THE ROLE OF IMMUNOHISTOCHEMICAL ANALYSIS IN THE DIAGNOSIS OF LUNG METASTASES

Ljuban Blanuša[1,2], Aleksandra Lovrenski[1,2], Marija Baroš Brkljača[1,3]

SUMMARY

Background: *Metastatic tumors are tumors whose primary origin is not the organ in which they are located, but have reached the target tissue by metastasis from the primary site of the tumor. The presence of metastases of the primary tumor in other organs is responsible for the highest number of cancer mortality, and in addition, their presence significantly changes the treatment of an oncologic patient in relation to a patient whom the primary tumor is not disseminated. The goal of this study was to determine the importance of immunohistochemistry in the diagnosis of metastatic lung tumors.*

Methods: *PA retrospective study included 84 patients with a pathohistologically proven metastatic lung disease at the Institute of Pulmonary Diseases of Vojvodina from April 1 2013 to March 31 2018. Material for pathohistological and immunohistochemical analysis was studied in the Institute for Histopathology and Molecular Diagnostics of the Institute.*

Results: *Out of a total of 84 patients, in 42 (50%) patients, the origin of pulmonary metastases was colorectal cancer, 15 (17%) renal cell carcinoma, 11 (13%) breast cancer, 4 (4%) malignant melanoma, 3 (4%) leiomyosarcoma of the uterus, and in the other 9 (11%) individual tumor cases. Antibodies used in the immunohistochemical assay are CK20 and CDX2 (colorectal cancer), CD10, RCC, Vimentin (renal cell carcinoma), PR, ER, Mamaglobin (breast cancer), HMB45, S100, Vimentin, MelanA (melanoma) SMA, Myosin, Desmin (uterine leiomyosarcoma).*

Conclusion: *For the purpose of faster and more precise diagnostic and timely treatment of patients with disseminated malignant disease, it is necessary to supplement the standard pathohistological analysis with immunohistochemistry analysis, which is an important method in determining the primary origin of metastatic tumors.*

Key words: *lung neoplasms; immunohistochemistry; pathology; metastases.*

Arch Oncol 2023; 29(2): 13-17

Published online first: 2023-10-26

https://doi.org/10.2298/AOO220825001B

[1] Faculty of Medicine,
University in Novi Sad,
Hajduk Veljkova 3,
Novi Sad 21000

[2] Institute for pulmonary diseases of Vojvodina,
Institutski put 4,
Sremska Kamenica 21204

[3] Institute of Public Health of Vojvodina,
Futoška 121,
Novi Sad 21000

Correspondence to:
Ljuban Blanuša
1404d20@mf.uns.ac.rs

Received: **2022-08-25**
Received in revised form: **2022-08-31**
Accepted: **2023-06-07**

INTRODUCTION

Secondary tumors, i.e. metastatic tumors, are tumors whose primary origin is not in the organ in which they are located, but have reached the target tissue by metastasizing from the primary focus of the tumor. The presence of primary tumor metastases in other organs is responsible for the highest number of deaths from cancer, and in addition, their presence significantly changes the way of treating an oncological patient compared to a patient in whom the primary tumor has not disseminated (1,2).

In order for a tumor to metastasize, certain steps must be met. First, malignant cells must have the ability to invade a blood vessel, and once they reach the bloodstream, they must evade the immune system and survive in the bloodstream. After they reach a blood vessel of a certain diameter, they cause microembolization of the blood vessel and after that extravasation of malignant cells, neoangiogenesis, micrometastases, and then macrometastases occur. The most common route of metastasis is hematogenous metastasis, where malignant cells carried by the bloodstream reach the target organ, where they remain in the capillary network. The second most important type of tumor dissemination from the primary focus is metastasizing via lymphatic vessels, where tumor cells are carried by the lymphatic current to regional lymph nodes, and from there by efferent lymphatic vessels to other lymph nodes (3,4).

The lungs, as an extremely well-blooded organ with a highly branched capillary network, located in the circulatory system between the right and left halves of the heart, represent a filter through which all venous blood from the body must pass in order to return to the systemic circulation. It has been calculated that the surface of the vascular network of the lungs is 100m ^ 2, which is much more than other organs. Because of this, the lungs, along with the liver, brain and bones, are the most common organ in which secondary tumors are located (1,2).

