The role of immunohistochemical evaluation in the diagnosis of malignant mesothelioma of the pleura

Milana Panjković*, Aleksandra Lovrenski*, Živka Eri*, Slavica Knežević Ušaj†, Dragana Tegeltija*, Jelena Krehedinač*

*Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; †Institute of Oncology of Vojvodina, Sremska Kamenica, Serbia

Abstract

Background/Aim. The final diagnosis of malignant pleural mesothelioma is made exclusively by histopathological examination of biopsy materials that are routinely complemented by the use of immunohistochemical analysis. The aim of this paper was to determine the significance of immunohistochemical analysis and application of certain antibodies in the diagnosis of malignant pleural mesothelioma. Methods. This retrospective analysis included clinical data of 32 patients with the histopathological diagnosis of malignant pleural mesothelioma made in the period 2004–2009 at the Institute for Pulmonary Diseases in Sremska Kamenica. The material was processed and analyzed at the Center for Pathology. Results. CK5/6 was positive, in 63% of the cases calretinin, in 94% and HBME-1 in 80% of the cases. CK7 was positive in 78%, and EMA in 83% of the cases. All the cases (100%) were negative for TTF-1, CEA, CD20, desmin and MOC31. Conclusion. Immunohistochemistry has become an essential diagnostic procedure for the diagnosis and determination of the type of malignant pleural mesothelioma, and due to the lack of individual antibodies a combination of antibody with different sensitivity and specificity is in use today.

Key words: plural neoplasms; mesothelioma; immunohistochemistry.

Introduction

Immunohistochemistry (IHC) is a highly sensitive laboratory method, used for identification and typization of tissues and origin of cells. It is a procedure of detecting cell surface antigen (Ag). It is based on the principles of an immune response, antigen-antibody reaction (Ag-Ab), in which antibody (Ab) binds only to specific antigen, giving a high specificity to this method. The antibodies, which are used, may be monoclonal or polyclonal. It is highly recommended to use monoclonal antibodies because they are more specific, and visualization of Ag-Ab reaction is performed with fluorescent, enzymatic, chemical or radioactive marked antibodies.

The development of immunohistochemistry overcomes many diagnostic problems today and allows adequate differentiation of malignant pleural mesothelioma (MPM). Immunohistochemistry is the most important, highly sensitive and specific assay that allows accurate determination of the type, and even subtype of the tumor. Its major role in establishing the final diagnosis of MPM is to provide a distinction in:
epithelioid MPM from adenocarcinoma, sarcomatoid MPM from primary or secondary pleural sarcomas and from secondary sarcomatous carcinoma, epithelioid MPM from mesothelial cells hyperplasia and desmoplastic MPM from secondary sarcomatous carcinoma, epithelioid MPM from primary or secondary pleural sarcomas and from secondary sarcomatous carcinoma.

Currently, there is no tissue-specific antibody with 100% specificity and high sensitivity, which can give certain confirmation or exclusion of MPM. The lack of this tissue-specific antibody induced the production and usage of a large number of mesothelial and epithelial antibodies with different sensitivity and specificity. Therefore, it is highly recommended to use a palette of antibodies that combines two or more positive mesothelial with two or more positive epithelial antibodies.

Regardless the shortage of tissue-specific antibody, immunohistochemistry is the most significant shift in achieving the differential diagnosis of MPM, and histopathology pattern in general. There is no other method, used in the past 50 years, which had such a strong influence on pathohistology. It is the last diagnostic step before the final diagnosis is made.

All modern histopathology laboratories require necessary immunohistochemical capacity for adequate functioning and providing correct diagnoses.

The aim of this study was to determine the significance of immunohistochemical analysis and application of certain antibodies in the diagnosis of MPM.

