# APPLICATION OF IMMUNOHOSTOCHEMICAL METHODS IN BONE MARROW TREPHINE BIOPSY

## ZASTOSOWANIE METOD IMMUNOHISTOCHEMICZNYCH W TREPANOBIOPTATACH SZPIKU

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*Summary:* The aim of this study was to demonstrate the role of immunohistochemistry in bone marrow trephine biopsy diagnostic protocol as tool for evaluation of primary tumor origin.
The study was based on materials collected from 39 patients diagnosed at Department of Tumor Pathology at the Greater Poland Oncology Centre. Material was collected from cases diagnosed in the period from 2002 to 2008. The average age of the patients was 42 years.
To achieve the aim of this project, immunoperoxidase methods were used based on Dako EnVision complex methodology. The studied material, the following markers were assessed: CK AE1/AE3, CK7, CK20, GCDFP15, PSA, TTF1, THY, CEA as well as ER and PgR receptors.
In 10 patients (25.6%) we revealed no infiltration of the marrow. Neoplastic infiltration of the bone marrow was found in 29 cases, representing 74.4% of the whole material. Among those 29 cases there were: 17 cases of metastases from the breast (58.6%), three cases of metastasis of adenocarcinoma (10,3%) and three cases metastases of undifferentiated cancers (10,3%), and one case of each the following metastasis of cancer such as: ovarian carcinoma (3.4%), from the urinary bladder (3.4%), from the prostate gland (3.4%), gastric cancer (3.4%), lung cancer (3.4%), non-epithelial neoplasm (3.4%).
On the basis of our results we could conclude that the use of immunohistochemical methods in the evaluation of bone marrow trephine biopsies could be of a great value. The use of appropriate panel of antibodies may put a significant impact on the diagnosis of hematological, as well as non-hematological neoplastic diseases found in the bone marrow.

*Keywords*: bone marrow infiltrations, tumor markers, diagnosis, immunohistochemistry

*Streszczenie:* Celem pracy było przedstawienie zastosowania metod immunohistochemicznych w diagnostyce szpiku kostnego jako narzędzie do oceny pochodzenia zmian nowotworowych (w tym przerzutów).

Badania były przeprowadzone na materiale pochodzącym od 39 pacjentów diagnozowanych w Wielkopolskim Centrum Onkologii w latach 2002-2008. Średnia wieku pacjentów to 42 lata.
Do osiągnięcia celu projektu, zastosowano metody peroksydazowe bazujące na protokole systemu Dako EnVision. W badanym materiale oznaczono następujące markery: CK AE1/AE3, CK7, CK20, GCDFP15, PSA, TTF1, THY, CEA, a także receptory ER i PgR.
U 10 pacjentów (25,6%) nie wykryto przerzutów do szpiku kostnego. Nacieczenie szpiku kostnego stwierdzono w 29 przypadkach, co stanowi 74,4% całego badanego materiału. Wśród tych 29 przypadków było: 17 przypadków przerzutów raka piersi (58,6%), trzy przypadki przerzutów nowotworu pochodzenia gruczołowego (10,3%), trzy przypadki przerzutów raka niezróżnicowanego (10,3%) oraz po jednym przypadku (tj. 3,4%) przerzutów: raka jajnika, raka gruczołu krokowego, raka pęcherza moczowego, raka żołądka oraz nowotworu nienabłonkowego.
Na podstawie naszych wyników można stwierdzić, że zastosowanie metod immunohistochemicznych w ocenie trepanobioptatów szpiku kostnego może mieć dużą wartość diagnostyczną. Zastosowanie odpowiedniego panelu przeciwciał, może mieć znaczący wpływ na rozpoznanie chorób hematologicznych, jak również niehematologicznych chorób nowotworowych zajmujących szpik kostny.

