

CHAPTER SEVEN
THE PATHOLOGIST
By Jonathan Oppenheimer, MD

Introduction by Aubrey Pilgrim

Ordinarily, this chapter should be included with the previous chapter on diagnosis. Most patients seldom come in contact with a pathologist. So many patients may not realize what an important role the pathologists play in the diagnosis, prognosis and treatment of their cancer.

Basically, the pathologist looks at the biopsy sample sent to him, then assigns a Gleason Score to the sample. Fig. 6-4 in the previous chapter is a drawing of Dr. Gleason's chart. The cells in a biopsy may be very much like all the samples shown in Dr. Gleason's chart. The pathologist must arbitrarily determine the most prevalent type of cells, then the second most prevalent and assign a score from 1 to 5 of the two types.

The determination of which Gleason type and score to assign to a biopsy sample is primarily subjective. Often different pathologists may not all agree on the type and score. It is a critical item that can help determine the treatment and prognosis of the cancer. In many labs, two or more pathologists review the biopsy sample to try to make sure it is correct. Often it may be beneficial to have the biopsy read at a different laboratory for a second opinion.

To do a biopsy, a hollow needle is inserted through the rectum or the perineum into the suspected area of the prostate. A few cells are picked up by the needle. Fig. 7-1 shows a biopsy gun at the top and a cutting needle at the bottom. Some cancers may present nodules that are so obvious that only one or two needles are needed to get a sample. But some may be fairly small and hidden. These may require six to eight or more samples.

The doctor may use a transrectal ultrasound (TRUS) probe to guide the needles to the suspected area. Fig. 7-2 shows a transrectal ultrasound probe. Even with a TRUS, a small cancer can be missed. The classic tumor is a hard mass of compacted cells or a lump that may be readily felt during a DRE. In this case an ultrasound may not be needed to get positive samples. But remember that the word cancer means crab, which is what the Greeks thought it looked like. So it may have a rather small tumor body with several extensions or legs that extend throughout the prostate. It is also possible that there may be as many as seven or more small foci or separate cancer colonies within the prostate. So it is quite possible to miss the cancer, even if several different samples are taken. Someone compared a biopsy as being similar to sticking a needle in an apple and trying to find a worm. If some of the cancer is missed a biopsy may not provide a true representation of the disease.

The biopsy core samples are sent to a pathology lab for analysis. Of course if the cancer is missed and you have a negative biopsy, then it could cause a real problem. One of the members of our support group had several biopsies, but each time with a negative report. But over the period of a couple of years, his PSA went from 10 up 15 ng/ml. BPH was ruled out, so at his insistence, they finally did a radical prostatectomy. He had stage B2 cancer with several small foci or colonies throughout the prostate.

There are some urologists who use a color Doppler ultra sound to do biopsies. Cancers usually have more blood vessels than ordinary tissues. The red blood vessels can be seen on a color Doppler ultrasound. Dr. Fred Lee of Crittendon Hospital in Rochester, Michigan, is an expert in finding cancers that are difficult to biopsy by using color Doppler ultra sound,

Dr. Oppenheimer is very active on the Internet and has been of enormous help to many patients. Some questions and answers are at the end of this chapter. Here is Dr. Oppenheimer's article.

The Pathologist

Jonathan Oppenheimer, M.D. completed his five-year residency in Anatomic and Clinical Pathology in Rochester, N.Y. After practicing as a general pathologist, Dr. Oppenheimer returned to Johns Hopkins Hospital for a post-doctoral Fellowship in Prostatic Pathology.

The pathologist is the physician who analyzes and diagnoses the tissue specimens (blood, biopsies, prostates) that your urologist or surgeon sends to the lab. From the perspective of the patient (and of most urologists) the pathologists and their labs are somewhat of a black box: in goes the specimen and out comes a diagnosis. In this article I will reveal some of the inner workings of this mysterious box and show how your pathologist may assist you in the diagnosis, prognosis, and treatment of prostate cancer.

Although the patient's prostate biopsy should be diagnosed by a pathologist who is proficient in interpreting this type of specimen, the patient (and increasingly the urologist) rarely has the opportunity to choose his pathologist. While the urologist may know of a pathologist with special competence in evaluating prostate samples, the insurance companies in some cases may have already contracted with a specific commercial laboratory to handle the specimen. This effectively removes the urologist's professional opinion from this decision.

Nevertheless most general pathologists are well-trained and are competent to read the great majority of prostate needle-biopsy and prostatectomy specimens. In general, pathologists see enough prostate specimens to gain familiarity with the subtle variations of normal tissue and the various forms of malignancy.

