

MORPHOLOGICAL DIAGNOSIS - GOALS, OBJECTIVES, OPPORTUNITIES**Tursunov Kh.Z., Nishanov D.A.**

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Abstract

The article provides general information about the goals, objectives and possibilities of morphological diagnostics in the practice of a pathologist. The procedure for preparing the drug in histological laboratories by conventional and immunohistochemical methods, their significance in the diagnosis of tumors is given in detail. The necessity and validity of improving the morphological diagnosis of diseases, especially oncological ones, using new technologies is indicated.

INTRODUCTION

Morphological diagnostics of pathological processes and diseases includes histological, histochemical and immunohistochemical studies carried out in the histological laboratory.

In fact, histological examination is the theoretical side of the diagnostic process, which determines the possibility of obtaining a comprehensive picture of the disease at different levels: systemic, tissue, cellular, subcellular, organ, etc.

It is difficult to imagine modern medicine without histology. After all, a microscopic study of the characteristics of tissues makes it possible to identify not only the disease, but also its characteristic features.

The following are sent for histological examination: biopsy material, tissues of the organs of the corpse during autopsy, all tissues and organs removed during surgical interventions (operations). A biopsy is a diagnostic operation in which tissue is taken from the body in vivo for diagnostic purposes. Nowadays, work with biopsies is 90% of the work of the histological laboratory. In connection with the development of non-invasive diagnostic methods, such operations are most often performed in order to confirm the diagnosis of a malignant neoplasm (tumor), but not only for this. There are certain rules for histological diagnosis regarding the volume of material and the order of its collection in various cases: prenatal diagnosis, gastrobiopsy, trepanbiopsy for bone marrow examination, skin biopsy, etc. These rules are reduced to a reasonable sufficiency of the material, provided that it is representative and informative.

The special role of histological diagnostics in oncological practice is due to the fact that specific methods of histological examination are the most suitable for determining the degree of tissue atypicality (cancer, precancer, etc.), determining the nature of the interaction of tumor tissue with healthy tissue (invasion, microinvasion, etc.), determining the tissue affiliation of

the material under study (important in determining metastases) and other types of studies that are fundamentally important for the diagnosis of tumors and the staging of oncological diseases. According to the regulations in force in Uzbekistan, specialized oncological therapy (chemotherapy, radiation therapy, etc.) cannot be prescribed without histological confirmation of the diagnosis of "malignant neoplasm (tumor)". Combining traditional histological methods with innovative methods, a pathologist can diagnose a tumor, verify it in accordance with international classifications, and evaluate prognostic and predictive factors. At the same time, a predictive assessment is understood as an assessment of the effectiveness of a specific preparation therapy for a specific patient (the principle of personalized medicine), which is critically important for an oncologist who is faced with the task of curing this patient, and, of course, for pharmaceutical companies producing anticancer preparations. The prospects for the use of histological methods in new, hightech areas of medicine are not limited to oncology. Already now they are in demand, for example, in transplantology, dermatology, gastroenterology.

THE WORKING PROCESS AND ORGANIZATION

The workflows in a histological laboratory represent an orderly path that incoming material must follow in order to draw up a histological conclusion. This path consists of a set of strictly obligatory and obligatory in some cases stages, operations at these stages are specific to the histological laboratory and are discrete (discontinuous). Not only does there not exist such a thing as a "histological conveyor", when one could place a container with material on one side of an imaginary device, and on the other side receive an answer in one form or another (verbal, digital or some other), but also many stages of histological examination are still not automated.

The histological material changes its shape twice

as it passes through the stages of the study. After the histological cutting step, representative sections travel around the laboratory in the form of histological blocks. A histological block is a preparation embedded in a special paraffin medium, usually in the form of an irregular cube (hence the name "block"). And after the stage of microtomy - a process that consists in cutting tissues for analysis with the required thickness of 2 to 5 microns - in the form of a preparation on a glass slide (the professional name for such a preparation is "glass"). In this case, the incoming material can be divided into several blocks, and several glasses can be made from each block.

The preparation ends its journey in a specialized archive, which is an important part of the laboratory. According to the regulations in force in our country, the patient's preparation (both the block and the glass) are subject to indefinite storage (the tissue in the paraffin block practically does not change its morphological, biological and chemical characteristics over time). The rationale for this is clear - the histological conclusion is confirmation of the need for surgery or the appointment of a specialized type of treatment, and it is not clear when exactly such confirmation may be needed. On the other hand, oncological patients, unfortunately, can return after primary treatment with relapses or complications, and to find out what is happening with the patient (recurrence of a previously treated tumor, new tumor, metastasis), it is necessary have a complete picture of the medical history, including previously collected material. Finally, with the development of new diagnostic technologies and new treatment technologies, the preparation archive plays the role of a kind of material bank with which scientific research, retrospective diagnostic studies, and patient samples for testing new types of treatment can be carried out.