*Słowa kluczowe:* przerzuty do szpiku kostnego, markery nowotworowe, diagnostyka, immunohistochemia

## INTRODUCTION

The use of immunohistochemistry in the diagnosis of bone marrow trephine biopsy is a part of daily work in departments of patology all over the world. The aforementioned methods have become widely used in pathological practice, in particular in the diagnosis of cancer. Those methods are used routinely in laboratories. When appropriate markers are used it allows the assessment of the histological type of the tumor, the differentiation of epithelial-type cancers, the diagnosis of soft tissue cancers, endocrine system changes and neuroendocrine tumors [10, 11, 12]. One of the advantages of using immunohistochemical methods in daily work is the need to determine the primary site of cancer.

In this study, we investigated the invasion of bone marrow by cancer cells. In primary hemato-oncological tumors, bone marrow biopsy diagnosis is the most important part in assessing disease diagnosis and classification and staging. The standard techniques used for appropriate diagnosis of those disorders include immunohistochemistry, flow cytometry, cytogenetic tests and histopathological examination of bone marrow biopsies. The last mentioned being obtained by trephine biopsy of the hip plate [15]. Additionally, such studies allow to determine the severity of the disease, to recognize remission and relapse, and are essential in monitoring the therapy [14].

In hematopoietic diseases, bone marrow biopsy is an important diagnostic procedure. The bone marrow can be obtained by aspiration through the needle (aspiration biopsy) or trephine biopsy.

Skeleton is the third place in terms of the incidence of cancer metastases. Very often, bone metastasis occurs after the diagnosis of the primary location of the disease. However, there are cases of bone metastasis, in which the metastasis can be the source of the first clinical symptoms of cancer. In this case, the speed and precision of the diagnostic procedure is very important. Proper and rapid diagnosis determines the beginning of the correct treatment, and this allows to achieve several years of survival or at least a significant improvement in the quality of life in the majority of patients [8].

Trephine biopsy becomes an increasingly frequent procedure. However, it is a procedure that carries with it a certain risk and therefore it should be performed only when there are clear indications. Trephine biopsy has become an indispensable procedure in determining marrow cellularity, actual bone marrow cell distribution, as well as in determining their spatial relationship to stromal cells [29, 30]. The indications for trephine biopsy among other reasons is previously done the so-called "empty punctures" during repeated aspiration biopsy of the marrow. Other indications include suspicion of diseases such as: bone marrow fibrosis, myelodysplastic syndrome, dissemination of myeloma and leukemia, bone marrow healing by malignant lymphoma, bone cancer metastases, monitoring of treatment and development of the disease; assessment of residual disease, before and after bone marrow transplantation, with particular emphasis on patients with acute leukemia, assessment of aplasia and bone marrow hypoplasia in pancytopenia or bicytopenia [6].

Bone marrow is usually taken from flat bones such as: sternum, spinous processes of lumbar spine, wing of ilium. In small children the bone marrow is taken from the tibia. In a single puncture between 0.2-0.3 ml bone marrow is being aspirated. If necessary, larger amounts of bone marrow (0.5-2 ml) could be taken. It could be used for cytology, as well as for other techniques such as: fluorescence, electron microscopy, tissue culture, parasite tests. Contraindications to perform trephine biopsy are hemorrhagic diathesis, including disseminated intravascular coagulation syndrome (DIC) [6].

One should remember, that trephine biopsy is not an alternative procedure to aspiration biopsy, but rather serves as complementary protocol. It allows the use of more bone marrow, allows to precisely determine the cellularity and architecture of the marrow, as well as to evaluate structures other than hematopoietic cells [31].

## EPIDEMIOLOGY OF METASTASIS TO THE BONE MARROW

Malignant neoplasms, depending on the clinical stage, are characterized by different frequency of bone metastases. The detailed epidemiological data are presented in table 1.