Methods

The study involved 32 patients, who had been admitted and treated in the Institute for Pulmonary Diseases of Vojvodina in the period from 1st January, 2004 to 31st December, 2009. All the patients had histopathologic confirmation of MPM. Surgery-obtained tissue was also processed and analyzed by immunohistochemical methods at the Pathology Department. Clinical and demographic data included in the study were: age, gender, smoking history, family cases of mesothelioma and symptoms of the disease. Surgical methods, for getting tissue samples were: video-assisted thoracoscopic surgery (VATS; 30 samples) and open lung biopsy (1 sample). In one patient with multiple pulmonary metastases of MPM, tissue sample for pathohistological analysis was obtained by transbronchial biopsy. All the samples were fixed in 10% neutral formalin, paraffin-embedded and sectioned in 4 microns thick slices for hematoxylin and eosin (HE) staining.

For IHC analysis the samples were glued to "Super-frost" (Men Glaser), positively charged glass plates already prepared for IHC reactions. After deparaffinization of slices, antigenic determination was demarked by the reaction with citrate buffer (pH 6, high temperature, two times for 10 minutes) and by further cooling in distilled water for 20 minutes. Subsequently, blocking of endogenous peroxidase with 3% hydrogen peroxide (H₂O₂) during 5 minutes was performed.

Beyond, preparations were treated with primary antibodies and then incubated for 30 minutes with biotinylated mouse antibody and then incubated for 30 minutes with streptavidin peroxidase complex system. As a chromogenic substrate diaminobenzidine-tetrahydrochloride (DAB) was used and contrastive analysis was performed with hematoxylin.

The microscopic examination of immunohistochemically processed samples was performed and based on their positivity or negativity to specific antibody, the final diagnosis of malignant pleural mesothelioma of certain type was concluded.

Results

The patients included in our study were 46 to 84 years old, the average age was 59.7 years. The females represented 41% of all the patients (13 patients) while 59% (19 patients) of them were the males. Smoking was reported in 50% of the cases (16 patients). All the patients denied cases of malignant mesothelioma in their family.

The most common clinical manifestations of malignant pleural mesothelioma in our patients were dyspnea, chest pain, cough and symptoms of general infection. Dyspnea was present in 66% of the cases (21 patients), chest pain in 56% (18 patients), cough was noted in 37.5% (12 patients), and symptoms of general infection syndrome were present in 8 patients (25%).

Epithelioid type had 69% of the patients (22 patients), 19% (6 patients) had sarcomatoid, and the remaining 12% (4 patients) had biphasic type of malignant pleural mesothelioma. All the patients had unilateral MPM, in 78% of the cases (25 patients) the tumor was positioned in the right hemithorax, and in 22% (7 patients) in the left.

The antibodies used in the research and the numbers of positive and negative results for each antibody are shown in Table 1. Antibody to cytokeratin 5/6 (CK5/6) was positive in 17 of 27 samples tested (63%), calretinin in 28 of 30 (94%), anti-human mesothelial cell, clone HBME-1 (HBME-1) in 20 of 25 (80%) cases. Antibody to vimentin was positive in all eight treated samples (100%), antibody to epithelial membrane antigen (EMA) in 5 of 6 samples (83%), and antibody to cytokeratin 7 (CK7) in 7 of the 9 treated samples (78%). Antibody to pancytokeratin (PanCK) was performed on one sample only, and it was positive (100%). Antibody to cytokeratin 20 (CK20) was negative in all the 4 (100%) cases. Antibody to thyroid transcription factor 1 (TTF-1) was applied in 9 cases, carcinoembryonic antigen (CEA) in 7, and desmin and epithelial specific antigen Ab-7, clone MOC-31 (MOC-31) in only one sample. These four antibodies showed negativity in each case.

In epithelioid type of MPM (Figure 1) antibody to CK5/6 proved positivity in 13 of 19 (68%) cases, while the antibody to calretinin (Figure 2) was positive in 18 of 20 cases (90%). Antibody to HBME-1 was used in 18 cases and expressed positivity in 15 (83%) cases. Positive findings were found in all the cases (100%) while applying antibodies to: vimentin (3 of 3), EMA (2 of 2) and CK7 (6 of 6). Antibody to TTF-1 was performed in 5 cases (Figure 3), CEA in 6 cases, CK20 in 4, and MOC-31 in one sample. These four antibodies showed negativity (100%) in all the cases.

