1
Clinical Pathology

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1.1
Introduction

Pathologists are referred to sometimes ironically as *doctors of dead persons*. This has roots in the common idea that pathologists are only responsible for doing autopsies and providing clues for the possible cause(s) of death. What accentuate such misconceptions are the equipment and methods of pathologic examinations. A pathologist never uses the usual tools of ordinary physical examinations such as stethoscopes or sphygmomanometers. He or she has also no direct encounter with patients. There is very little similarity between the image that people have in mind of a physician and of a pathologist.

In this chapter, we try to provide a realistic image about the territories of working of a pathologist. We begin this chapter with a brief definition of the science of pathology and the history of contemporary surgical pathology. Then the reader can find general information about the frequent types of specimens that are handled by pathologists as well as the ordinary diagnostic methods that are applied by them for making an accurate diagnosis. This section is followed by a brief review of the ancillary and more sophisticated diagnostic methods in the field of pathology. In the next section, we will introduce a list of basic definitions that are used frequently by pathologists to describe specifically the different groups of pathologic processes. Finally, we provide some examples of the limitations in the field of diagnostic pathology.

1.2
Pathology as a Medical and Research Discipline

In the study of medicine, pathology functions as a bridge between basic and applied medical sciences and in this way it plays a very substantial role not only in the understanding of the pathophysiologic basis of diseases but also in translating it into the practical management of patients and disease samples.

Ex-vivo and In-vivo Optical Molecular Pathology, First Edition. Edited by Jürgen Popp.

There are two basic schools of thought about the practice of pathology. In most European countries, a pathologist deals principally with microscopic evaluation of tissue specimens (small biopsy samples as well as large resection specimens) and cytological material. As an adjunct to this histologic and cytological evaluation, a pathologist uses some ancillary methods (such as immunohistochemistry (IHC) or molecular and genetic examinations) for more accurate diagnosis, classification, and prognostication of diseases. In the United States, a pathologist is, in addition, responsible for all laboratory investigations that are elsewhere covered by disciplines such as microbiology and laboratory medicine. These analyses are carried out on body fluids (blood, serous fluids, urine, feces, etc.), secretions of organs (exocrine secretions of pancreas), or other materials that are taken out from or expectorated by a patient (sputum, coverings of skin ulcers, etc.). They cover a broad spectrum of diagnostic methods apart from microscopy, including microbiologic, serologic, biochemical, and microscopic examinations. In this chapter, wherever we use the term *pathology*, it refers mainly to the macroscopic and microscopic evaluation as well as molecular assessments of tissue samples.

1.3 Historical Perspectives

The microscopic analysis of cells and tissue (e.g., cytology and histology) appeared for the first time in the nineteenth century as an important method for research and diagnosis in the field of medicine. Generally, Xavier Bichat is considered in most publications as the founder of pathology. The branch of histopathology appeared some years later, with Müller publishing a book on the structural characteristics of cancer cells and their growth. Virchow, a student of Müller, introduced the important correlations between cells, which are the smallest units of vital organisms and tissues, disease states, and related disease mechanisms. He became famous worldwide for his cellular pathology studies and his claim that every disease originates from diseased cells or, according to him, ''Omnis cellula e cellula.'' This statement is valid also today in the era of molecular pathology. The introduction of more innovative techniques, such as the microtome in the year 1839, enabled the pathologist to have better and thinner sections from tissues and had a great influence on the development of pathology. Gradually, the application of frozen section examination found its place in the routine practice of pathology for rapid as well as intraoperative evaluation of the suspected tissues. Another important development was the invention of standard hematoxylin and eosin (H&E) staining in the year 1875. The Carl Zeiss Company developed the first fluorescence microscope in the year 1965 in Göttingen. During the 1980s, the immunohistochemical analysis of tissues developed rapidly, which even today continues to be an invaluable diagnostic tool in pathology laboratories around the world.

Cytopathology is one of the important branches of pathology. By this method, it is possible to analyze all body fluids for the presence of tumor cells and evidences

of inflammatory changes. In the middle of the nineteenth century, Virchow introduced the cell as the basic functional element of the body and hence the basic element in the development of diseases. This way he deserves to be considered as the founder of cytopathology. But the development of cytopathology as a diagnostic tool took, in fact, more time. The first important development took place under the influence of the Greek physician Papanicolaou. He introduced in the year 1928 a method of staining cytology smears, which was named after him as the Pap test. His knowledge and works had a great influence on the routine performance of gynecologic cytology and led to a considerable reduction in mortality due to uterine cervical carcinoma by early diagnosis.

1.4 Specimens

1.4.1 Biopsies, Resections, and Cytology

One of the main duties of a pathologist is to provide the clinicians with a precise tissue-based diagnosis, particularly in cases with a complicated disease process or in situations in which there are uncertainties with the clinical diagnosis. In these situations, the pathologists receive a small biopsy sample from a relatively large lesion or organ. Most of the time the questions asked are as follows:

- Is there any pathologic change in this specimen?
- If yes, is it a preneoplastic, neoplastic, or non-neoplastic lesion?
- If it is a (pre)neoplastic lesion, is there any sign of dysplasia or malignancy?
- If yes, which type of tumor is it? Is it invasive or noninvasive? What is the grade of the tumor?
- If it is a non-neoplastic lesion, which type of disease process can it be? Is it an inflammatory process? Is it an infectious disease? If yes, is there any sign of the responsible infectious agent? If no, which type of inflammatory reaction can it be?

The notable improvement of endoscopic devices and imaging techniques has enabled physicians to gain access to the mucosal coverings of most internal organs and to take samples from them. Accordingly, pathologists encounter these days more frequently small biopsy samples. The most frequent areas of endoscopic samplings are mucosal coverings of the upper and lower intestinal tracts, respiratory tract, acoustic sinuses, female genital tract, urinary tract, and joint spaces.

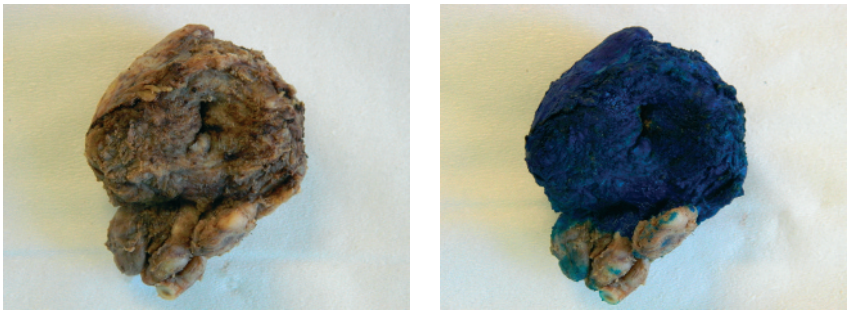
The same set of questions can be answered by pathologists using other types of specimens that are obtained for cytologic examinations. The fluid accumulations in serosal spaces (pleura and abdominal spaces), secretions of some organs (nipple discharge), expectorated sputum, and voided urine can contain single as well as small aggregates of detached epithelial cells or suspended inflammatory cells, whose morphologic evaluation can serve as a basis for diagnosis. After collection,

these fluids are centrifuged. The supernatant fluid, which is usually cell-poor or near completely acellular, can be used for chemical or serologic laboratory examinations. By preparing a direct smear, staining, and microscopically evaluating the cell-rich sediment, a pathologist or cytopathologist can provide an appreciable amount of diagnostic information. It is also possible to prepare a cell block from the sediment and to examine their sections microscopically. Other alternative methods to obtain specimens for cytologic examinations are brushing and washing of the mucosal (respiratory tract, esophagus) and serosal surfaces (washing cytology of Douglas pouch) or extracting fine tissue particles by aspiration using a narrow (fine pore size) needle. Fine needle aspiration (FNA) is a rapid and relatively noninvasive method of sampling, particularly when the target organ is superficial or palpable (thyroid, breast). With the guidance of sonography or computerized tomography (CT), FNA or fine needle biopsy (FNB) can also be used safely to obtain material from more deeply located organs such as pancreas, mediastinal structures, lungs, and liver.