Laboratory assistants are needed to perform histological processes (except cutting and analysis) in the histological laboratory. Unfortunately, the reality is that specialized training for a histological laboratory technician can only take place at the workplace in the laboratory itself. Domestic medical technical schools do not train such specialists.

A few words about the organization of the diagnostic process in the histological laboratory. To make an accurate diagnosis, it is absolutely necessary to know as many details as possible about the analyzed material and its characteristics, including those that are formed during the work with the material itself: identification with the patient (demography, clinical data), identification with the block (from which part of the

material taken), the integrity of the material (quality of the biopsy), the quality of the histological wiring, orientation during the stage of histological filling, and others. When passing through discrete, not always automated steps of a large number of preparations, this information can be lost. In the worst case, it can be confused - that is, the preparation on the coverslip can be mistakenly identified with another patient. In this case, both patients receive an incorrect diagnosis (oncological, for example) - someone is treated for a "foreign" disease, and someone is not treated, despite the disease. Traditionally, paper journals were used to track the passage of the preparation through the stages of histological examination, and with the development of modern technologies in the laboratory, barcoding technologies, LISs and special programs for organizing (managing) the process in the histological laboratory began to arrive. Process management tools are especially important in the context of an acute shortage of pathologists and histological laboratory assistants, which has become a common problem area not only in domestic, but also in world practice.

In the process of working with the received material, errors in its processing may occur - artifacts (from Latin *artefactum* - artificially made). In the case of some artifacts, the preparation (block) can be restored and the material still analyzed; in some cases, it is necessary to perform an additional diagnostic operation on the patient (biopsy) to obtain new material. In any case, artifacts greatly complicate the task of analysis for the pathologist and can cause an incorrect diagnostic conclusion, which will further affect the nature of the patient's therapy.

ANALYSIS AND STAINING

The impossibility of obtaining an auto-mated "answer" follows from the fact that all stages of the histological examination, in fact, are the preparation of material for analysis, which is also not automated and is performed literally through the eyes of a pathologist. The pathologist analyzes the preparation, distinguishing between healthy and pathological organ tissues, normal and atypical (cancer) cells in the same way that ordinary people recognize their relatives and friends at home or colleagues at work - by their appearance and the characteristic features associated with it. An integral part of the analysis is the consideration of the patient's clinical data.

The pathologist should take part in two stages of histological examination - histological cutting and, in fact, analysis. Histological cutting is the stage at which representative areas for examination are literally cut out from the incoming material. Since only a doctor

can reliably assess the representativeness of a particular area for research, as well as the required number of such areas, the work is carried out at this stage by a couple: a doctor and a laboratory assistant or two doctors. The analysis itself, as mentioned above, is performed only through the eyes of a trained specialist - a pathologist. In popular rumor, the pathologist is ironically called the "best diagnostician", hinting at the fact that the diagnosis based on the autopsy results: a) is obvious; b) can no longer help the patient. In fact, a pathologist during the autopsy of corpses is faced with the whole spectrum of pathologies, in contrast to a doctor of a narrow specialty. His work is an analysis of cause-and-effect relationships that led to a sad outcome. Working with life-time diagnostics (surgical material, biopsies) also requires knowledge of various pathological processes, from inflammatory and parasitic to tumors. The profession of a pathologist, on the one hand, is not easy, and on the other hand, it is not very prestigious because of the "halo" of the specialty. On the other hand, the histological research segment is steadily growing along with the growing attention to oncological diseases and new scientific data in this area. Under these conditions, the insufficiency of pathologists is a worldwide trend, in Uzbekistan 35% of the positions of pathologists from those available in our medical facilities are employed (in Russia it can be defined as acute - in fact, 40% are employed).