**TABLE 1.** The incidence of bone metastases depending on the severity of the clinical cancer

| MALIGNANT NEOPLASM | FREQUENCY OF APPEARENCE |
|---|---|
| Soft tissue sarcoma | 25% |
| Malignant melanoma | 15% |
| Prostate cancer | > 80 % |
| Breast cancer | > 70 % |
| Thyroid cancer | 70 % |
| Non-small-cell lung carcinoma | > 30 % |
| Bladder cancer | 25 % |
| Kidney cancer | > 20% |
| Endometrial cancer | > 20% |

Taking into account the incidence of particular cancers in clinical practice, bone metastases are most often diagnosed in the course of breast cancer, non-small-cell lung cancer, prostate cancer, kidney cancer, colorectal cancer, stomach cancer, and endometrial cancer. Bone metastases are most often located in: spine of 61.8%, femur 10.4%, ribs 9.5%, skull 8.8%, pelvic bones 4.7%, sternum 2.1%, humerus 1, 3%. In other bones, there is only 1.4% of metastases.

The spread of cancer cells to bone marrow tissues is a phenomenon that occurs in the terminal stages of the disease. The primary site of neoplasm that gives metastases to the bone marrow is most often tumors of the breast, prostate, lung, thyroid, kidney and stomach. Bone marrow examination should be carried out in patients who have been diagnosed with or suspected to be a neoplastic disease in which the assessment of the severity of the disease affects the choice of treatment. The trephine biopsy is more likely to be detected than the aspiration of fine needle aspiration, but it is recommended to perform both procedures simultaneously in order to increase the probability of detecting cancer cells (tab. 2). Trephine biopsy allows the assessment of the structure of the collected tissue and using immunohistochemical analysis one can infer about the type of cancer and determine the starting point of the metastasis. For this purpose, one should use markers that enable detection and determination of the origin of neoplastic metastases occurring most often in the bone marrow. These markers are presented in table 2.

Before analyzing the patient's bone marrow, one should consider what treatment is planned and whether the detection of metastasis will affect the therapeutic treatment [17, 29, 31, 34, 35, 36, 37].

**TABLE 2.** List of tumor markers determined in the bone marrow

| TYPE OF CANCER | MARKERS |
|---|---|
| Breast | GCDFP15, ER, PGR, CK AE1/AE3, CK 7, CK 20, |
| Prostate cancer | PSA PSAP, CK AE1/AE3, CK 7, CK 20, |
| Lung cancer | TTF1, CHR, NSE, CK AE1/AE3, CK 7, CK 20, , |
| Thyroid cancer | THY, Kalcytonina, TTF1, CK AE1/AE3, CK 7, CK 20, |
| Kidney cancer | CD10, RCA, CK AE1/AE3, CK 7, CK 20, |
| Stomach cancer | CEA, CK AE1/AE3, CK 7, CK 20, |

## AIM OF THE STUDY

The aim of this study is to determine the basic panel of markers used to detect cancer cells in the bone marrow and to determine a panel of markers to establish cancer histogenesis.

## MATERIAL AND METHODS

### CLINICAL MATERIAL

The material for the study was collected from 39 patients of the Greater Center of Oncology in Poznan treated in years 2002-2008. The average age of patients is 42 years; the oldest patient was 76 and the youngest was 31 years old.

### IMMUNOHISTOCHEMICAL METHODS

Two-stage immunoperoxidase methods with the En Vision complex were used. The characteristics of the antibodies used are shown in table 3.