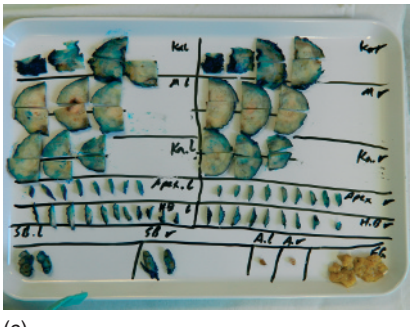
Alternatively, pathologists receive large specimens, for example, resections, which can be different in size and extent from a part of an organ to complete removal of one or many organs together as well as limb amputations. Not infrequently, the reason for such an extensive operation is tissue necrosis and gangrene due to problems of blood supply (ischemia). But most of the time, such a large resection is performed for the complete removal of a malignant tumor as in curative surgery or for the reduction of the size of a tumor as in palliative surgery. Particularly in the case of curative surgeries, a pathologist should thoroughly examine the specimen at both the macroscopic and microscopic levels. The frequently asked questions about such specimens relate to the reconfirmation of diagnosis, grading of the tumor (i.e., degree of malignancy), the extent of tumor infiltration, and the evaluation of resection margins (i.e., if they are tumor-free or affected by the tumor). There are many different recommendations and guidelines for standardization of sampling and for reporting tumor resections (Figure 1.1).

1.5 Conventional Diagnostic Methods in Pathology

After taking a tissue sample from a patient by any of the above-mentioned methods, it is necessary to fix it. Fixation is a way of treating a tissue using specific kinds of chemicals, usually in the form of fluids. The process of tissue decay and organ destruction begins as soon as the tissue is detached from the body and has lost its source of blood supply. It is a self-destruction and autolytic process that can continue up to the complete destruction of the sample. In the case of inappropriate and untimely fixation, the tissue consistency will be lost and it will not be possible to examine the tissue at both the macroscopic and microscopic levels. In some cases, it is very important that the pathologist provides the clinicians with some information about the characteristics and composition of the constituting cells at the molecular level. Such molecular evaluations are exceedingly difficult if not



(a) (b)



(c)

Figure 1.1 A radical prostatectomy specimen with both seminal vesicles (a). The outer surface of prostate (resection margin) is marked with blue ink. Such an approach makes the decision about the presence of tumor infiltration at resection margins easier (b). The specimen is completely sectioned in a systematic fashion in small pieces. All tissue fragments are processed and examined microscopically (c).

impossible to carry out on improperly fixed samples. The most universally used fixative solution in most of the pathology laboratories around the world is buffered 4% formalin solution.

There are many other fixatives that can be used in specific situations. Most of them suffer from one or more drawbacks such as high costs, problems with disposal, need for specific methods of tissue processing after fixation, too long a fixation time, and effects on the results of immunohistochemical or molecular examinations.

1.5.1 Cytology

As described above, the specimens that are received for cytologic examinations are usually in the form of an aspirated, expectorated, or washed fluid. One or more smears are usually prepared from the sediment of a centrifuged fluid. Depending on the desired staining method, these smears can be fixed by chemicals or are air-dried. The rest of the sediment can be processed similarly as for a tissue sample

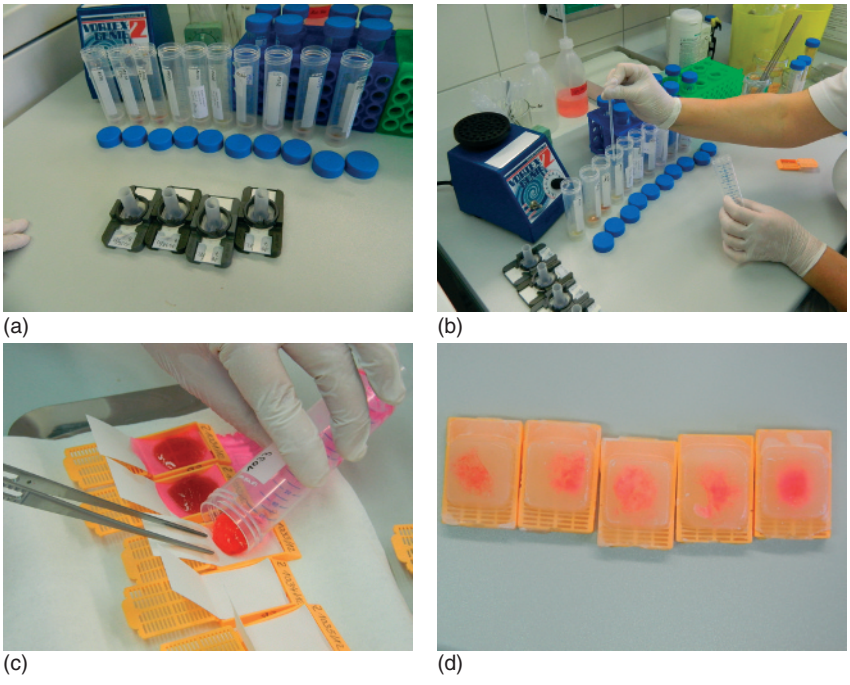


Figure 1.2 Preparation of cell block from liquid samples. The fluid is centrifuged. A part of the sediment is used for the preparation of cytologic smears by cytocentrifugation (a). The remaining sediment is coagulated by adding plasma and thrombin (b). The coagulated sediment is drained into a plastic basket and processed as a tissue fragment in a tissue processor (c). The paraffin blocks that were prepared from a liquid sample are ready for sectioning by a microtome (d).

by transferring the cells into a network of protein material, for example, protein glycerin or plasma, followed by coagulation with thrombin. The cells are then fixed in formalin followed by paraffin embedding just like a tissue sample. If these cell blocks contain a sufficient number of cells, they serve as a very helpful reserve for further examinations such as immunohistochemical or sometimes molecular genetic tests (Figure 1.2).

1.5.2 Histology

Immediately after submission to a pathology laboratory, every tissue sample is given a numerical code. Different methods of labeling, such as bar coding, can be used for coding the samples.

The process of tissue examination by a pathologist begins by naked eye examination. A lot of information can be obtained after a careful macroscopic tissue examination or grossing. For small biopsy samples, these pieces of information are usually limited to the dimensions, as well as the number, color, and amount of the

sample. It provides basic information regarding the adequacy of the specimen for further evaluations. In the case of some specific types of specimens, it is the duty of the pathologist to examine the small specimens by a hand lens or by a low-power microscope before subjecting it to complete formalin fixation and ordinary tissue processing. The best example is the renal needle biopsy. By low-power microscopic examination, the pathologist can tell the clinician whether he or she was successful in obtaining an adequate amount of renal tissue. On the other hand, the pathologist may need to divide the sample appropriately into three portions. Each portion is then handled differently for different methods of examination, that is, fresh tissue for immunofluorescent examination, fixation in glutaraldehyde for electron microscopy, and fixation in buffered formalin for conventional tissue microscopy and specific chemical staining. The last option represents the standard procedure that is applicable in all cases.

The most important role of grossing is in the evaluation of large resection samples. It is evident that microscopic evaluation of a whole resection sample, for example, the complete removal of an organ or extremity, is neither possible nor necessary. There are specific guidelines from which a pathologist can obtain information on how a resection specimen should be sampled and examined for microscopy. In most cases, these resection samples are those that contain a malignant tumor. In this situation, the clinicians might want to know the extent of the tumor and the completeness of its removal. The macroscopic examination defines the exact location, size, shape, and configuration of the tumor, the depth of local invasion (in tumors of luminal structures such as intestinal tract), the relationship with adjacent normal tissue, and the distance from surgical resection margins. It is also necessary to look for lymph nodes to examine them for possible metastatic foci. According to the guidelines, a pathologist takes small tissue fragments from the tumor, resection margins, and lymph nodes, which should not be less than a minimum recommended number. In some types of specimens, for example, radical prostatectomy specimens, it is recommended to completely embed the specimen in thin sections. To maintain the orientation during the microscopic examination, it is sometimes necessary to paint the specific areas such as resection margins by the different colors of specific dyes.