The Lab

The lab may be one of many types, ranging from a small hospital-based operation with one pathologist, to a multiple-partner group in a large community hospital or university teaching institution, to a large commercial corporation employing many pathologists. Generally, the larger and the more academic the laboratory, the better the chance that one of the pathologists will have a particular interest in prostate pathology. Some labs may even specialize in urologic diagnosis.

A thorough evaluation of tissue takes time as well as expertise. Some larger commercial labs may focus too much on profit margins and provide less than optimal evaluations. Such is the fate of medicine at the close of the twentieth century. If you prompt your urologist with the right questions, he or she may ask the pathologist to re-evaluate your tissue and thereby identify additional diagnostic or prognostic factors.

Tissue Processing of Biopsy Specimens

Immediately after obtaining the needle biopsy, the urologist places each of the tissue core samples into a small vial containing a fixative (usually 10% formalin) which acts as a “pickling solution,” preventing the specimen from degrading and preserving it for further processing. During fixation the tissue shrinks by about one-third, a factor to take into account if the pathologist attempts to calculate the size or volume of the tumor.

After further chemical processing the fixed tissue is embedded in paraffin cubes (“blocks”) which are then cut with a very sharp knife to create translucently thin sections. These sections are stained with colored dyes and placed on glass slides for viewing under the microscope. Occasionally the pathologist finds it necessary to confirm or rule out carcinoma (cancer) by applying special immunoperoxidase stains which highlight a specific protein (CK-903 or low-molecular-weight cytokeratin) which surrounds benign, but not malignant, glands. These special studies may be performed on material that is still in the paraffin blocks. The amount of time that the laboratory is required to store these blocks varies from state to state and ranges from five to twenty years.

At the time of the pathologist’s original diagnosis, or later using tissue remaining in the saved paraffin blocks, he or she may perform additional tests such as ploidy analysis, which measures the chromosomal content of malignant cells. Ploidy analysis on prostatectomy specimens has been shown to be an important prognostic factor in predicting the spread of tumor outside the prostate and thus the chance of recurrence after surgery. This test also helps to predict the responsiveness of the tumor to hormonal treatment.

Making the Diagnosis

Fortunately, there are objective criteria which pathologists use to make the diagnosis of cancer, but these are most easily applied on the largest and most-

easily-diagnosable lesions. Many of the older pathologists now practicing were trained before the current popularity of thin-needle-core biopsies. Many of them may not have had formal training in the interpretation of such specimens. This lack of training partly explains the considerable variation in the ability of pathologists to diagnose small lesions.

Fortunately, (for themselves as well as their patients), pathologists learn to be conservative in their diagnoses. There is nothing more embarrassing (or costly to the pathologist) than making a diagnosis of cancer only to find that the subsequently removed prostate is free of disease. Therefore, pathologists who lack the confidence to be definitive may under call a lesion, using words like "atypical," "suspicious," and "can't rule out". A repeat biopsy may well provide the evidence necessary to make an unequivocal diagnosis, but may have been avoided if the original biopsy had been reviewed by someone with more experience. Caution is indeed the better part of valor and even the most expert of prostate pathologists must occasionally offer less than definitive diagnoses.

The same psychology that may lead a pathologist to hesitate in making a diagnosis of cancer may be responsible for the problem of under calling the Gleason score (see below) when a definitive positive (cancer) diagnosis is made on a very small lesion. My experience at Johns Hopkins Hospital was that most "2+2=4" biopsies sent for evaluation were actually "3+3=6" biopsies that had been under graded by a general pathologist. This phenomenon decreases the predictive value of aids such as the Partin and Narayan tables. These tables are based on information (typically Gleason score, clinical stage, and PSA level), and can assess the degree of severity of the tumor prior to surgery.

Another reason for sub-optimal diagnosis is that the pathologist might miss a microscopic focus of carcinoma, prompting an additional biopsy to make the diagnosis or giving false assurance that cancer has been ruled out.

Fortunately, there are many fewer misinterpretations of a benign entity as malignant, since cases have become more widely publicized in pathologic circles. A much more common occurrence is the failure to identify prognostic factors (such as extensive intravascular, extraprostatic, or perineural tumor involvement) that might rule out or at least call into question aggressive treatments designed to be curative.

Interpretation of Your Pathology Report

You should obtain a copy of your pathology report(s); it is your information and you have every right to have it. A careful reading of its contents will make you a more informed patient, better able to formulate rational treatment decisions with the aid of your urologist, surgeon, and oncologist.