**TABLE 3.** List of applied antibodies

| ANTIBODIES | CLONE | CATALOG NUMBER | DILUTION |
|---|---|---|---|
| Monoclonal mouse antibodies against the Cytocreatin | Clone AE 1 i AE 3 | M 3515 DAKO | 1/50 |
| Monoclonal mouse antibodies against the CK 7 | Clone OV-TL 12/30 | M 7019 DAKO | 1/100 |
| Monoclonal mouse antibodies against the CK 20 | Clone $K_s$20.8 | M 7019 DAKO | 1/20 |
| Polyclonal rabbit antibodies against the PSA | - | A 0562 DAKO | 1/3000 |
| Monoclonal rabbit antibodies against the TTF-1 | Clone 8G7G3/1 | M 3575 DAKO | 1/10 |
| Polyclonal rabbit antibodies against the CEA | - | A 0115 DAKO | 1/3000 |
| Polyclonal rabbit antibodies against the MPO | - | A 0398 DAKO | 1/6000 |
| Polyclonal rabbit antibodies against the THY | - | A 0251 DAKO | 1/2000 |
| Polyclonal rabbit antibodies against the CD3 | - | A 0452 DAKO | 1/100 |
| Monoclonal mouse antibodies against the CD10 | - | (N Novocastra – CD10, 56C6, NCL-CD10-270) Novocastra United Kingdom | 1/100 |
| Monoclonal mouse antibodies against the CD20 | Clone L26 | M 0755 DAKO | 1/300 |
| Monoclonal mouse antibodies against the estrogen receptor | Clone 1D5 | M 7047 DAKO | 1/50 |
| Monoclonal mouse antibodies against the progesteron receptor | Clone PGR 636 | Catalog number M 3569 DAKO | 1/100 |
| DAKO En- Visio+® System Labelled Polimer- HRP Anti- Rabbit | - | K 4003 | - |
| DAKO En- Visio+® System Labelled Polimer- HRP Anti- Mouse | - | K 4001 | - |

In order to verify the correctness of the performed staining, appropriate controls were used (in accordance with the methodological recommendations). A list of positive controls for performed staining is given in table 4.

**TABLE 4.** A set of positive controls used in research

| ORGAN | EVALUATED ANTIGEN |
|---|---|
| Salivary glands | CK AE1/AE3 |
| Large intestine | CK 20 |
| Kidney | CK 7 |
| Gastric mucosa | CEA |
| Prostate | PSA |
| Tonsills | CD 3, CD 10, CD 20, MPO |
| Nipple | ER, PgR |
| Thyroid | TTF-1 |

Tissue material was fixed in 10% buffered formalin, dehydrated in a series of alcohols (50%, 70%, 96%, 99.9%) and in xylen. Next, material were immersed in paraffin at a temperature of 58 °C according to classic histological methods. The paraffin blocks were cut into 4-5 μm thick sections and applied onto slides.

## IMMUNOPEROXIDATIC METHODS

For detection of antigens: cytokeratins AE1 / AE3, cytokeratin 7, cytokeratin 20, CD 3, CD 20, TTF-1, estrogen receptors, progesterone receptors, methods from the DAKO En-Vision complex were used.

For the detection of antigens: CD10, MPO, methods from the DAKO En-Vision complex were used. For the detection of antigens: PSA, THY, CEA, methods were used from the DAKO En-Vision complex.

## RESULTS

In the years 2002-2008, 39 trephine biopses of bone marrow were selected for the further studies. They were patients of the Greater Poland Cancer Center in Poznan in whom immunohistochemistry of bone marrow biopsy was performed. A detailed results are presented on chart 1.

In the diagnosis of bone marrow metastases the basic panel of tumor markers was used, namely a panel of antibodies directed against CK AE1/AE3, CK 7, CK 20; the results are given in table 5.