The most common tumors that metastasize to the lungs are tumors originating from the gastrointestinal tract, among which the most common are colon tumors, followed by kidney and breast tumors, as well as melanomas. Since there is a wide variety of tumors that can metastasize to the lungs, it is necessary to find a way to determine the primary origin of the tumor. As it is not possible to type the origin of the secondary tumor based only on the histological image, it is necessary to include immunohistochemical analysis in the diagnostic algorithm. Immunohistochemistry is a method based on the fact that cells in certain organs express certain proteins and macromolecules specific to the cells of that organ, which allows the origin of those cells to be identified. In immunohistochemistry, antibodies are used that will bind to specific antigens on cells and lead to a staining reaction that can be read microscopically (1).

The aim of the work was to determine the number of patients with pathohistologically confirmed metastatic lung tumors, operated on at the Institute of Pulmonary Diseases of Vojvodina. To determine the importance of immunohistochemistry in the diagnosis of metastatic lung tumors.

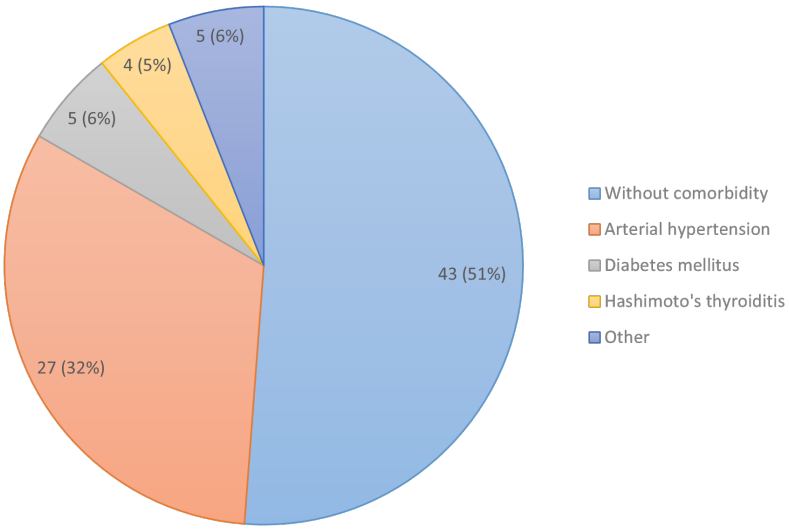

Chart 1. Comorbidities in our patients.

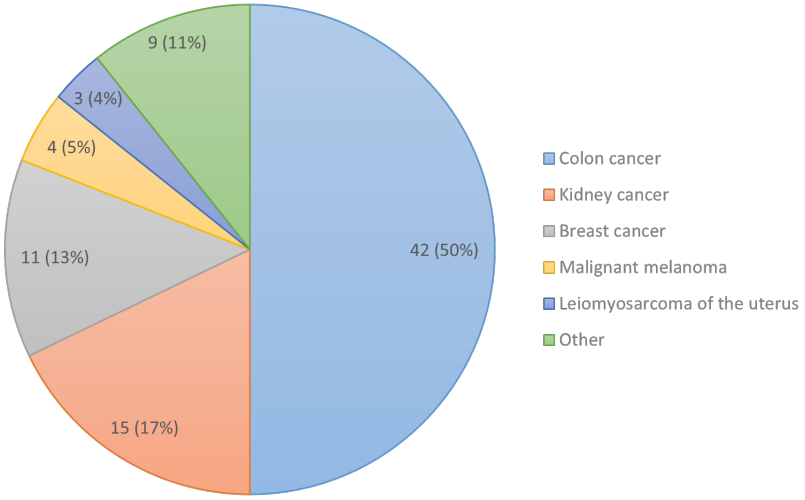

Chart 2. The origin of metastases in the lungs.

METHODS

Sampling and processing of material for pathohistological analysis

Biopsies for pathohistological analysis were obtained surgically - thoracotomy with lobectomy and video-assisted thoracoscopy with atypical lung resection.