Furthermore, asking the laboratory or the urologist for a copy of the report serves other purposes. It demonstrates your interest in being an active participant in the

important decisions that must be made, perhaps fostering better discussion between you and your doctor. In addition, the request will often cause the pathology lab to review the slides, and can give the pathologist the opportunity to identify additional diagnostic and prognostic factors.

The Biopsy Report

A complete report should include:

- *Your name and associated individual identifiers (age, patient number, etc.)
- *The accession number of the case (Usually in the form of “S-year- number”, e.g., S-00-16258).
- *A gross description of the specimen (including the number and size of the tissue cores) removed from the prostate and received by the laboratory.
- *The diagnosis, which reduced to its most basic form is either benign (normal), atypical/suspicious, or malignant (cancer).
- *The name and signature of the responsible pathologist along with the name and address of the lab.

It is often of value if the pathologist mentions any finding he sees (e.g., marked inflammation or signs of infection) that may explain an elevated PSA. While such findings cannot prove that no cancer is present in an unsampled portion of the prostate, this might indicate benign conditions. In this situation, the urologist might prescribe a course of antibiotics to lower the PSA value before he or she performs another biopsy. Although the PSA level may rise as the result of the biopsy, it should decrease to baseline levels in 4 to 6 weeks.

Many benign conditions mimic the appearance of prostate cancer. The two most common are infection and prostatitis. Your urologist can explain them to you if they appear in the pathologist’s report.

High-grade prostatic intra-epithelial neoplasia (HGPIN), although itself a non-malignant condition, is often a precursor to cancer. A repeat biopsy on men with a previous diagnosis of HGPIN demonstrates cancer in up to half of the cases. Low grade PIN is not important and probably should not even be mentioned in the biopsy report.

If a malignant diagnosis is made, it is imperative that a Gleason grade be assigned. The grade describes how closely the malignant glandular microstructures resemble normal ones, with a lower number being closer to normal and describing a tumor with less potential to spread. The Gleason score is the sum of the two Gleason patterns which the pathologist believes best characterize the tumor. Thus a score of $3 + 4 = 7$ means that the pathologist sees predominantly pattern 3 and a secondary pattern of 4. An accurate assessment of Gleason grades and score is the single most useful factor in predicting the course of the disease and the probable outcome. For this reason, pathologists should provide these numbers. Terms like “low-grade tumor” or

“moderately differentiated adenocarcinoma” are not as reproducible and do not substitute for Gleason grade and score.

The pathologist should indicate the amount of tumor present on each core (measured as percent of core involvement and length in millimeters) as well as the particular location involved (apex, base, transition zone, side of prostate, etc.) since this information may be useful in estimating the total size of the tumor and as one factor in formulas to predict the extent of tumor (staging) found after possible prostatectomy.

Seminal vesicle involvement and extraprostatic extension may occasionally be identified in needle cores. Certainly the pathologist should note the presence of perineural invasion - a worrisome sign for extension of tumor beyond the prostate. Furthermore, a pathologist who has had experience correlating routine slides with formal ploidy studies can often make a good estimate of cellular ploidy (a determination of gross chromosome abnormalities) merely by looking at the routinely-stained glass slide.

If your biopsy report contains the phrase “outside consultation,” the pathologist recognized the case as difficult and sent it to another institution for a second opinion.

Pay particular attention to words such as “atrophy,” “atypical hyperplasia,” “atypia,” or “atypical glands,” all of which indicate that the pathologist may have seen something abnormal in the specimen.

Don't be concerned about mention of “rectal” or “colonic” tissue; such a finding is irrelevant. Small fragments of bowel lining (mucosa) are very common in needle core biopsies since the needle has to punch through this tissue to get to the prostate. The body quickly produces more mucosa to plug up the tiny hole.

Perineural involvement (tumor surrounding a small nerve) is a warning for probable extension of the tumor outside the prostate. It is a significant factor that the pathologist should note in any biopsy specimen containing tumor.

Reports after RP

One of the most important pieces of information to be obtained from the post-operative pathology report is the assessment of surgical margins and of capsular penetration. Organ-confined tumor is within the confines of the anatomical prostatic capsule. Established capsular penetration means that more than a few cells are found outside the normal confines of the prostate.