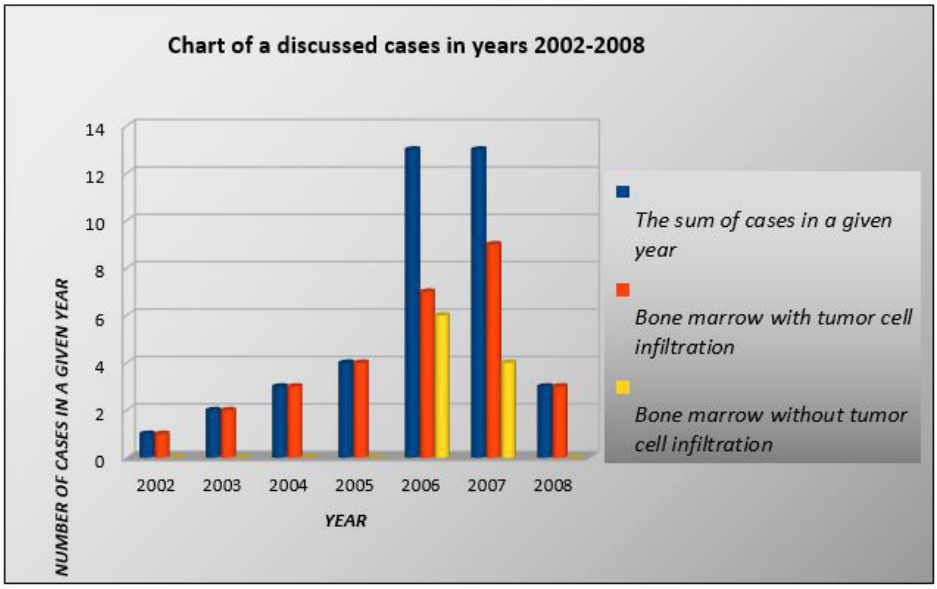



**CHART 1.** Number of discussed cases of trephine biopsy between years 2002-2008

**TABLE 5.** Comparison of cytokeratin reactions depending on the type of metastasis

| MARKER / TYPE OF METASTASIS | CK AE1/AE3 | CK 7 | CK 20 |
|---|---|---|---|
| Metastasis of breast cancer | + | + | - |
| Metastasis of cancer from the ovary | + | + | - |
| Metastasis of cancer from the bladder<br>Metastasis of cancer from the bladder | + | + | + |
| A metastasis of cancer from the prostate gland | + | + | + |
| A metastasis of cancer from the stomach | + | + | + |
| Metastasis of cancer from the lungs | + | - | - |
| A metastasis of glandular cancer | + | - | - |
| A metastasis of undifferentiated cancer | + | + | - |
| Metastasis of non-epithelial cancer | - | - | - |

By use of a immunohistochemistry method in the bone marrow trephine biopsy, the cancer starting point was diagnosed (representative examples are presented on Figure 1-19). Types of bone marrow infiltrating infiltrates as well as tumor markers are shown in table 6 and figures 1-19.

**TABLE 6.** Non-lymphoid metastases detected in the bone marrow

| TYPE OF CANCER | NUMBER OF CASES OF BONE MARROW OCCUPANCY | MARKERS MARKED |
|---|---|---|
| Breast cancer | 17/29 (58,6%) | CK AE1/AE3, CK 7, CK 20, P15, Er, PGR |
| Ovary cancer | 1/29 (3,4%) | CK AE1/AE3, CK 7, CK 20, Ca 125 |
| Blader cancer | 1/29 (3,4%) | CK AE1/AE3, CK 7, CK 20, PSA, LCA |
| Prostate cancer | 1/29 (3,4%) | CK AE1/AE3, CK 7, CK 20, PSA |
| Stomach cancer | 1/29 (3,4%) | CK AE1/AE3, CK 7, CK 20, CEA |
| Lung cancer | 1/29 (3,4%) | CK AE1/AE3, CK 7, CK 20, TTF1 |
| A metastasis of adenocarcimona | 3/29 (10,3%) | CK AE1/AE3, CK 7, CK 20, śluz, CEA, MPO |
| A metastasis of undifferentiated cancer | 3/29 (10,3%) | CK AE1/AE3, CK 7, CK 20, |
| Metastasis of non-epithelial cancer | 1/29 (3,4) | CK AE1/AE3, CK 7, CK 20, wimentine |

39 bone marrow biopsies were studied using immunohistochemical methods. In 29 cases, bone marrow involvement by cancer cells was observed. In 10 cases the bone marrow was free from tumor cell infiltration. As control we used 10 trephine biopsies without overt disease. The results are shown in table 7.

In individual cases where it was difficult to determine the primary source of metastasis due to the very small amount of material, it was only possible to determine the origin of the tumor. The results are shown in table 8.