Thoracotomy is an operative technique in which an incision is made on the chest that allows us to access and visualize the organs and other structures inside the chest for diagnostic and therapeutic purposes (5,6).

Video-assisted thoracoscopy (VATS) is a minimally invasive operative method in which a thoracoscope is introduced into the pleural space, which enables the visualization of organs and structures in the chest (5).

All obtained samples were fixed as a whole in 10% neutral formalin and, after molding in paraffin, cut with a microtome into tissue sections 4 microns thick, and then stained with the hematoxylin-eosin (HE) method.

Immunohistochemical analysis

Immunohistochemistry (IHH) is a sensitive microscopy-based technique for visualizing cellular components, such as proteins and other macromolecules in tissues. This method of visualization is based on the antigen-antibody reaction, where the antigenic proteins and other macromolecules are on the surface of the examined cells, and the antibodies we use for antigen recognition (primary antibodies) can be monoclonal or polyclonal. Polyclonal antibodies are specific for several epitopes and their potency is higher than monoclonal antibodies, but monoclonal antibodies are more specific than polyclonal ones because they have a clearly defined epitope to which they bind. Secondary antibodies are labeled enzymatically, radioactively, fluorescently or with some other marker, bind to the Fc fragment of primary antibodies and give a colored reaction that can be observed microscopically. This will happen if the primary antibodies have bound to the antigens on the cells of the preparation, if they have not, they will be washed out of the preparation and the secondary antibodies will have nothing to bind to and they will also be washed out of the preparation (7).

For IHH analyses, samples were glued onto “Superfrost” (Men Glaser) positively charged glass slides prepared in advance for IHH reactions. After deparaffinization of the sections, antigenic determinants were unmasked by boiling the preparation in citrate buffer (pH=6) twice for 10 minutes and cooling in distilled water for 20 minutes. Endogenous peroxidase was then blocked with 3% hydrogen (H2O2) for 5 minutes. Preparations processed in this way were treated with primary antibodies. After that, the sections were incubated for 30 minutes with a biotinylated mouse antibody, and then incubated for another 30 minutes with the streptavidin-peroxidase complex system. DAB (diaminobenzidine-tetrahydrochloride) was used as a chromogenic substrate and contrast was performed with hematoxylin.

RESULTS

The research covered a period of five years, from April 1, 2013 to March 31, 2018. In that period, the total number of patients in whom a secondary lung tumor was pathohistologically determined was 84, and in 73 out of 84 patients, this was determined using immunohistochemical staining. All patients in this study were older than 40 years, and the age range was from 41, for the youngest, to 85 years for the oldest patient. The average age was 66, and the standard deviation was +/-10 years. The largest number of patients was in the age range of 60 to 79 years, as many as 73%, or 63 out of 84 patients.

Regarding the gender distribution among our patients, of the total number, 44 (52%) patients were male, while 40 (48%) patients were female. As we can see, the ratio of men and women among the patients is almost equal.

When we talk about the harmful habits of patients in our research, almost the same percentage of patients were

those who consume cigarettes (41 patients - 49%) and those who do not consume cigarettes (43 patients - 51%). In addition to having an oncological disease, the patients in our study had no other comorbidities in 51% of cases (43 patients). Among other patients, 27 (32%) had hypertension as a comorbidity, then 5 (6%) patients had diabetes mellitus, 4 (5%) Hashimoto's thyroiditis, and of the other 5 (6%) two patients had asthma, two ulcers and one schizophrenia (Chart 1).

In 13 (15%) of our patients, there is a family burden of cancer, while in the other 71 (85%) patients there is no history of cancer in the family.

The most common way material was sampled for pathohistological analysis was VATS with atypical lung resection performed in 54 (65%) patients, while classic thoracotomy with lobectomy was performed in 9 (10%) patients. In 23 (25%) patients, samples were obtained by bronchoscopy, with the fact that in 15 patients a bronchobiopsy (BB), in 4 patients a catheter biopsy (KB), and in 4 a transbronchial biopsy (TBB) was performed.