When the surgeon removes the prostate, he often includes a thin rim of non-prostatic soft tissue that surrounds the prostate. The outer aspect of this soft tissue constitutes the surgical margin. A report of negative margins means that the pathologist found no evidence of cancer at the outer edge of the tissue the

surgeon removed. Conversely, a finding of positive margins means that cancer cells were present outside the capsule.

One may take such information as the presence of capsular penetration and the status of surgical margins and combine it with Gleason score and PSA to estimate whether the cancer was or was not completely removed by surgery. Such a determination may suggest the early use of additional salvage treatments such as radiation and/or hormonal therapy.

If hormonal therapy has preceded the surgery (neoadjuvant therapy) careful examination of the removed tissue will tell if the therapy was successful in stopping the growth of the cancer, or whether hormone-insensitive cancer cells have continued to proliferate. Pathologists should not attempt to establish Gleason grades on such hormonally-treated tissue; the changes resulting from the hormone treatment cause an artificial increase in perceived grade.

Other items of note that may appear on the post-surgical pathology report include seminal vesicle involvement, intravascular involvement, involvement of nerve twigs at the periphery of the gland, size of tumor nodule(s) with calculation of volumes, presence of intraductal features, and the percentage of poorly differentiated tumor (Gleason pattern 4 and above) within the tumor.

Recuts are additional slides, prepared exactly like the original ones, from tissue remaining in the paraffin blocks. They are made either because a pathologist needs to see more tissue “deeper in the block” to confirm or rule-out a malignant diagnosis, or because slides are to be sent to a different pathologist for a second opinion (i.e., an outside consultation).

Pathologists may help you and your care-givers in the interpretation of laboratory tests such as free PSA, PSA velocity and prostatic acid phosphatase (PAP). They may also help to evaluate the ultra-sensitive PSA tests that warn of early tumor recurrence. This information can help you and your urologist to initiate early adjuvant therapy when it is most effective.

Understanding your pathology and laboratory reports will help you become a more active and informed participant in the medical decisions that will affect your future. Your pathologist can help you in this process.

PLOIDY and Chromosomes

Remember that your body is made up of several trillion very tiny cells. The cells have a central nucleus which contains the chromosomes, protoplasm and other structures. The chromosomes carry the 100,000 genes that determine the characteristics of the person. There are various numbers of chromosomes in the cells of different plants and animals. In the human, there are 23 pairs. These pairs of chromosomes are called diploid, which simply means twofold. Diploid means that the cells have two sets of homologous

chromosomes. Homologous means that they are similar, such as your two hands are similar.

Some cancer cells do not have the characteristic pairs of chromosomes. Some of them may be aneuploid, (the prefix an- means without or not, eu means good), which means that the cells are not good and do not have the normal pairs of chromosomes. A single cancerous tumor may have several different ploidy types of cells. Ploidy tests can be done on biopsied material from a tumor to determine the ploidy. Those tumors that have a high percentage of aneuploidy usually have a poor prognosis. (Prognosis is Greek meaning foreknowledge. It usually means a prediction of an outcome of a disease).

Here is a question about how to interpret a ploidy report that was answered by Ralph Valle, an expert in all things regarding prostate cancer:

Question- I just got a copy of my ploidy report. Anyone know how to interpret this:

DNA Index, Main Peak	1.56
Cell Count	54
% Diploid	29.64
% Aneuploid	53.70
% Tetraploid	16.6
C.V. of important peak	11.78
Reference Range of DNA Index	
Diploid Cells:	0.9-1.1
Aneuploid Cells:	1.1-1.8 & over 2.2
Tetraploid Cells:	1.8-2.2

Answer - Your main peak at 1.56 is in the aneuploid DNA index range. The diploid cell count was 29.64% and the non-diploid cells were 70.36% of the cells counted. The locations (indices) of the peaks refer to the quantity of DNA in a cell. The reference range is 0.9 through 1.1 for diploid cells. Cells ready to divide have double the amount of DNA, so that peak is at 1.8 through 2.2. (Typically up to 15% of cells are in the tetraploid state, ready to divide.)

Aneuploid is anything outside those ranges. There will always be some level of aneuploidy because there are cells in the process of preparing for cell division. However, if you have a peak at some other location, it means you have a cell population that is reproducing with an abnormal amount of DNA, or an abnormal set of chromosomes. This is characteristic of an aggressive cancer. The cells causing this peak are sometimes called a "cell line".

Both flow cytometry and static image analysis have been used to determine DNA content (ploidy) of CaP. DNA studies have shown that, as a group, patients with diploid cancers have longer disease free intervals and survival times than those with non-diploid tumors. However, they may not be so helpful in predicting stage

for an individual patient. Approximately 30% of all organ-confined tumors are non-diploid and almost half of the tumors with region lymph node metastases are diploid (Epstein 1992).