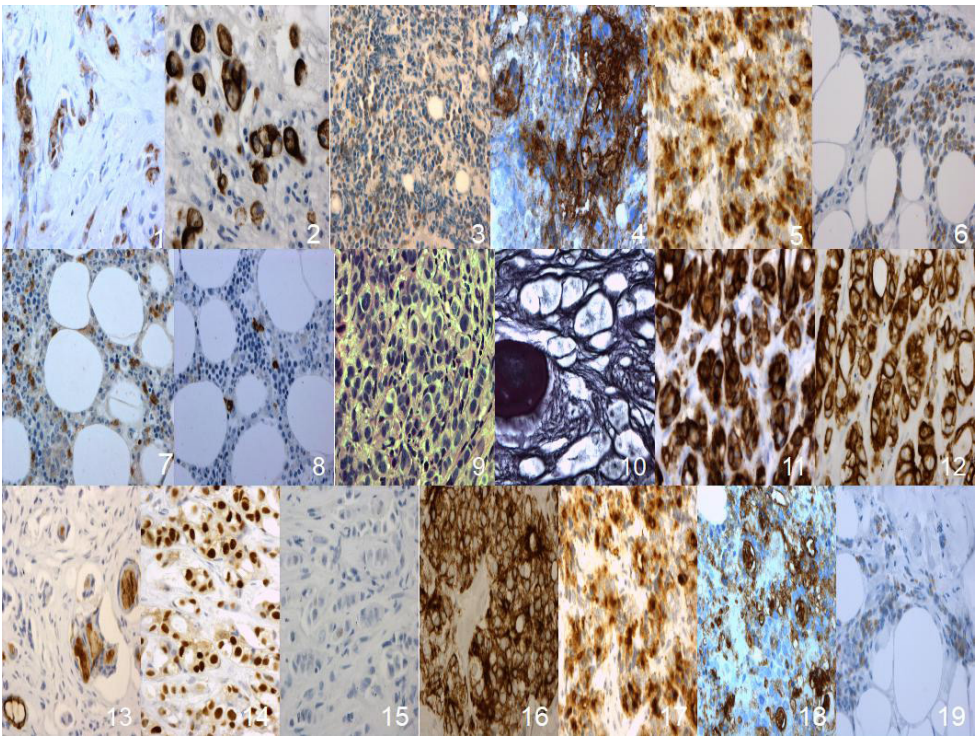


**FIGURE 1.** Positive expression of GCDFP15 for metastasis of breast cancer
**FIGURE 2.** Positive CK AE1/AE3 expression in the case of metastatic ovarian cancer
**FIGURE 3.** Positive expression of the TTF1 marker in a lung cancer metastasis
**FIGURE 4.** Positive reaction of the CEA marker in the case of gastric cancer metastasis
**FIGURE 5.** Positive expression of the PSA marker in the case of metastatic prostate cancer
**FIGURE 6.** Positive expression of the CK 20 marker in the event of bladder cancer
**FIGURE 7.** Positive expression of the CD3 marker in the bone marrow in which no cancer cells were detected
**FIGURE 8.** Positive expression of the CD20 marker in the bone marrow in which no cancer cells were detected
**FIGURE 9.** Bone marrow with infiltration of breast cancer cells; hematoxylin-eosin stain
**FIGURE 10.** Bone marrow with infiltration of breast cancer cells, staining with silver-absorbing fibers
**FIGURE 11.** Bone marrow with infiltration of breast cancer cells, positive expression of CK AE1/AE3
**FIGURE 12.** Bone marrow with infiltration of breast cancer cells, positive expression of CK7
**FIGURE 13.** Bone marrow with infiltration of breast cancer cells, positive expression of GCDFP15
**FIGURE 14.** Bone marrow with infiltration of breast cancer cells, positive expression of the estrogen receptor
**FIGURE 15.** Bone marrow with infiltration of breast cancer cells, positive expression of the progesterone receptor in single cells
**FIGURE 16.** Bone marrow with infiltration of bladder cancer cells, positive expression of CK20
**FIGURE 17.** Bone marrow with infiltration of prostate cancer cells, positive PSA expression
**FIGURE 18.** Bone marrow with glandular adenoma cell infiltration, positive expression of CEA
**FIGURE 19.** Bone marrow with infiltration of breast cancer cells, positive expression of the progesterone receptor in single cells