In the patients in our study, half of them, ie 42 patients, had metastases in the lungs of primary origin from the large intestine. In second place was clear cell carcinoma of the kidney, which was diagnosed in 15 (17%) patients. In 11 (13%) patients, lung metastases from the breast were diagnosed. In 4 (5%) patients, the metastases originated from melanoma, in 3 (4%) patients, they originated from leiomyosarcoma of the uterus (Chart 2). In the other 9 (11%) patients, metastases originated from different individual tumor cases.

As each tumor has its own ability to metastasize, it is important to determine the time period in which secondary tumor deposits appear in other organs.

In patients whose metastases originated from colon cancer, in the first 5 years metastases to the lungs occurred in as many as 86% of patients, i.e. in 36 patients out of 42. In the period from 6 to 10 years, metastases occurred in 5 (12%) patients, and after 10 years only in one (2%) patient (Chart 3).

When it comes to patients whose metastases originated from renal cancer, lung metastases occurred in the first 5 years in 73% of patients, that is, in 11 out of 15 patients. In the period from 6 to 10 years, metastases occurred in one (7%) patient, while lung metastases occurred in 3 (20%) patients over a period of more than 10 years (Chart 4).

In breast cancer, lung metastases occurred in 3 (27%) patients in the first 5 years. In the period from 6 to 10 years, metastases occurred in 2 (18%) patients, and in the period after 10 years, they occurred in 55% of patients, that is, in 6 to 11 patients (Chart 5).

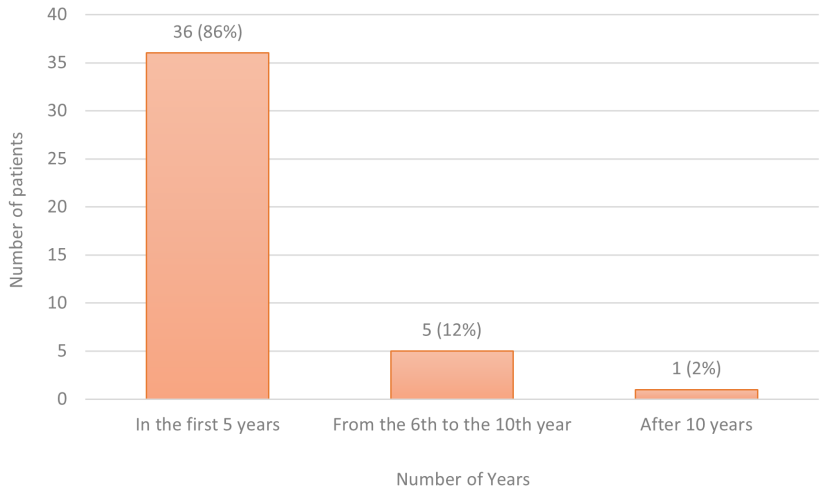

Chart 3. The time of occurrence of lung metastases in patients with colon cancer.

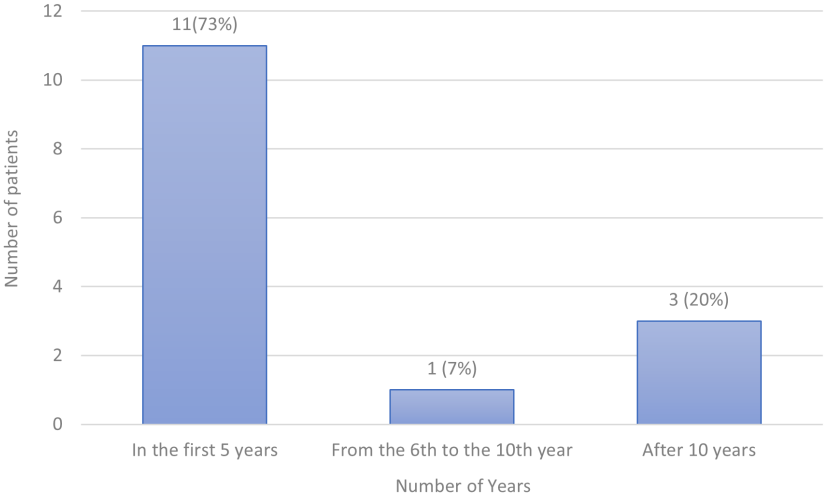

Chart 4. The time of occurrence of lung metastases in patients with kidney cancer.