The prepared tissue slices are then placed in a plastic cassette. On this cassette, the code number of the specimen and if necessary the specific code of the area of sampling are written or typed. Now the tissue slices are ready to be processed. Tissue processing is a vital step for preparing the tissue slices for microscopic examinations. This task is performed automatically by a ''tissue processor.'' The device consists of vessels containing specific chemical compounds (mainly alcohol and xylene) at a previously determined and graded concentration. The processing of tissue is enhanced and accelerated in new-generation tissue processors by the application of microwave energy or vacuum. The tissue processing ends with embedding the tissue in a paraffin block. Now the tissue is ready to be cut to obtain thin slices for microscopic examinations. Using specific sharp blades and a precisely designed device, it is possible to cut the paraffin blocks into very thin sections (preferably 3–5 μm in thickness). The sections are placed on a glass slide,

stained, and finally cover by a cover slip. They are now ready for microscopic examination by a pathologist.

1.5.3 Microscopy

A physician collects the necessary information by examining a patient and observing the signs and symptoms of the disease. Then he or she makes a list of differential diagnoses and tries to reduce the size of this table by the application of specific laboratory tests. The final target is to reach an accurate diagnosis. Pathology as a practice has similar components. By careful examination of the microscopic changes on a slide, a pathologist tries to gather specific morphologic signs and symptoms (in this situation the key morphologic findings) in order to have a list of differential diagnoses. The basic forms of pathologic changes (with few exceptions) more and less resemble each other in the different organs and body tissues. For example, an acute or a chronic inflammatory reaction is accompanied almost always by a predominantly neutrophilic or lympho-plasmacytic inflammatory cell infiltration, respectively. The basic microscopic examinations are performed almost always in the first step on H&E stained slides. By using two different acidic (eosin) and basic (hematoxylin) stains, the basophilic components of the cell structure (mainly RNA and DNA) gain a deep blue color and the acidophilic components (cell cytoplasm and interstitial stromal materials) gain a pale to deep pink appearance. Some specific cell components or specific cell types can be amphophilic (neither eosinophilic nor basophilic). Although the basic structure of the tissue and basic forms of pathologic processes are in most cases easily appreciable during this primary microscopic evaluation, it is sometimes necessary to stain new tissue sections to answer specific questions. Some examples of chemical-specific tissue stains are as follows:

- *Periodic acid-Schiff* (*PAS*): Using this stain, we can see a better reaction of chemicals or structures with a high content of carbohydrates or glycoproteins. It shows better intracytoplasmic or interstitial accumulations of mucinous secretions (for example, in mucin-secreting adenocarcinomas). Some specific forms of microorganisms, for example, fungi, are better recognizable by this type of staining. A pathologist can find more easily the megakaryocytes in a closely packed and hypercellular bone marrow tissue. It is also a very good staining of the basement membrane in different epithelial coverings, and its application plays a crucial role in the microscopic investigation of glomerular diseases in the field of renal pathology (Figure 1.3).
- *Giemsa*: It is one of the basic special stains and is regularly used in the microscopic examination of lymphoid and hematopoietic tissues (lymph node or bone marrow biopsies). In addition to providing better nuclear morphology, application of this staining is a single histologic method for the evaluation of tissue infiltration by mast cells. Most of the microorganisms, particularly bacteria, are better recognizable by this method of staining. A modified Giemsa staining is routinely used in gastric mucosa biopsies for the evaluation of *Helicobacter pylori* infection.

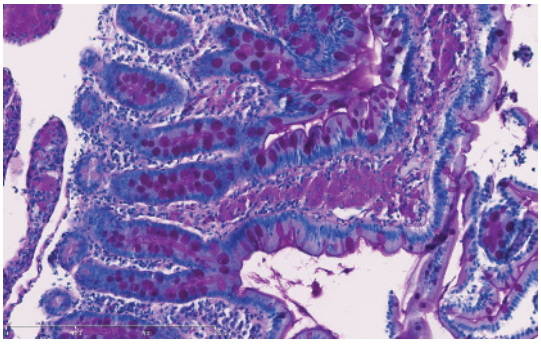


Figure 1.3 Collection of macrophages with deeply PAS-positive cytoplasm in the lamina propria of a duodenum mucosa biopsy is a characteristic feature of Whipple's disease.

- *Masson trichrome*: It provides a better evaluation of the extent and severity of tissue fibrosis. In liver pathology, its routine application is one of the bases of diagnosis of advanced liver fibrosis or liver cirrhosis. Its application in medical renal biopsies as adjunct to other specific chemical stains (such as Jones staining) is extremely useful in judging the presence of fibrinoid necrosis, glomerulosclerosis, and abnormal depositions in the mesangial spaces and basement membrane.
- *Iron staining*: Iron depositions in tissue are recognized in routine H&E staining as coarse dark-brown crystalloid materials. Although an experienced pathologist can recognize iron deposition by noticing the background histologic features and morphologic characteristics, in the liver tissue, for instance, it can be often mistaken for the intracellular bilirubin (a product of hepatocytes) or lipofuscin (a final metabolite of fat in senescent or hypoxic injured cells). In one of the specific iron staining methods (Prussian blue), the iron crystals gain a deep blue stain, while the other two remain unstained. The same staining can help a pathologist to differentiate between iron-laden intra-alveolar macrophages (i.e., heart failure cells) from pigment-laden macrophages with ingested coal particles. The estimation of iron stores in bone marrow specimens is important to differentiate pathologic situations with increased iron stores (for example, myelodysplastic syndromes, sideroblastic anemia, and anemia of chronic disease) from situations with low iron stores (such as iron deficiency or chronic hemorrhagic anemia) (Figure 1.4).

1.5.4 Intraoperative Assessment (Frozen Section Examination)

Preparation of formalin-fixed paraffin-embedded (FFPE) tissues is the usual method of tissue processing before microscopic examination, but it is not the only one. In some instances, the pathologists receive the sample in a fresh state (without any fixative or other chemical additives). The interested part(s) of the sample can be embedded in special media and rapidly frozen by immersing the sample in liquid

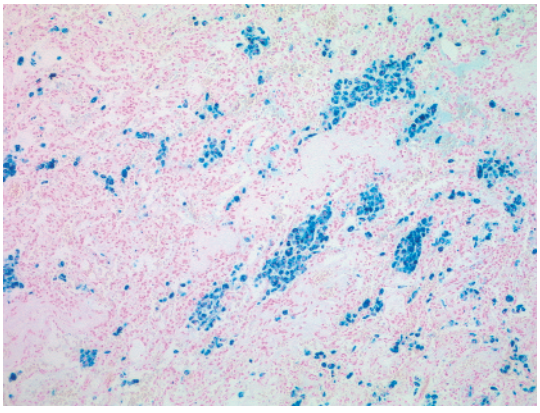


Figure 1.4 Iron staining of a lung tissue shows large number of intra-alveolar iron-containing macrophages (blue stained cells). This is an indication of recurrent and chronic intra-alveolar hemorrhage due to blood congestion. Such a situation happens, for example, in patients suffering from heart failure. Accordingly, these cells are called *heart failure cells*.

nitrogen. Using a microtome mounted inside a freezer (cryocut microtome), thin tissue sections are prepared and stained. Such type of examination is called *frozen section examination* and is usually performed for the following purposes:

- *Intraoperative consultation*: To provide surgeons with an accurate diagnosis or as accurately as possible, the impression about the nature of a pathologic change to avoid a second surgical intervention and reducing the risks of reoperation. In this way, the pathologists are usually asked about the biologic (benign or malignant) behavior of the sampled tissue. This method of diagnosis was more frequent in earlier years. Nowadays, it is performed less frequently because of improvements in preoperative diagnostic methods and nonoperative invasive sampling techniques. Even in cases with confirmed diagnosis of cancer, a pathologist can be asked to determine the extent of a tumor or its grade. These types of information can influence the extent and method of surgery. Another application of frozen section examination during a surgery of a malignant neoplastic process is the evaluation of surgical margins.
- *Adequacy of sampling*: Even when the pathologist is not specifically asked by a surgeon to provide an accurate intraoperative diagnosis, by using this method the former can assess the adequacy of sampling for further examinations.
- *Molecular testing*: Although some specific types of tissue fixative solutions have been shown to protect the tissue structures, fixation with most of the commercially available and routinely used fixatives can hamper molecular testing. Well known is the masking of many antigenic epitopes and false negative immunohistochemical results in FFPE tissues. On the other hand, some sensitive molecules such as RNA can be damaged (partially or completely) during chemical fixation. Particularly for research purposes, there is a trend to archive a small, rapidly frozen tissue fragment (Figure 1.5).

