoplasm appear to result in clinically significant cancers after many years.

The zonal distribution of prostate cancer is important in deciding the type of biopsy required for diagnosis. The anatomic division of the prostate can be divided into central, transitional, and peripheral zones (38). The majority of prostate cancers arise in the peripheral zone (38,39), a region not usually included in most TUR specimens. Only rarely do cancers arise in the central

zone, and some evidence shows that these may have a different biologic behavior (38). The transitional zone may serve as a barrier to neoplasms arising in the peripheral portion of the gland. Only after extensive invasion is the transitional zone infiltrated and the central zone involved by cancer. Rarely, neoplasms arise in nodules of hyperplasia and develop primarily in the transitional zone of the gland (38). Thus, TURP does not resect portions of the gland in which the majority of cancers arise. Continued improvement in identifying asymptomatic "early" cancers requires demonstration of nodules by physical examination and ultrasound, with directed needle biopsies.

### DNA Analysis

The biologic behavior of prostatic adenocarcinoma is highly varied (40). Pathologic staging and histologic grading are the traditional means of predicting prognosis for patients with prostatic carcinoma (3,8). Poorly differentiated carcinomas progress rapidly, but patients with well differentiated neoplasms may have prolonged survival. With moderately differentiated neoplasms, some patients do well but some die from their tumors (41).

Chromosome analysis and DNA quantitation studies in various tumors show that malignancy is often associated with devia-

tions from normal ploidy (42,43). The normal human somatic cell contains 46 chromosomes (23 pairs) and is referred to as diploid. A cell with fewer or more than 46 chromosomes is described as aneuploid (hypodiploid or hyperdiploid, respectively). Although identification of individual chromosomes is possible only during metaphase, nuclear DNA content can be measured on interphase cells, independent of the proliferative activity of the tumor. Quantitative measurement of nuclear DNA content is accomplished by one of two methods, the Feulgen-Schiff technique or the use of fluorescent dyes such as propidium iodide (43). These stains bind to normal DNA in a stoichiometric fashion, with the intensity of staining proportional to the DNA content. Thus, the DNA content in tissue sections can be determined by static cytometry using computer assisted image analysis. DNA content in tumor nodules can be measured by flow cytometry (FCM) using disaggregated tumor specimens of single cells in suspension.

Digital image analysis is a new, evolving approach to quantitative DNA cell analysis. Nuclear DNA content can be determined on archival pathologic specimens as well as on small tissue samples. The variable amount of tumor often admixed with nonneoplastic tissue in needle biopsies makes microscopic image analysis an effective means of assessing DNA content in prostatic adenocarcinoma. Nuclear DNA content assessed by image analysis has also been shown to correlate well with flow cytometric DNA measurements (44-46).

Several studies have utilized FCM to assess nuclear ploidy whereas others have utilized static cytometry or image analysis for assessment of nuclear DNA content. Using Feulgen-stained nuclei and slide cytophotometry, Zetterberg and Esposti (47) found that well differentiated tumors were predominantly diploid and that poorly differentiated tumors were primarily hyperdiploid or aneuploid. Patients with moderately differentiated tumors had either 1) diploid tumors or 2) aneuploid or hyperdiploid tumors. These investigators subsequently examined tissues from 43 patients diagnosed with prostatic carcinoma up to 15 years earlier (48). All patients had been treated with estrogen therapy. Patients with diploid range DNA content had a good response to estrogen, whereas those with aneuploid DNA tumors had a poor response to estrogen and thus decreased survival. Similar findings have been reported by Tavarres et al (49) and Seppelt and Sprenger (50).

FCM was applied to prostate cancer in 1977 by Bichel et al (51). Nuclei obtained from fine needle aspirates were examined in 50 patients with BPH or prostate carcinoma. They found primarily diploid or diploid plus tetraploid populations in patients with BPH. In patients with prostate carcinoma, well differentiated tumors were primarily diploid and poorly differentiated tumors had a higher DNA content with cell populations in the tetraploid and octoploid range. Moderately differentiated tumors fell into two groups: those with no or few tetraploid cells (similar to well differentiated carcinomas), and those with a high percentage of tetraploid and octoploid cells (similar to poorly differentiated tumors).

Ronstrom et al (52) studied 500 patients with suspected prostate carcinoma who underwent transrectal fine needle aspiration biopsy. The aspiration cytology revealed 301 specimens interpreted as benign, 33 suspicious for carcinoma, and 166 diag-

nostic of carcinoma. The 166 carcinomas revealed 45 (27%) diploid tumors, 75 (45%) tetraploid tumors, and 46 (28%) aneuploid tumors. The incidence of aneuploidy was inversely related to tumor differentiation. Thus poorly differentiated tumors were most likely aneuploid (77%), and well differentiated tumors were most likely diploid (56%). As expected, moderately differentiated tumors had an intermediate incidence of diploid and aneuploid populations.

Stephenson et al (53) studied 82 patients with stage D-1 disease by FCM using cells from the lymph node metastases. Approximately 10% of patients had uninterpretable histograms. The median survival was five years for patients with aneuploid tumors and 8.8 years for those with diploid tumors. Winkler et al (54) evaluated prostatic tissue from 91 patients with stage D-1 disease undergoing radical prostatectomy. A total of 87% of the tumors were diploid (and/or tetraploid) and 13% were aneuploid. Only 15% of the diploid tumors progressed, whereas 75% of aneuploid tumors progressed. In a similar study, Lee et al (55) evaluated 88 patients undergoing radical prostatectomy with negative lymph nodes. Flow cytometric DNA quantitation showed 42% of the tumors to be diploid and 58% aneuploid. The probability of disease-free survival at 60 months was 85% for diploid tumors and 9% for aneuploid tumors. In addition, aneuploidy correlated with a greater likelihood of seminal vesicle invasion by tumor and subsequent development of recurrent disease.

At Henry Ford Hospital we have studied 44 patients with localized stage A or B prostate cancer who were surgically staged and uniformly treated with <sup>125</sup>Iodine implantation. Feulgen-stained nuclei were evaluated using image analysis. Twelve patients (27%) developed stage D-2 disease, with a mean follow-up of 69.5 months. The DNA pattern was diploid in 35 patients (80%) and aneuploid in eight (18%). All of the aneuploid tumors progressed to stage D-2 disease, whereas only 11% of the diploid tumors progressed ( $P < 0.001$  unpaired *t* test). Determination of nuclear DNA content using image analysis provides objective information that is directly related to prognosis. This confirms the previously mentioned studies correlating tumor cell DNA content with tumor progression (54,55).

Image analysis has many advantages compared to FCM. It allows DNA quantitation on small cell samples, as well as on paraffin-embedded archival samples or fresh tissue (56). Image analysis is ideal for studying solid tumors since single cell suspensions are not necessary as in FCM. FCM of paraffin-embedded material is inferior to that obtained with fresh tissue, with 5% to 20% of histograms reported as uninterpretable (54,57). Using image analysis of Feulgen-stained nuclei, all histograms were evaluable with no cases excluded for inadequate staining or preservation. Small amounts of tumor often admixed with normal glands can be identified by traditional morphologic observations and DNA quantitation restricted to the malignant cells.

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