In sarcomatoid type of MPM, positivity to calretinin (6 samples), vimentin (3 samples) and PanCK (1 sample) was found in 100% of the cases. Antibody to CK5/6 was positive in 1 of 5 cases (20%) and HBME-1 in 60% (3 of 5 cases). Antibody to EMA showed positivity in 1 of 2 cases (50%). Antibodies to TTF-1 and desmin were performed on one sample with a complete negativity (100%), while the antibody to CK7 showed negativity in both cases done.

In biphasic type of MPM, antibody to CK5/6 was applied in 3 cases and calretinin in 4 cases with 100% positivity in all the samples. Positive findings (100%) were found while applying antibodies to HBME-1, vimentin and EMA (in 2 of 2 cases made). Antibody to CK7 was performed on one sample with absolute positivity. The negative findings (100%) were observed when antibodies TTF-1 (3 of 3 cases) and CEA (1 of 1 case) were used.

The percentages of the results for the applied antibodies in MPM expected to be positive were as follows: antibody to CK5/6 was positive in 68% of the cases with epithelioid, 20% cases of sarcomatoid and 100% of cases in biphasic type. Antibody to Calretinin was positive in 90% of the cases of epithelioid type and in 100% of the cases in sarcomatoid and biphasic type. Antibody to HBME-1 showed positivity in 83% of the cases of epithelioid type, 60% in sarcomatoid and 100% in biphasic type.

The percentage of the results for the applied antibodies in MPM expected to be negative was: antibody to TTF-1 was negative (100%) in all types. Antibody to CEA was performed in epithelioid and biphasic type and in both cases it was negative.

Discussion

The final diagnosis of MPM is performed exclusively by pathohistological examination of biopsy-tissue samples, but frequently immunohistochemical analysis is necessary as additional method. Immunohistochemistry is the most reliable method that allows adequate differentiation and overcoming the diagnostic problems in the diagnostic algorithm.
of malignant pleural mesothelioma \(^6\). \(^7\). Due to the lack of tissue-specific positive or negative antibody it is now strongly recommended to use palettes of antibodies that combines two or more mesothelial positive antibodies with two or more epithelial positive antibodies \(^3\)–\(^5\).

During the last decade, production and usage of a large number of antibodies, with different sensitivity and specificity, placed MPM to serious research activities hoping to discover a winning combination of antibodies.

Keratin antibodies are among the most commonly used antibodies when there is a suspicion on MPM, especially for distinguishing MPM from adenocarcinoma and sarcoma \(^8\). CK5/6 is the most adequate antibody used for this purpose \(^9\). This antibody was performed in 27 of our patients and showed positivity in 63% of the cases. The highest positivity was shown in biphasic type (100%), followed by epithelioid (68%) and lowest in sarcomatoid (20%) type of MPM. A research performed by Soomro et al. \(^6\) showed high positivity of this antibody in biphasic (60%) and epithelioid type (100%), and somewhat less in sarcomatoid type (28.6%), as well. Although not showing 100% positivity for all types, antibody to CK5/6 is considered one of the most specific antibodies and most sensitive in the diagnosis of MPM. It is proved to be useful in differentiation of sarcomatoid type of MPM from most sarcomas \(^10\), as well as in differentiation of MPM from lung adenocarcinoma \(^9\). It is believed that if CK5/6 and anti-mesothelial antibodies are positive, diagnosis of MPM should be made \(^11\). Antibody to CK7 was positive in 78%, while antibody to panCK was applied only in one tissue sample, and the result was positive. A positive finding to these antibodies is extremely useful concerning the diagnosis of epithelioid type of MPM because it confirms the process of epithelialization in the sample treated \(^12\). Antibody to CK20 was performed in 4 cases of the epithelioid type of MPM and it was negative in all of them.

Calretinin belongs to a large family of calcium-binding cytoplasmic proteins. Antibody to calretinin was positive in more than 95% of the patients with epithelioid and biphasic type of MPM. Other authors state that the sensitivity of this antibody depends on the clones used and ranges from 73% to 100% \(^2\). All the authors agree that it provides an excellent positivity in epithelioid and biphasic type, but in terms of sarcomatoid type, there are conflicting data. Kayser \(^13\) confirms good positivity (more than 50%) to calretinin in sarcomatoid type of tumor \(^13\), but there are also data suggesting that this antibody is negative in sarcomatoid type \(^10\). In our research, we concluded that the antibody to calretinin was positive in 90% of epithelioid and in all the cases (100%) of sarcomatoid and biphasic type, thus agreeing with the majority of authors that it is one of the most useful antibodies in the diagnosis of MPM.