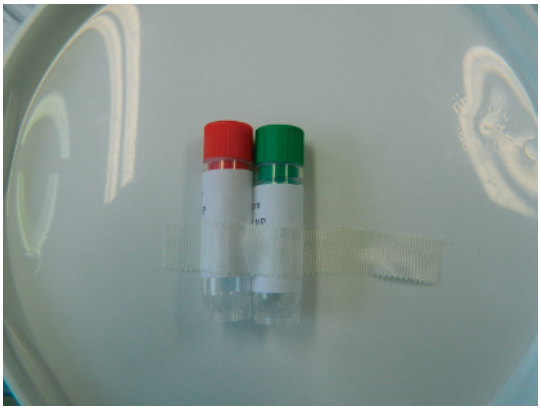


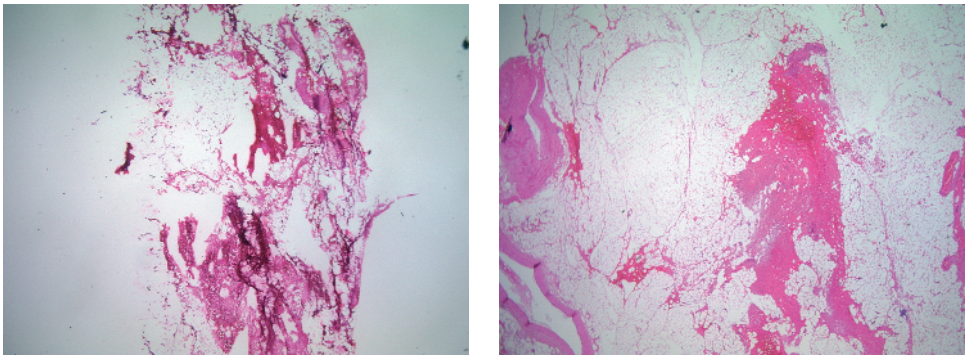
Figure 1.5 Two small vessels with patient identification are attached to the tissue container. The specimen has been sent in fresh state (without fixative solution). In patients with a macroscopically identifiable tumor, small fractions of tumor and nontumoral tissue are sampled, transferred into red-top and green-top vessels, respectively, and kept in a −70 °C freezer for further research or diagnostic molecular examinations.

- *Specific chemical staining*: Some basic chemical components of the tissue structure, such as fat, can easily dissolve in the chemicals that are used for tissue processing. For qualitative and semiquantitative assessment of these materials, there is also a need for fresh frozen samples. A good example is the confirmation of fatty deposition in some genetic metabolic storage diseases to differentiate them from other intracellular accumulations, such as watery changes.

1.5.5 Why Pathologists Do Not Like Frozen Tissue Examination?

For many reasons, the assessment of a frozen tissue during a surgery is not a favored way of tissue examination for most pathologists. Some of the reasons are as follows:

- An intraoperative consultation is an emergency situation. That means that, during the preparation of sections and microscopic examination for an intraoperative consultation, all the normal working activities have to be stopped. This takes a relatively long time, which affects the daily practice.
- The quality of tissue sections that are prepared during an intraoperative consultation is usually not comparable with that of the slides that are processed in the normal way by FFPE. Seeing such slides is sometimes similar to seeing through fogged glasses. Taking decisions that have a great influence on the patients' situation by examining frozen sections is a very difficult and sometimes risky task. A pathologist can defer his diagnosis until the examination of permanent sections, but such a delay is not acceptable for many clinicians and surgeons.



(a) (b)

Figure 1.6 Frozen section of a tissue fragment rich in adipose tissue (a). Compare the quality of this section with the section from the same sample that is prepared after formalin fixation and paraffin embedding (b).

- Some tissue samples, particularly those rich in fat, are difficult to cut after freezing. Preparing an appropriate interpretable section from these samples is not a simple task (Figure 1.6).
- The results of frozen sections examination can be partially or sometimes totally different from those of tissue examination on permanent paraffin-embedded blocks. This can have legal as well as moral consequences.

1.5.6 Microdissection

The purpose of microdissection is to provide a group of cells consisting as pure as possible of target cells for a specific examination. This method is particularly important for some diagnostic molecular assessments. For example, during the assessment of microsatellite instability in colon carcinoma, it is necessary to compare the length of microsatellite DNA polymorphism in normal and tumoral tissues. For this reason, these two cell elements have to be examined separately. The simplest way for microdissection is to look for normal and neoplastic cell components on a routinely stained H&E slide and marking the area occupied by neoplasm on the slide. This area can then be extrapolated on a thicker and unstained section from the paraffin block. The demarcated area is dissected using a sharp knife, suspended in specific solutions, and then examined by a desired method (for example, by polymerase chain reaction (PCR)).

For more accurate assessments and especially for research purposes, it is also possible to use more accurate methods of microdissection that will let the scientists even to cut a single or a small and purified group of desired cells. One of these methods is laser capture microdissection.

1.6 Nonconventional (Ancillary) Diagnostic Methods in Pathology (Molecular Assessment of Tissues)

The molecular methods of tissue assessment can be divided into two broad groups. The first group of assessments is performed on tissue sections and provides information that is interpretable in conjunction with the location of the reaction. The results of these methods provide a color signal that can be seen under ordinary light microscopes (i.e., IHC) or by specifically designed microscopes such as fluorescence microscopes (for example, fluorescent *in situ* hybridization or FISH). The examiner can judge whether the reaction is mainly in the tumoral or normal cells. He or she can also evaluate the reaction in relation to the location of the reaction in different parts of the cell structure, for example, nucleus, cytoplasm, or cell membrane. These groups of tests are accordingly categorized as *in situ* methods.

In the second large group of molecular testing, the subject of examination is an extracted and usually amplified sequence of the DNA of tumoral or normal cells. As in these methods there is no possibility for assessing the relation of results with morphologic parameters, they are categorized as non-*in situ* methods.

1.6.1 *In Situ* Reactions

1.6.1.1 Immunofluorescence (IF)

It is a simple, fast, and usually cheap method of *in situ* molecular assessment. Briefly, a microscopic section of a frozen tissue is exposed to an antibody. In case the tissue section contains the material of interest, a specific antigen–antibody reaction ensues. Any excess unbound antibody is washed away. In the next step, the tissue section is exposed to a secondary antibody which is conjugated with a fluorescence material. A specific antigen–antibody reaction is detected by looking at the slides under a fluorescent light. By using two different fluorophores, it is possible to carry out simultaneously two different immunofluorescence (IF) examinations on a single slide. There are some drawbacks for IF. Before describing these drawbacks, it is worth pointing out that, despite the following problems, IF has kept its role in the assessment of abnormal glomerular depositions in the field of nephropathology:

- The results of IF examinations on FFPE tissues are usually unsatisfactory. It should be carried out on fresh frozen tissues. As in the normal workflow of a pathology laboratory, most of the samples are FFPE, they cannot be used appropriately for IF examinations.
- IF needs a specific type of microscope and a dark room for interpretation.
- By using IF, it is only possible to see a shadow of the background tissue. In fact, IF cannot be precisely categorized as an *in situ* method.

- Because of the short half-life of fluorophores, it is not possible to see the reactions after few days. This hampers the archiving of the IF-stained slides and assessment of reproducibility of IF examinations.