During the analysis, the pathologist uses an optical microscope. In order to visualize the structure and details of the studied tissues and cells, the preparation is passed through histological staining. At the same time, there are many types of histological staining, since different objects (bacteria, fungi, mucin, metals, etc.) can be clearly visualized using different chemical compounds. In addition to traditional histochemical stains (in which staining occurs due to the properties of chemical affinity, that is, the attachment of certain dye molecules to certain molecules of tissue and cellular structures), modern histology uses immunohistochemical stains (in which staining occurs due to the interaction of proteins, the "antigen-antibody"), allowing to study the immunomorphological properties of the preparation. Immunohistochemical studies in modern times have become a powerful engine for the development of clinical (lifetime) histology, since they can only be performed in a histological laboratory (it is necessary to reliably determine the structure and type of the analyzed tissue: normal cells, tumor cells, invasive tumor component) and provide a fundamentally new, more detailed information on the properties of the studied objects (cells and tissues) compared to

histochemical methods.

It is almost impossible to make an accurate diagnosis of oncology without special studies. Currently, medicine has such opportunities. Therefore, if there is a suspicion of cancer, then an immunohistochemical study is performed. This study means the study of a tissue sample under a microscope. They are obtained by biopsy and pre-treated with specific antibodies. When antibodies interact with cancer cells, a fluorescence phenomenon can be observed. This gives grounds to be almost 100% sure that oncology has a place. Antibodies to almost all neoplasms have already been developed and actively introduced into medical practice. Immunohistochemical examination in cancer allows:

- ▣ Recognize the neoplasm and determine its type.
- ▣ Find out the prevalence of the primary focus in the body.
- ▣ When taking biological material from secondary foci, it is possible to determine the source of spread of metastases.
- ▣ The study allows you to evaluate the effectiveness of therapy.
- ▣ With the help of the analysis, it is possible to find out at what stage of development a cancerous tumor is.
- ▣ Immunohistochemical study also allows you to find out the growth rate of neoplasms.

This research method is considered more informative in comparison with the usual histological one. If you use both methods of research, you can get a complete picture, so in practice, doctors most often do just that.

Almost all tissues of the human body can be examined using this method, but most often such an analysis is prescribed for suspected oncology:

1. To determine primary neoplasms.
2. To detect metastases.
3. This analysis helps to determine the prognosis for the development and course of pathology.
4. Analysis serves as one of the methods for studying receptors for a number of hormones.
5. IHC study allows you to detect microorganisms.
6. The research method is used to determine the sensitivity of cancer cells to chemotherapy and radiotherapy.

It must be borne in mind that only a doctor who has a certificate confirming special training in conducting analyzes using the IHC method has the right to examine the prepared tissue samples.

The conclusion should indicate:

1. Indicators of antibodies to which the tropism of

the studied tissue was determined.

2. Type of cancer cells and their number.

3. The identified antigens are indicated, which help to establish the type of oncology.

Thus, we can conclude that immunohistochemical examination is effective in various types of oncology. The analysis provides exhaustive information and allows you to recognize the beginning pathological process at the cellular level.

Along with immunohistochemical methods, genetic methods are also used in the study of histological preparations - for example, in situ hybridization, with which it is possible to study variations in the number of copies of genes in cells (using the same optical microscope), - the advantage of which is carried out in a histological laboratory again, this is an accurate (visual) identification of the cell under study (for example, a tumor cell), as well as information about the interaction of normal tissue and the tumor component.

Usually, the material in the form of a preparation on a glass slide ("glasses") passes through routine staining with the histochemical dye hematoxylineosin (abbreviated as "HE" or English "H & E"), and then, according to the results of the primary review of the results of this staining, the pathologist prescribes additional stains either to search for a diagnosis within the framework of differential diagnosis, or to clarify the presumptive diagnosis. Thus, a modern pathologist must have the skills to analyze histological stains of various nature, as well as be able to compare the staining results and available clinical information (demographic data, an extract from the medical history) to draw up a histological conclusion. In this complex and responsible work, pathologists, in addition to their own education and experience, can be helped by specialized atlases, illustrated with images of characteristic and rare, artifactual results of staining of certain tissues. Serious help can be a consultation with another pathologist, who will share his thoughts on the analyzed case, or suggest the direction of the search for further stains in the framework of differential diagnosis.