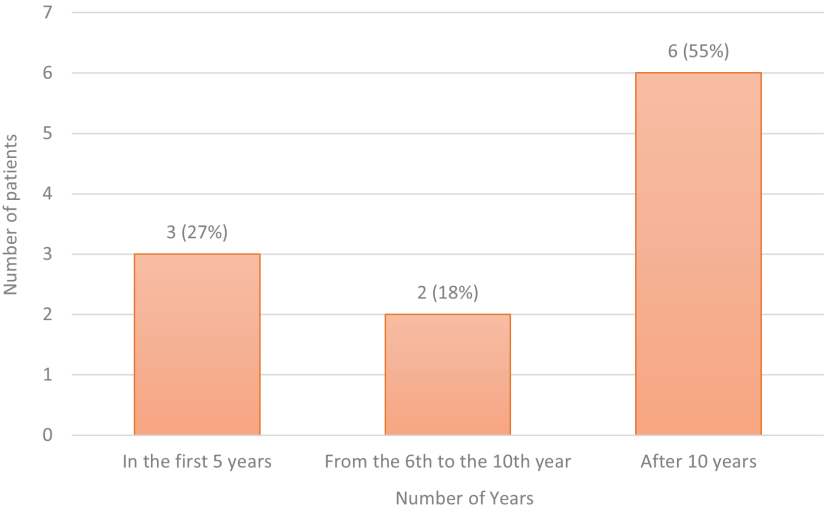

Chart 5. The time of occurrence of lung metastases in patients with breast cancer.

DISCUSSION

Metastases are a far more common source of lung malignancies than primary malignant lung neoplasms. This is expected considering the position of the lungs in the body's circulatory system and their abundant vascularization. In addition to the lungs, the most common sites of metastases are the brain, liver and adrenal glands, also thanks to their abundant vascular network, while the most common sources of metastases in the lungs are colon, kidney, breast cancers, as well as melanoma (1,3).

A special problem is represented by tumors of unknown primary origin (cancer of unknown primary - CUP), which are metastatic tumors whose primary origin cannot be determined at the time of diagnosis. When it comes to their epidemiology, they account for 5% of the total number of cancers, and in some cases the primary place of origin of the tumor is never determined. These metastases represent a big problem from the point of view of therapy because tumors of different origin have different therapy. Since radiological methods can only prove the presence of a tumor mass in an organ, for definitive tumor typing, it is necessary to perform a pathohistological and immunohistochemical analysis of the biopsied tissue (8,9).

When starting with immunohistochemical analysis, it usually starts with the use of antigens that are less specific and are expressed in more tumors, thus reducing the spectrum of tumors that are the primary source of metastatic deposits. Perhaps the most important broad-spectrum antigens are cytokeratins: 7 (CK7) and 20 (CK20). Cytokeratin 20 is expressed on tumor cells of the lower digestive tract, urothelium, Merkel cells, while cytokeratin 7 is expressed on tumor cells of the lung, ovary, endometrium, and breast (9).

After narrowing down the spectrum of potential tumors, specific tissue antigens are determined that are specific for a certain tissue, and thus we can determine the exact primary origin of the tumor. For example, along with CK7-/CK20+ for the diagnosis of colorectal cancer, we also used the marker CDX-2, which when positive represents a highly specific finding for colorectal cancer (9).