1.6.1.2 Immunohistochemistry (IHC)

Principally, the basis of IHC is very similar to that of IF. In IHC examination, instead of conjugation with a fluorescent material, the secondary antibody is conjugated with an enzyme. This enzyme changes a chromogene to a chromatic substance, and produces a color signal wherever a specific antigen–antibody reaction takes place on the slide. This means that the final reaction can be seen under an ordinary light microscope as a brown or red color change, depending on the type of chromogenic material and conjugated enzyme. During the past three decades, IHC has gradually become an integral part of routine diagnostic pathology as well as the basis for most researches in the field of biology of neoplastic and non-neoplastic disease processes. In routine practice of clinical pathology, IHC examinations are used to confirm a line of differentiation in undifferentiated tumors (e.g., lymphoma vs small-cell carcinoma vs sarcoma in the so-called small blue round cell tumor), the original tissue of a metastatic carcinoma in metastases of undefined origin (or the so-called carcinoma of undefined primary (CUP)), classification of lymphomas (e.g., B-cell vs T-cell lymphomas as well as subclassification of B- and T-cell lymphomas), assessment of tumor prognostic factors (e.g., hormone receptors in breast carcinoma), and assessment of tumor predictive factors (e.g., Her-2-neu expression in breast and gastric carcinomas) (Figure 1.7). In comparison with IF, IHC has many advantages:

- Most IHC reactions are satisfactory enough to be performed on FFPE tissues. This means they can be carried out on normally processed tissue sections. On the other hand, it can be applied on archived paraffin blocks, irrespective of the age of the blocks.
- The IHC-stained slides can be archived and reassessed any time, because the color signals of IHC reactions do not fade rapidly.
- The IHC reactions can be seen and assessed under normal ordinary bright-field microscopes.
- Using counterstaining for cell nuclei, it is possible for pathologists to localize the reactions (normal vs neoplastic cells; membranous vs cytoplasmic vs nuclear).
- It can be performed by automated IHC stainers.

1.6.1.3 Fluorescent *In Situ* Hybridization (FISH)

The basis of FISH is the attachment of a fluorescent labeled array of nucleotides (a probe) to its complementary genetic structure of a normal or an abnormal gene. After washing the excess unbound probes, any microscopically detectable fluorescent signal evidences the presence of the investigated target gene or specific arrangement of nucleotides. The main application of FISH in daily practice is the assessment of gene amplification (e.g., Her-2-gene amplification in case of questionable Her2 IHC results). With some modifications, FISH has been used successfully for the assessment of some frequent forms of translocations or

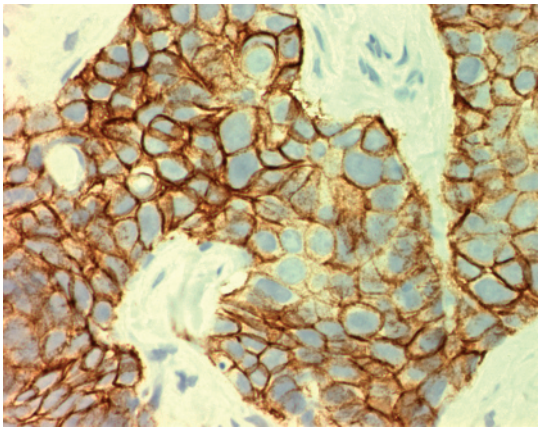


Figure 1.7 Circumferential, continuous, and strong immunohistochemical membranous reaction against Her-2-neu in an invasive ductal carcinoma of the breast. It serves as a predictive factor and determines the likelihood of effectiveness of anti-growth factor receptor immunotherapy.

fusions in specific types of tumors (Figure 1.8) (e.g., BCR-ABL/t(9;22)(q34;q11) in chronic myelogenous leukemia (CML) or IgH-gene translocations in follicular lymphoma). Another modification of this method is the labeling of the probes with chromogenic substances similar to IHC detection systems, which is called *chromogene in situ hybridization* (*CISH*), or labeling with silver dyes (*silver in situ hybridization* or *SISH*). The main advantages of these two methods are the possibility of interpretation of the results by a conventional bright-field light microscope and the possibility to assess the location of the reaction (i.e., tumoral vs nontumoral tissue).

1.6.2 Non-*In Situ* Methods

In many instances, the pathologist might want to know the presence or absence (or sometimes the quantity) of a specific chemical compound or gene in the assessed tissue regardless of the localization of that compound. During the past decades, several methods have been developed that enable the pathologist to carry out such measurements even on small biopsy samples. The list of such methods is already long and still growing rapidly. One of the breakthroughs in such molecular assessments was the introduction of PCR. This method was first introduced by Mullis, an American biochemist, and has found rapid and widespread applications in different fields of science, particularly diagnostic and research medicine. The inventor was awarded the Nobel Prize in the year 1993. The basis of this method is cycling thermal melting and enzymatic replication of DNA. By using small known DNA sequences (primers), it is possible to amplify the target gene. The number of amplified genes reaches a sufficient level after a few cycles,

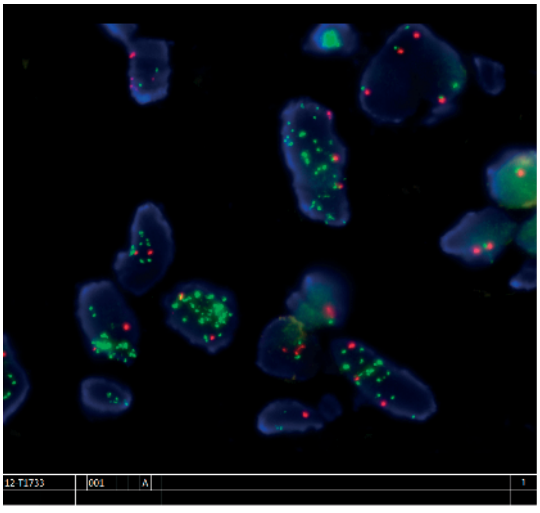


Figure 1.8 FISH analysis of a soft tissue tumor with evident amplification of MDM2-gene (green signals) in comparison with the centromere of chromosome 12 (red signals).

allowing qualitative or (semi)quantitative measurements. Other than in research activities, the most frequent applications of PCR in daily pathology practice are the following:

- Detection of the presence and subclassification of infecting microorganisms (viral particles, fungal elements, mycobacterial infections, etc.).
- Detection of mutations (e.g., KRAS mutation in metastatic colorectal carcinomas, platelet-derived growth factor receptor (PDGFR) mutations in lung adenocarcinomas, and c-kit mutations in gastrointestinal stromal tumors (GISTs)).
- Assessment of the monoclonality of B- or T-lymphocyte proliferations in cases of suspected B- or T-cell lymphomas.

The other more advanced technologies are based partly on the PCR and gene amplification (gene sequencing). Some other methods, such as gene expression profiling, are expensive and time consuming, and are rarely used for diagnostic purposes.

Other than genes, the chemical compositions and other cell structure constituents such as proteins are among the attractive targets for specific measurements. Although IHC is a rather inexpensive and readily available method for this purpose, it is not sometimes accurate enough for research purposes. Blotting and proteomics are two examples of fine measurement methods for assessing the protein composition of tissues.

A schematic presentation of work flow in a pathology laboratory is illustrated in (Figure 1.9). Performing autopsies, teaching pathology to medicine and other students and research activities are not displayed in this simplified schema.