Because diploid tumors are more responsive to hormonal therapy (Zinke, 1992), the Prostate Cancer Working Group (sponsored by the College of American Pathologists) has found that DNA ploidy studies are useful in patients with T3 or node-positive disease. The Working Group does not recommend DNA analysis in T1, T2, or node-negative disease because of conflicting results in the literature (Grignon 1995).

Studies demonstrating the utility of DNA ploidy status have been performed on whole prostates obtained after RP. Since CaP is often a heterogeneous, (heterogeneous means different grades of cancer cells), and multifocal disease, it doesn't necessarily follow that a tiny piece of tissue obtained on needle biopsy is representative of the lesion as a whole. Two reports have confirmed that ploidy status differs in different parts of an involved prostate (Greene, 1991; O'Malley, 1993).

This intratumor variation in DNA ploidy suggests that multiple site sampling (possibly by fine needle aspiration) may be necessary to obtain accurate DNA measurements. Thus it may be misleading to assume that a tumor is entirely diploid when only a small fraction of it is sampled. Nevertheless, three studies have shown correlation between ploidy on needle biopsy and subsequent RP material (Leung, 1994; Takai 1994, Ross 1994).

The lack of mutually accepted standards limits the usefulness of ploidy analysis. For example, the percentage of non-diploid cells that are necessary to call the test results "non-diploid" varies from study to study (Epstein 1992). Another glitch is that cells normally found in the seminal vesicle are normally tetraploid and will yield a false positive unless these cells are first recognized as non-cancerous (Hardt, 1994).

Both flow cytometry and image analysis techniques suffer from limitations. Flow cannot be performed when only a small amount of tumor is present on needle biopsy. Neither can it distinguish tumor from non-tumor cells so a small number of non-diploid cells may be diluted to insignificance by larger numbers of benign diploid cells. This may explain why flow is less sensitive than image analysis to non-diploid cell populations (Pindur 1994).

Static image analysis allows determination of ploidy in tissue sections with relatively small amounts of tumor, but the interpretation of results is still hampered by the lack of standard methodologies (Grignon, 1995; Hardt, 1994; Falkmer, 1992). This problem will likely resolve with increased use of image analysis; it is the favored technique of DNA analysis within large commercial labs.

Should DNA ploidy be determined from needle biopsy or RP tissue in order to determine prognosis? Two studies using RP material have shown that while DNA ploidy analysis can be used to predict stage, it does not add any additional information to that provided by grade (Epstein 1992, Dejter, 1989). A more recent paper using needle biopsies (Ross 1994) came to the opposite conclusion; ploidy analysis was more important than grade. This last study showed a ten-fold increase in risk for metastasis and a three-fold risk for extracapsular spread if the initial needle biopsy demonstrated non-diploidy. These results have not been duplicated.

In conclusion, it appears premature to place too much emphasis on DNA ploidy analysis. While groups of diploid patients have better prognoses than groups of non-diploid patients, ploidy status may have uncertain prognostic value in individual patients. A small biopsy demonstrating diploid tumor may be missing a significant underlying non-diploid component. Published studies have reached different conclusions concerning prognostic value. Technical standards and methodologies differ from lab to lab. Correlation with histologic criteria remains essential.

Additional tests such as ploidy analysis, which measures the chromosomal content of malignant cells, may also be performed on prostate tissue. This test may be ordered either at the time of the original diagnosis or afterwards from tissue remaining in the saved paraffin blocks. Ploidy analysis on prostatectomy specimens has been shown to be an important prognostic factor in predicting the spread of tumor outside the prostate and thus the chance of recurrence after surgery. This test may also help to predict the responsiveness of the tumor to hormonal treatment. An experienced pathologist can often estimate the ploidy by examining the stained slide, thereby saving the cost of formal DNA ploidy analysis.

Pathologists

Here is Dr. Oppenheimer's phone number and the phone numbers of a few other pathologists:

Jon Oppenheimer (Tennessee) [888] 868-7522 <http://www.ourlab.net>

David Bostwick (Virginia) [800] 214 6628

Jon Epstein (Hopkins) [410] 955-2405

John McNeal (Stanford) [650] 725-5534

Gary Miller (Colorado) (303) 315-5408

Dianon Laboratories 1 [800] 328-2666

UroCor, Inc. 1 [800] 411-1839