**TABLE 7.** Evaluation of bone marrow involvement depending on the type of metastasis

| | |
|---|---|
| Metastasis of breast cancer | 17/29 (58,6%) |
| Metastasis of cancer from the ovary | 1/29 (3,4%) |
| Metastasis of cancer from the bladder | 1/29 (3,4%) |
| Metastasis of cancer from the prostate gland | 1/29 (3,4%) |
| A metastasis of cancer from the stomach | 1/29 (3,4%) |
| Metastasis of cancer from the lung | 1/29 (3,4%) |
| A metastasis of adenocarcimona | 3/29 (10,3%) |
| A metastasis of undifferentiated cancer | 3/29 (10,3%) |
| Metastasis of non-epithelial cancer | 1/29 (3,4%) |

**TABLE 8.** List of positive reactions for markers determined in the bone marrow depending on the origin of the tumor

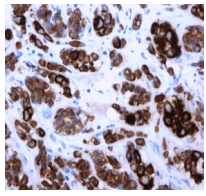
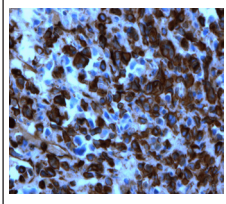
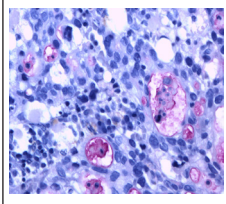
| **TYPE OF METASTASIS** / **POSITIVE REACTION** | Undifferentiated cancer | Non-epithelial cancer | Adenocarcinoma |
|---|---|---|---|
| CK AE1/AE3 | 3/3 (100%) | 0/1 (0%) | 2/2 (100%) |
| CK 7 | 3/3 (100%) | 0/1 (0%) | 0/2 (0%) |
| CK20 | 0/3 (0%) | 0/1 (0%) | 0/2 (0%) |
| WIMENTYNA | | 1/1 (100%) | |
| THY | | | 0/2 (0%) |
| CEA | | | 1/2 (50%) |
| MUCYKARMIN | | | 1/2 (50%) |
| Selected examples (40x lens) |  Positive expression of CK AE1 / AE3 |  Positive expression of vimentin |  Positive reaction in mucicarmine staining |

**TABLE 9.** List of positive markers reactions determined in the marrows without tumor cell infiltration

| CK AE1/AE3 | CD 3 | CD 20 |
|---|---|---|
| 0/10<br>(0%) | 10/10<br>(100%) | 10/10<br>(100%) |

In 10 cases, i.e. in the control group, lymphoid cells were observed. In order to detect them, a panel of markers including such markers as CD 3, CD 20, MPO, Hb. Was used (tab. 9).

## DISCUSSION

Immunohistochemical methods in the study of bone marrow biopsies allow not only the proper diagnosis, but also allow to assess the severity of the disease, the effects of treatment and also predict the further consequences of the disease. In the diagnosis of bone metastases to the bone marrow, the basic panel of markers, namely the anti-CK AE1/AE3, CK7, CK 20 panel, proved to be useful in the study. Use of this panel allowed to assess the nature of epithelial or non-epithelial tumors. In the case of non-epithelial cancer, it was necessary to detect the following markers: desmine, vimentine, neuron specific enolase, synaptophysin. After this assessment, it was possible to propose an extended panel, which enabled further, detailed diagnostics.