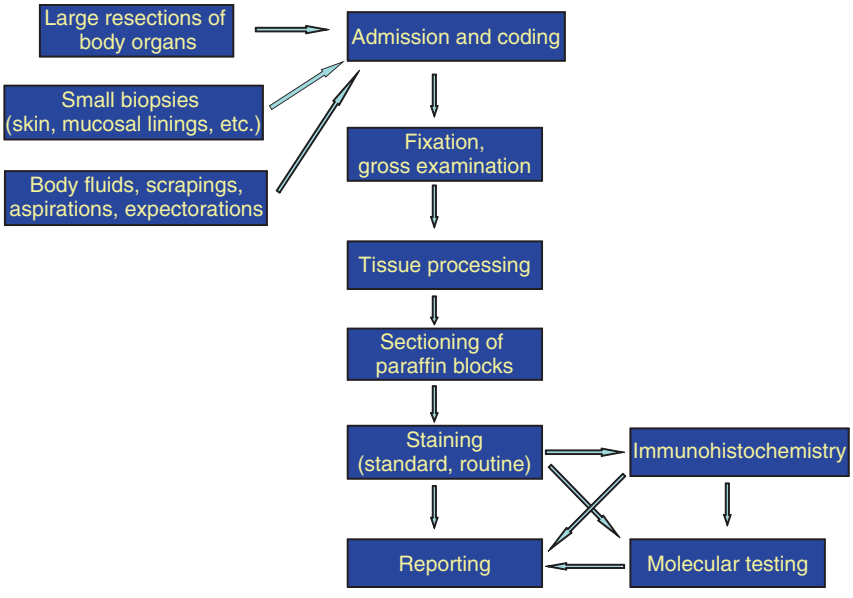


Figure 1.9 Schematic presentation of work flow in a pathology laboratory. In many pathology institutes, teaching and research activities constitute a large part of the work load.

1.7 Summary of Major Terms in Clinical Pathology

1.7.1 Injury and Adaptation

- *Homeostasis*: A steady state in which the cells, a tissue, or an organ is in balance with their microenvironment.
- *Cell injury*: Any external or internal insult that deranges the homeostasis.
- *Injurious* (*noxious*) *agent*: Any type of energies, physical stresses, chemical compounds, or invading microorganisms that can affect homeostasis. Examples: Toxins, radiation, burning, trauma, and bacterial infection.
- *Adaptation*: A series of cell changes at the molecular and cellular levels that serve to protect the cells from cell death when they are exposed to a noxious agent. These changes result in a new steady state in which the cell molecular composition, microscopic appearance, or macroscopic features are different from the original ones.
- *Reversible adaptations*: A couple of changes in the chemical composition and structure of injured cells that enable them to protect themselves against an injurious event. The changes can disappear after the cessation of exposure to the injurious agent. The microscopically and macroscopically detectable morphologic changes in reversible adaptation are usually of one of the following forms:

  - *Hypertrophy*: Increase in the size of tissue due to an increase in the size or the number of constituting cells. Examples: heart muscle hypertrophy in blood hypertension and hypertrophied muscles in an athlete.
  - *Atrophy*: Decrease in the size of the tissue due to decrease in the size of the constituting cells. Examples: Disuse atrophy of the limb muscle after long-term immobility and endometrial atrophy in postmenopausal, women. Thus, atrophy is the opposite of hypertrophy and hyperplasia. Sometimes, the term *atrophy* is accompanied by an attribute to differentiate between its two major causes, that is, numerical atrophy (in apposite to hyperplasia) and simple atrophy (in apposite to hypertrophy).
  - *Hyperplasia*: Increase in the size of a tissue or an organ due to increase in the number of constituting cells and reactive cell proliferation. Examples: Endometrial hyperplasia in patients with estrogenic excess and hyperplasia of the parathyroid glands in patients with chronic hypocalcemic states.
  - *Metaplasia*: Substitution of a mature form of tissue with another mature form of tissue that is not normal for that position. Examples: Squamous metaplasia of the columnar epithelial cells of the respiratory tract and columnar metaplasia of the normal squamous epithelium of distal esophagus (Barrett's esophagus) (Figure 1.10).
- *Irreversible injury*: Specific forms of tissue change that happen when the effect of injury is extensive, stark, and long, or the capacity of the injured cell for adaptation is low. It appears in two basic morphologic forms:
  - *Tissue necrosis*: Typical intravital form of cell death (cell death in a fixed tissue or autolytic changes in an unfixed tissue are not categorized as cell necrosis).
  - *Apoptosis*: Specific morphologic form of cell death that happens normally in tissues with high turnover (endometrial tissue and lymph nodes) as well as neoplastic or pathologically changed tissues.

1.7.2 Inflammation and Repair

Inflammation: A series of vascular and cellular changes in response to invading microorganisms or injurious agents, which specifically happen in multicellular and vascularized organisms.

Acute inflammation: An inflammatory response with sudden onset and short (usually 24–48 h) duration. The typical morphologic changes in an acutely inflamed tissue are vascular dilatation, blood congestion, intercellular edema, and acute inflammatory cell (neutrophilic) infiltration.

Chronic inflammation: An inflammatory response of longer duration (on the order of days). The typical morphologic features that differentiate the chronic from acute inflammation are more pronounced tissue destruction, beginning of tissue fibrosis, and infiltration of chronic inflammatory cells (lymphocytes, plasma cells, and macrophages).

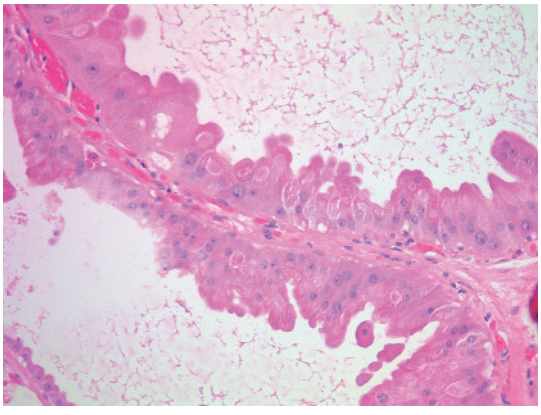
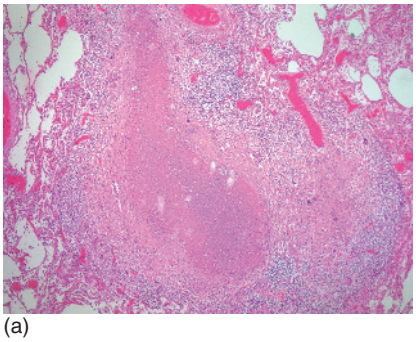
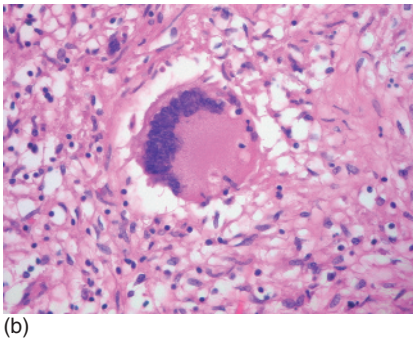


Figure 1.10 Epithelial lining of dilated ducts in a sample of a female breast tissue. The cells show abundant eosinophilic cytoplasm, a relatively small nucleus, and prominent basophilic nucleolus. These features characterize normal apocrine sweat glands and cannot be detected in a normal breast tissue. This type of change is called *apocrine metaplasia* and is characteristic of the fibrocystic changes of the breast.

Granuloma: A typical structure representative of a typical form of chronic inflammation (e.g., granulomatous inflammation) composed basically of aggregation of epithelioid (epithelial-like) macrophages. Other usually present components are a central area of necrosis (typical for tuberculosis granulomas), multinucleated giant cells (dispersed in between histiocytes), and peripheral layer of lympho-plasmacytic infiltration and fibrosis. Examples: Specific forms of infections (tuberculosis and mycotic infections), reaction to foreign bodies, and autoimmune diseases (sarcoidosis) (Figure 1.11).



(a)



(b)

Figure 1.11 Lung tissue with a typical structure of a granuloma. From center to periphery, we can recognize a central area of necrosis, layers of epithelioid histiocytes, lymphocytic inflammatory cells infiltration, and reactive fibrosis (a). In between the epithelioid histiocytes, there are scattered histiocytic giant cells with a horse shoe arrangement of nuclei, which is a characteristic feature of Langhans-type giant cells (b). The combination of these two features (central caseating necrosis and Langhans-type giant cells) is highly suggestive of an infection with *Mycobacterium tuberculosis*.

1.7.3 Neoplastic Diseases

Neoplasia: An autonomous and usually uncontrollable proliferation of cells. The autonomy of proliferation is a key point in the definition of a neoplasia. It has to be differentiated from reactive hyperplasia in which the proliferation is usually dependent on a stimulatory factor. A neoplasm usually leads to the formation of a tumor (a mass lesion that can be differentiated from the background organ tissue by its different consistency, appearance, or shape). A neoplasm (or a tumor) can be benign or malignant.