In our study, the HBME-1 antibody was positive in 83% of epithelioid, 60% of sarcomatoid and 100% of biphasic type of MPM. Although this antibody shows a high degree of sensitivity for MPM \(^14\), today it is not considered as a specific antibody for MPM. Recent studies have shown a good reactivity of HBME-1 antibody in tissue with adenocarcinoma of the lung, kidney, thyroid and other tumors of the female genital tract \(^15\). However, because of its sensitivity, it is still used in the diagnosis of MPM, as one of the most potent antibody.

In our patients, antibody to vimentin showed 100% positivity in all three types of MPM, but concerning the fact that only 8 samples were treated with this antibody, we cannot rely on its sensitivity. Kayser \(^13\) ranged the positivity of vimentin antibody from 8% to 100%, depending on the type of MPM, while other authors listed positivity to vimentin in all samples treated in all three types of MPM \(^1\)–\(^5\). Together with antibodies to CK5/6 and/or HMBE-1, antibody to vimentin can be used as a very good indicator for MPM when it is necessary to distinguish it from metastatic carcinoma of the pleura \(^2\).

Epithelioid and biphasic type of MPM in our patients showed 100% positivity for antibody to EMA, while the positivity of sarcomatoid type was 50%. Many papers argue that positivity of this antibody is expressed in MPM and metastatic adenocarcinoma, while the other recorded its negativity in mesothelial hyperplasia \(^2\). From our point of view, both questions are open for further research and discussion. In everyday laboratory work, antibody to EMA showed excellent results in the diagnosis of MPM, especially in the differentiation of epithelioid type MPM from metastatic adenocarcinoma. It was often used in many laboratories combined with antibody to CEA for making the differential diagnosis of MPM \(^1\)–\(^12\).

Antibody to CEA is one of the most commonly used and probably the best antibody in distinguishing MPM from adenocarcinoma \(^11\), \(^12\). Nevertheless, in our study negative results were obtained in all the cases. There are a few documented cases with MPM positive to CEA, but it is assumed that this positivity is due to the nature and chemical composition of antibody \(^2\). Our results supported the usage of monoclonal antibody to CEA and agreed with the findings of the majority of authors. This contributes the fact of CEA negativity in diagnosis of MPM.

Antibody to TTF-1 was positive in 75%–85% of lung adenocarcinoma and adenocarcinoma, while in cases with MPM in all histological types negativity is expressed in 100% \(^2\). In our study, this antibody proved negativity in all the cases and all types of MPM confirming its major role in the differential diagnosis of MPM.

Antibodies to MOC-31 and desmin were performed in only one case showing the expected negativity. Concerning the fact that analysis was done in only one patient, major conclusions about the role of these antibodies cannot be made, but their negativity contribute to differentiation of MPM from other tumors \(^10\), \(^14\).

**Conclusion**

Immunohistochemistry has become an essential diagnostic procedure in the diagnosis and determining the type of MPM. The deficiency of tissue-specific antibody for MPM ensures the usage of combinations of antibodies with different sensitivity and specificity.
Antibody to CK5/6 (with the expressed positivity) has excellent sensitivity and specificity in differentiating epithelioid type MPM from adenocarcinoma of the lung and sarcomatoid type of MPM from most sarcomas.

Calretinin antibody is one of the most useful and most commonly used antibodies, which provides excellent positivity in epithelioid and biphasic type of MPM, but less in sarcomatoid type.

Antibody to HBME-1 has a low specificity in the diagnosis of MPM, but on the other hand it shows excellent sensitivity and is now widely used as a positive antibody in the diagnostic algorithm of MPM.

Antibodies to TTF-1 and CEA are the most important negative antibodies which are of great confidence in the differential diagnosis of epithelioid type MPM from lung adenocarcinoma.

REFERENCES


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