In detailed diagnostics, an extended panel was used, which included markers characteristic for a given organ. And so for the gland, they were antibodies against GCDFP 15, as well as receptors such as estrogen receptor alpha and progesterone receptor. In the case of the ovary, Ca 125 was determined, and antibodies to PSA were detected for the prostate; for the bladder, these were antibodies against PSA and LCA (CD45); in the case of the lung it was an anti-TTF1 antibody, for the stomach against CEA and for the liver anti-CEA and MPO antibodies. In the diagnosis of bone marrows without tumor infiltration, a panel of markers was used, which consisted of antibodies directed against CD 3, CD 20 ,. CK AE1 / AE3.

However, it should be remembered that immunohistochemical analysis requires a lot of experience. A well-chosen panel of markers does not always make it possible to make an accurate diagnosis. The result largely depends on the histopathological development of the material.

The preparation of the material has a very large impact on the quality and success of the study. Poorly prepared material will cause difficulties in the evaluation of preparations, and thus will not give a definite answer about the patient's condition.

Based on immunohistochemistry in 39 bone marrow trephine biopsy, bone marrow infiltration by tumor cells was found in 29 cases, which is 74.4%. Whi-

le in 10 cases (25.6%), i.e. in the control group, no cancer cells were detected. Among cancerous infiltrations, metastases were found in the breast, stomach, prostate, bladder and lung (79.3%) cancers. The remaining cases, i.e. 6 cases (20.7%), were cases in which it was only possible to determine the origin of the metastasis. Thus, 3 cases are metastases of glandular origin, which is 42.9%; 1 case is metastasis of non-epithelial cancer (14.3%) and another 3 cases are metastases of undifferentiated cancer (42.9%). The basic panel of antibodies that was determined in all cases included CK AE1 / AE3, CK7, CK20 antibodies. In the case of CK AE1 / AE3, a marker of epithelial-derived tumors, positive expression was not only present in non-epithelial tumor metastases and in the control group. Positive expression of CK 7 occurred in most cases excluding prostate cancer and lung cancer. In contrast, positive expression of CK 20 occurred in the case of bladder cancer and stomach cancer. In addition to the basic panel of markers, a marker characteristic for each organ was chosen for each type of cancer, where positive expression occurred. And in the case of breast cancer in addition to the detection of the primary panel, GCDFP15 protein was also detected, as well as receptors such as estrogen and progesterone receptors. In the case of GCDFP15 protein, positive expression occurred in 6 cases out of 17, which is 35.3% and in the case of receptors: estrogen 11/17 (64.7%), progesteron 7/17 (41.2%). For ovarian cancer, the detected markers were cytokeratins, where positive expression was obtained only in CK 7. In addition to cytokeratins, Ca 125 was detected, where negative expression was obtained. In stomach cancer, positive expression was obtained for the entire base panel, as well as for additional markers detected such as CEA, CAM 5.2 and chromogranins. For the prostate gland, positive expression occurred in the case of CK AE1 / AE3, and the additional marker was PSA, where positive expression was also obtained.

In the case of lung cancer, a positive expression occurred in the case of CK AE1 / AE3 as well as in the case of TTF-1 and synaptophysin, which are markers detected in such cancer. For the bladder, in addition to the primary panel cytokeratins, whose expressions were positive, markers such as PSA, where negative expression and LCA were detected, were obtained positive.

The remaining cases are people who were able to determine the origin of the tumor due to the small amount of material without specifying the source of the metastasis. In the case of glandular tumors, positive expression was obtained with CK AE1 / AE3. In this case, a positive reaction to mucicarmins was also obtained. For undifferentiated cancers, positive expression was obtained for CK AE1 / AE3 and CK 7, whereas for non-epithelial cancers, positive expression was obtained only in the case of vimentin detection. In bone marrows without tumor cell infiltration, CD20 expression was detected in B and CD3 lymphocytes in T lymphocytes. Expression was positive in all cases. Expression of CK AE1 / AE3 was also detected. In the cases discussed, it was negative.

In conclusion, it could stated, that immunohistochemistry has become a method that is irreplaceable in diagnostic protocols on the daily practice protocol. It allows the diagnosis of many neoplastic, hematological and non-haematological diseases, which in the case of clinical cases has been demonstrated in this work.

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