Tumor differentiation: In tumor pathology, the term *differentiation* is usually used to define the ''level of differentiation'' which signifies the level of morphologic similarity of the constituting tumor cells with the normal cells of counterpart tissue. The higher the similarity, the higher the differentiation. That means that most benign tumors are well differentiated. A similar concept can be expressed in an opposite way. Anaplasia is used to define the level of dissimilarity of tumor cells with presumed normal counterparts. The higher the anaplasia, the lower the differentiation. Morphologically, at the microscopic level most anaplastic tumors, regardless of the tissue of origin, are similar to each other. It is very hard to recognize the tissue of origin of an undifferentiated anaplastic tumor when microscopic examination is the only method of evaluation.

In some anaplastic undifferentiated tumors, it is sometimes very hard, if not completely impossible, to determine the ''line of differentiation'' without the application of ancillary methods such as IHC or electron microscopy. That means that it is hard to define whether this tumor is a carcinoma (a malignant tumor with epithelial differentiation/origin), a sarcoma (a malignant tumor with mesenchymal differentiation/origin), or even a lymphoma (a malignant tumor with lymphoid differentiation/origin).

Tumor grade (*of malignancy*): A concept providing a scale of the level of aggressiveness of a tumor and its potential for lymph node or distant organ metastasis. One of the most important parameters in defining the grade of malignancy of a tumor is the ''level of differentiation.'' In fact, the grading of malignancy is multifactorial and, other than the level of differentiation, is related also to some other parameters such as the number of mitosis as well as the presence and extent of necrosis. For most malignant tumors, a three-tiered grading system is applicable, for example, G1 = low grade of malignancy ~ high differentiation; G2 = medium grade of malignancy ~ moderate differentiation; and G3 = high grade of malignancy ~ low/poor tumor differentiation. Highly anaplastic and undifferentiated tumors are considered as G4. In these cases, the ''level of differentiation'' is very low and the line of differentiation during routine microscopic examination will be uncertain.

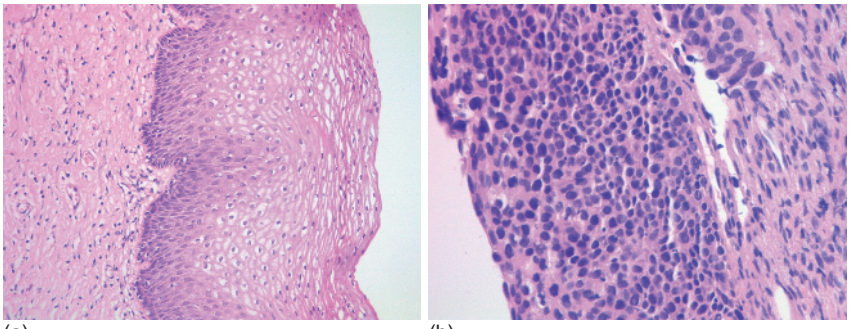
Benign tumors: They are well-differentiated tumors with little, if any, anaplasia. Benign tumors usually have a very slow growth rate. That in turn let the host tissue to react to this new growth and produce a fibrous wall. The presence

of a fibrous capsule is considered by surgeons and pathologists as one of the indicators of a benign tumor. There are exceptions to this general rule. For example, hemangiomas are benign neoplasms of vascular tissue that have no regular boundaries and no fibrous capsule. Most benign tumors are named by addition of the suffix "-oma" at the end of the tissue name. Examples: Lipoma (benign tumors of adipose tissue) or leiomyoma (benign tumors of smooth muscle tissue). Adenoma is the name of benign neoplastic proliferations originating from a glandular tissue or with glandular differentiation. There are also exceptions to this rule of nomenclature. Melanoma is a highly malignant tumor originating from melanocytes (pigment-containing cells in the skin and mucosal surfaces). Lymphoma is a group of usually highly malignant neoplastic proliferations of lymphocytes or lymphoid cells. In the gastrointestinal tract, the term *adenoma* is usually used to describe the polypoid epithelial proliferations with some attributes of malignant proliferations but without tissue invasion.

Dysplasia or dysplastic changes: They are defined as morphologically detectable preneoplastic changes. That means that they have the capacity to progress to a (malignant) neoplasm, but in principle, they can also revert to the normal state. The dysplastic changes represent morphological alterations at the cellular microscopic level and in the nuclei of the composing cells. These morphologic features are to a large extent common between malignant neoplastic and preneoplastic (dysplastic) lesions and include nuclear hyperchromasia, nucleomegaly (increase in the size of the nucleus), irregularity of nuclear border, clumped chromatin pattern, nuclear pleomorphism (different shape and size of the nucleus from one tumor cell to another), loss of polarity, and nuclear pseudostratification. For many of the frequently encountered dysplastic lesions, such as dysplasia of the uterine cervix epithelium (Figure 1.12) or adenomatous polyps of gastrointestinal tract, there is a grading system. Grading a dysplastic lesion provides a basis for their classification according to the probability of development of a neoplasia. For example, the chance of progression to cancer and local recurrence after resection of an adenomatous polyp with low-grade dysplasia is much lower in comparison with a polyp with high-grade dysplasia.

It is noteworthy that, according to the latest edition of WHO classification of gastrointestinal tumors, in addition to changes in nuclear features, the architectural changes also play an important role in the grading of dysplastic changes in an adenomatous polyp.

Malignant tumors: An autonomous proliferation of cells with the potential for local invasion of background normal tissue or metastasis to regional lymph nodes or distant organs. In comparison with benign tumors and dysplastic changes, malignant tumors show a substantially low level of differentiation (higher anaplasia) and increased mitotic activity. There are again a few exceptions to this rule. Glioblastoma multiforme (a malignant brain tumor) and basal cell carcinoma (a malignant skin tumor) are locally aggressive tumors that never metastasize. On the other hand, chondroblastoma is generally considered a



(a) (b)

Figure 1.12 Normal stratified squamous epithelium of uterine cervix (a) and uterine cervix mucosa with histologic features of a high-grade dysplasia (high-grade cervical intraepithelial neoplasia, high-grade CIN) (b). Note the presence of cells with large hyperchromatic nuclei in all layers of the epithelial covering in high-grade dysplasia.

benign bone tumor. But some cases show well-documented metastasis in the lung tissue. Malignant tumors of epithelial origin or with epithelial differentiation are named by adding the suffix "carcinoma" to the name of the normal tissue cells. Examples: Adenocarcinoma of the lung (malignant epithelial proliferation of lung tissue with the predilection to form glandular elements), renal cell carcinoma (malignant epithelial tumors of the kidney tissue), and colon carcinoma (malignant epithelial tumors of colon). Malignant tumors of mesenchymal origin or with mesenchymal differentiation are named by adding the suffix "sarcoma" to the name of the background or differentiated tissue. Examples: Liposarcoma (malignant tumors of adipose tissue) or osteosarcoma (malignant mesenchymal tissue with the potential to form osteoid or background osseous substance).

Tumor metastasis: A unique capability of malignant tumor cells to detach from parent tumoral mass, invade the lymphatic or blood vessel wall, circulate with lymphatic or blood flow to regional lymph nodes or distant organs, and finally produce a new tumoral mass in the new location. It has been considered as the most reliable criterion of malignancy of a neoplasm. Two other important characteristics of a malignant tumor are the invasion into and the destruction of local normal tissue.

Tumor stage: The term that defines the extent of tumor expansion in the body of the patient. The staging of almost all carcinomas is performed according to the assessment of three basic parameters: the local extension of the primary tumor (T), lymph node metastasis (N), and distant (hematogenous) tissue metastasis (M). For any kind of carcinoma, there are a set of precisely defined definitions of various levels of the above-mentioned parameters that let the physicians to categorize the tumor stage for each patient. This system of staging is called

TNM staging and is considered a standard approach in the evaluation of every carcinoma. At the same time, there might be other specific staging systems for a same tumor that can be applied in parallel or independent of TNM staging. Some examples for such a specific staging systems are FIGO staging (abbreviation of the French name for International Federation of Gynecology and Obstetrics) and Astler–Coller and Duke staging systems for colon carcinomas. It has been recommended that the TNM system be used for staging soft-tissue and bone sarcomas. As, in general, the lymph node metastasis of sarcomas is a very rare event, in practice, the staging of these tumors depends mainly on two parameters (T and M). Other than a few exceptions, lymphomas are considered generally as a systemic disease and their staging is dependent not on the local manifestations but on the extent of involvement of different areas of the body and possible extranodal manifestations such as bone marrow, spleen, or liver infiltration.