CD10 marker, which is a metallopeptidase and is expressed on the epithelium of the proximal tubules, but is also expressed on hematopoietic cells and is important for diagnosing leukemia and lymphoma, was used for the diagnosis of clear cell renal carcinoma (10). Then the RCC marker (Renal Cell Carcinoma marker) was used, which is also expressed normally on the epithelium of the proximal tubules and on tumor cells of the origin of the tubule epithelium (11). In addition to these two, the palette for the diagnosis of clear cell carcinoma also uses vimentin, which is normally expressed on mesenchymal cells (for example, on fibroblasts and endothelial cells), but is also expressed on the cells of some cancers, such as clear cell renal carcinoma (12). Mammaglobin, a glycoprotein that belongs to the secretoglobin-uteroglobin family of glycoproteins, is used in the diagnosis of breast cancer, it is highly specific for breast tumors (13,14). In certain studies, its role as a serum marker for breast tumor is studied (15,16). Progesterone receptors (PR), normally expressed in the breast on the surface of epithelial cells, are extremely useful in the diagnosis of metastatic tumors, as they are a specific marker for tumors originating from the breast. It is important to determine the degree of PR expression in the tumor because it gives us insight into whether the tumor will respond to tamoxifen therapy (17). Estrogen receptors (ER) are also significant data, the level of its expression in the tumor due to the response to therapy with tamoxifen and other anti-estrogens. In addition, it is significant as a prognostic marker of survival in people with breast tumors (higher expression of ER is a positive prognostic parameter) (18).

In the case of malignant melanoma, vimentin is used in the palette for IHH diagnostics, as in clear cell carcinoma of the kidney, followed by HMB45 (Human Melanoma Black), a membrane protein necessary for the formation of melanosomal fibrils. This marker is used for the diagnosis of invasive melanomas, in addition, it is also significant for the diagnosis of the presence of metastases in "sentinel" lymph nodes, but also for the diagnosis of epithelioid cell tumors (for example, angiomyolipoma) (19). S100 is a protein that plays a role in regulating cell growth and differentiation, it is expressed on cells of neural crest origin (melanocytes, glia cells) and therefore it is also expressed on melanoma cells (20). MelanA (Melanoma Antigen) is an antigen that cytotoxic T lymphocytes recognize on melanoma cells, it is used to diagnose melanoma metastases, it is more sensitive than the HMB45 marker (21).

In uterine leiomyosarcoma, we used the following markers: SMA (smooth muscle actin), desmin and myosin. SMA (smooth muscle actin) is used to identify smooth muscle cells, myofibroblasts, myoepithelial cells and pericytes in tumors of the breast, uterus, sweat and salivary glands (22). Desmin serves to identify tumors of muscle tissue origin, as well as to differentiate between smooth muscle cell tumors (desmin+) and gastrointestinal stromal tumors (almost always desmin-) (23). Myosin is found in the smooth muscle and striated muscle cells (24).

Among our patients, 50% of patients had diagnosed metastases of colorectal cancer origin, 17% of clear cell renal carcinoma origin, 11% of breast cancer origin, 5% of melanoma origin, 4% of uterine leiomyosarcoma, and the remaining 11% of other rare tumors. In a study by Younes et al. of 440 patients who underwent lung surgery to remove metastases, in 11% of patients the primary source of metastases was colorectal cancer, in 13% breast cancer, in 3% renal cancer, and in 10% melanoma. In their study, 53% of all metastases were adenocarcinomas, while in our study, even 85% were adenocarcinomas (25).

In a study by Crow et al., where an autopsy was performed on 56 people who died of cancer, 30 (54%) had metastases in the lungs, and of these 30, in 5 patients

the metastases were of origin from the large intestine, in 2 from the breast, and in one patient from the kidney and from the uterus (26).

CONCLUSIONS

The role of immunohistochemistry is great because it is a very reliable way of determining the origin of metastases in the lungs, identifying the primary tumor and applying therapy, because the way the patient is treated depends on the origin of the primary tumor.

The antibodies utilised for immunohistochemical analysis in our research to ascertain the source of metastatic tumours included: CK20 and CDX2 (colorectal cancer), CD10, RCC, Vimentin (clear cell kidney cancer), PR, ER, Mammaglobin, (breast cancer), HBM45, S100, Vimentin, MelanA (malignant melanoma), SMA, Myosin, Desmin (leiomyosarcoma).

For the purpose of faster and more accurate diagnosis and timely treatment of patients with disseminated malignant disease, it is necessary to supplement the standard pathohistological analysis with immunohistochemical analysis, which represents an important method in determining the primary origin of metastatic tumors.

Declaration of Interests

Authors declare no conflicts of interest.

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