Such consultation can be obtained remotely by sending the glass for analysis physically, or by sending the image of the preparation via electronic telemedicine communication channels. Taking a photograph of the analyzed image has been possible since the beginning of the 90s of the 20th century, when trinocular tubes for microscopes appeared, on which you can install a camera and photograph the field of interest when viewing the specimen. However, already in the 21st century, the technology of full scanning of

the preparation on a cover glass (glasses) with high resolution has become widespread. Such a "virtual preparation" is fundamentally different from a photograph of the field of view precisely in that it is completely scanned and represents a complete analogue of a physically existing preparation. Scanning is done using a special device - a histological slide scanner. Glass scanners differ in their characteristics: scanning speed, number of simultaneously loaded glasses, scanning method (technology), resulting image format, etc. Histological slide scanners can be used for the following purposes: formation of a digital archive of laboratory preparations, training of students and novice pathologists using scanned images (including examination tasks), remote consultation using a scanned image of the preparation, automatic analysis of the preparation. The latter function is probably the most promising for the development of all histological practice. New devices successfully cope with various tasks when calculating image morphometric parameters (the number of cells, the percentage of positively stained cells, etc.), and can also perform the rudiments of analysis using built-in algorithms for evaluating predefined variants of immunohistochemical stains, helping the pathologists.

In recent years, along with histological laboratories in the state pathological and anatomical institutions of the republic, private histological laboratories («Ipsun pathology», Uzlab, «PREMIUM DIAGNOSTICS» etc.) have been organized and operate, where immunohistochemical methods of research are more successfully used in tumors.

Starting a conversation about the prospects of commercial histology in our country, it would be appropriate to determine the size of the market for these studies. In Uzbekistan, about 23,000 new cancer patients are diagnosed per year (in Russia - 520,000) and about 110,000 are registered at the dispensary. The total number of biopsy studies performed in our country per year is estimated at more than 1000,000 (for comparison, this figure is estimated at 30 million in Russia and 300 million biopsy studies per year in the USA). At the same time, only 90% of cancer patients have histological confirmation of an oncological diagnosis. It turns out that 1 million biopsy studies are not enough to confirm 100% of oncological diagnoses and the actual size of the biopsy research market is 10 percent more (taking into account the fact that not all biopsies are done to confirm oncological diseases), that is, about 1,5 million studies biopsies per year. At the same time, there is every reason to believe that, as in other areas of domestic medical practice, the

number of prescribed studies is lower than the real need for these studies and, thus, the potential volume of pathoanatomical studies is even greater.

The existing infrastructural and organizational limitations of the state healthcare system naturally encourage patients to turn to commercial organizations for the provision of necessary services.

What are the trends in this market and assumptions about the dynamics of demand for histological examinations? According to the World Health Organization, in 2002, 10.9 million cancer patients were diagnosed world-wide. By 2020, this organization predicts an increase in the number of diagnosed cancer patients to 16 million people, which corresponds to an increase of about 50%. It is logical to assume that, based on the growth in the number of cancer patients, the demand for histological studies in oncology will increase.

The number of histological studies will grow at an accelerated pace, especially in the field of immunohistochemistry and in situ hybridization. The fact is that modern oncological science is on the path of ever greater detail, expanding the nomenclature of the classification of tumor types. This happens due to the polymorphism of oncological pathology as such. An expanding classification requires the production of an increasing number of immunohistochemical reactions per analyzed case, since in many respects the immune properties of the tumor are a classification criterion. Following the immune characteristics in the classifications, genetic characteristics are increasingly appearing, and the most accessible method for the pathologist to diagnose them is in situ hybridization. At the same time, PCR and genetic sequencing methods are already being used in specialized histological laboratories to diagnose rare cases that go beyond the capabilities of immunohistochemistry and in situ hybridization methods.

Finally, the transition of the health care system to dispensary and screening programs inevitably increases the amount of research in applications such as:

1) cervical cancer screening - biopsy of all suspicious areas and biopsy with a suspicious cytological result

2) all gastroscopy, since the diagnosis of gastritis is morphological

3) colonoscopy - biopsy of any polypoid formations

4) fluorography of the lungs - a biopsy of all suspicious areas, etc.

Thus, we can assume that there is a demand for histological studies in our country, and it will grow. An important criterion for the prospects of any services is

traditionally the analysis of international experience in a particular area.

CONCLUSION

Pathological anatomy, in addition to its complexity and diversity, is also differs in that this medical specialty, like no other, is located "at the junction" of clinic and science. Therefore, firstly, to describe all the subtleties of technology and organization in the limited framework of a journal article is a rather difficult task. And, secondly, pathological anatomy (histopathology) is constantly evolving along with the development of scientific knowledge. New knowledge, new methods, new tasks, new equipment come to the histological laboratory, which organically flow into clinical research that works for the benefit of a particular patient. The solution of responsible tasks entails the growing responsibility of the histological laboratory, the growing attention from the attending physician, the organizers of medicine, the patient and people who are not indifferent to him. This attention is the key to the development of various forms of histological diagnostic services.

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