1.8 Limitations of Clinical and Diagnostic Pathology

One of the reasons why diagnostic pathology is sometimes called *surgical pathology* is the fact that the first generations of pathologists were surgeons. They could diagnose different disease processes during the operation only by looking at the organ by the naked eye. The invention of the microscope and the application of microscopic examination was a great breakthrough. It showed the surgeons and pathologists that the assumptions that are made during gross examination of organs can be completely wrong. Further developments provided important tools that have changed the practice of pathology for ever. Some of these methods (IHC, IF, PCR, and FISH), their applications, and their influence on pathology practice have been comprehensively described previously in this chapter. Although the achievements have been tremendous, they are not enough.

In routine practice of diagnostic pathology, it can be assumed that at least 50–60% of specimens can be diagnosed solely on the basis of gross examination and microscopic evaluation. For a large proportion of remaining cases, it is necessary to apply further supplementary methods. In many of these cases, additional information is needed to provide support to the primary histology-based diagnosis or to exclude possible and frequent differential diagnoses. Nevertheless, there is always a minor but very important group of cases that, even for experienced pathologists, are very challenging and sometimes cannot be solved. Subspecialization in specific diagnostic fields can help resolve the diagnostic problems of a group of these complicated cases. Even in this situation, the diagnoses of expert pathologists are sometimes rather subjective, difficult to formulate, and somehow inspirational. The following situations exemplify some difficulties and problems encountered in the field of diagnostic pathology.

1.8.1 Prediction of Tumor Behavior

The prediction of tumor aggressiveness, velocity of progression, and even sometimes discrimination of benign and malignant neoplastic proliferations from each other are some of the most important tasks of a pathologist. Sometimes, this task cannot be accomplished accurately even with the help of IHC or molecular genetic methods. For instance, in pheochromocytoma (a sort of tumor of the adrenal medulla), the level of nuclear atypia, nuclear pleomorphism, and abnormal nuclear features (which basically are good microscopic indicators of malignancy in a neoplasm) are very high. These changes can easily impact the diagnosis of a malignant neoplasm. It has been shown that such nuclear changes in endocrine glands, in contrast to other organs, play no essential role in making the diagnosis of malignancy. A similar situation can happen in the parathyroid gland. Gastrointestinal stromal tumors (GISTs) are a group of mesenchymal tumors of the gastrointestinal tract and the peritoneal cavity. Potentially, all of these tumors can be aggressive. By definition, there is no ''benign'' or ''malignant'' GIST, simply because the pathologists cannot predict the tumor behavior accurately solely on the basis of macroscopic and microscopic features. To predict the behavior of a GIST, a constellation of clinical and pathological findings, including tumor size, tumor location, tumor encapsulation, local aggression, presence of necrosis, the number of mitotic figures, and the evaluation of the proliferation index using IHC evaluation of mitosis associated molecules (Ki67), is used to provide a sort of risk stratification.

There is no single marker or a combination of IHC markers that can discriminate accurately a benign tumor from a malignant one. Application of clonality analysis in the assessment of T- and B-lymphocyte proliferations is a very helpful tool for the discrimination of neoplastic (predictably monoclonal) and non-neoplastic or reactive (polyclonal) processes. Even such a valuable tool is not always reliable. Other than false positive (for example, in samples with a few analyzable target cells) or false negative results, there are situations in which a truly monoclonal population of B lymphocytes (e.g., monoclonal B-cell lymphocytosis) or plasma cells (e.g., monoclonal gammopathy of undetermined significance (MGUS)) is present in the bone marrow, without any further indications of a malignant behavior. There is no need for immediate therapeutic intervention in these types of changes.

1.8.2 Diagnosis of Tumor Origin in a Case with Metastatic Tumor Disease

Detection of a metastatic carcinoma in a patient with no known primary site (the so-called CUP) is always challenging not only for clinicians but also for pathologists. IHC (for example, application of a set of low and high molecular weight cytokeratins) can provide helpful information to narrow down the list of differential diagnoses. In fact, there are some tables in which the metastatic carcinomas are classified according to their cytokeratin expression pattern (CK7+/CK20−, CK7−/CK20+,

CK7+/CK20+, and CK7−/CK20−). Unfortunately, in many cases, the spectrum of differential diagnoses is too wide. Until now, there is no organ- or tissue-specific IHC marker. Many of the allegedly ''specific'' markers are not totally specific. For instance, the expression of prostatic specific antigen (PSA), which is supposed to be restricted to only normal prostatic tissue and the tumors of prostate, is also detected in some salivary gland tumors. Thyroid transcription factor 1 (TTF1) can be expressed other than in thyroid follicular epithelial cells by lung tissue.

This is the reason why all these markers need to be evaluated within the context of conventional histology. It may also explain why the comments of pathologists remain speculative in many cases and are sometimes disappointing for the clinician.

1.8.3 Individualized Medicine and Targeted Therapy

Although tumor grading and staging are very helpful tools for the prediction of prognosis and can determine the need for supplementary treatments, they are no longer the only satisfactory pieces of information that a pathologist can and have to report in the evaluation of malignant tumors. Alongside the vast improvement of our knowledge about the pathogenesis and progression of different types of tumors, the treatment of cancer patients has been revolutionized. Instead of blind application of high doses of strong toxic chemotherapeutic drugs, nowadays the predilection is to provide treatments that can act effectively and specifically on tumor cells and destroy them without affecting normal tissues with as little side effects as possible. These new treatment options are expensive. It is the job of the pathologist to find the cases that benefit maximally from targeted therapies. For example, all breast cancers are examined immunohistochemically and, if indicated, by FISH for the evaluation of overexpression or amplification of a growth factor receptor, Her2 protein and/or Her2 gene. Only those with evident overexpression of protein and/or those with evident gene amplification will get the maximum benefit from treatment with herceptin (blockers of Her2 protein on the cell surface). All GISTs, and to some extent other soft tissue tumors, are examined for the overexpression of CD117 (c-kit) to identify those patients who can benefit from targeted treatment against this protein.

The growing number of prognostic and predictive factors in different tumor types is fascinating. By pinpointing the examination at the molecular level, it seems that classification of a tumor under one of the well-characterized diagnostic entities is rather arbitrary. It has been shown that the tumor cells in two different patients with a microscopically similar form of cancer at the same stage can have different genetic and/or chemical characteristics. This difference is sometimes critical and determines the likelihood of response to specific forms of treatment. Some examples are the KRAS mutation in colon carcinoma, BRAF mutation in malignant melanoma, and epidermal growth factor receptor (EGFR) mutation in lung adenocarcinoma. But these genes and their products are only one of tens or even hundreds of factors whose over- or underactivity results in tumor

formation or accentuates its progression. It is imaginable that, in order to achieve the best therapeutic result, it is necessary to have a complete view of the chemical composition and pathogenetic pathways of tumor cells in each individual case. Use of the contemporary routine methods makes it cumbersome to assess easily and cost and time effectively all the relevant parameters in every case. Such an assessment needs rapid and reliable methods with the integration of a set of assessments for diagnostic, prognostic, and predictive factors in a few steps or, optimally, simultaneously.

Many other situations in diagnostic pathology can be added to the above list (preneoplastic and preinvasive neoplastic conditions, infectious organisms and their possible relation to carcinogenesis, manifestations and complications of non-neoplastic diseases in the elderly, such as atherosclerosis and diabetes mellitus, etc.). The pathologists hope that new breakthrough technologies such as spectroscopic examinations can help them to resolve some of these processes and assist them in providing an explicit and reliable set of clinically relevant information.

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