

Research Article



Prognostic Value of Individual Tumor Cells in Bone Marrow, Lymph Nodes in Patients with Non-small Cell Lung Carcinoma

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Received: 22-04-2017; Revised: 18-05-2017; Accepted: 14-07-2017.

ABSTRACT

The results of the examination of the bone marrow and distant lymph nodes for the presence of circulating tumor cells in 83 patients with non-small cell lung cancer T2-3N0M0, who had been radically operated, are given. Isolated tumor cells were determined by immune cytochemical and immune histochemical methods with the monoclonal antibodies to cytokeratins. Isolated tumor cells were detected in bone marrow in 30.1% and in lymph nodes in 20.5% of patients. The simultaneous presence of isolated tumor cells in bone marrow and lymph nodes was found in 18.1% of patients that significantly reduced the 3-year survival rate. Isolated tumor cells can be considered as a negative prognostic factor that must be taken into account when choosing the adjuvant treatment of lung carcinoma.

Keywords: Lung carcinoma, lymph nodes, Bone marrow.

INTRODUCTION

Lung carcinoma (LC) is the most widespread human tumor. Results of treatment for patients with LC are unsatisfactory due to late diagnosis and high aggressivity of tumor. The latter possessing high metastatic potential, which is realized yet at an early stage of disease as metastases in lymph nodes (LN) and hematogenic metastases in all, without exception, human organs and systems. Bone marrow (BM) is also targeted organ for distant metastases of LC and other tumors /1/.

The main prognostic factors in LC are the size of primary tumor, quantity of affected LN, and degree of differentiation of tumor cells. Therefore, the question of circulating tumor cells (CTC) detection in LN and BM in patients with LC and their effect on disease prognosis remains actually today.

Light microscopy method has a limit of their capacity and allows identifying 1 tumor cell to 100 healthy BM cells. Advances in molecular biology have made possible CTC detection in biological samples. In recent years such molecular biology methods as immune cytochemical and immune histochemical have been widely used for CTC detection. These methods are based on the use of monoclonal antibodies (MAB) for cytokeratins, which are expressed in membranes of tumor cells. The reaction allows to detect 1 tumor cell to 500 000 healthy cells.

Due to implementation of these methods in laboratory practice, in recent years many reports appears about CTC detection in BM in patients with breast, gastric, colorectal, prostate and other types of cancer / 2,3,4 /.

X.F. Deng and coauthors /5/ have described data of BM test for presence of tumor cells in patients with non-small cell LC, and micro metastases were detected in 22% of patients.

With the help of molecular biological testing methods also have been detected CTC in distant LN with LC where under light microscopy metastases were not found /6/. Various studies using immuno histochemical method have estimated, that in 27-53% patients, which had been operated for LC, were found CTC in histological tumor negative LN / 7 /.

Many authors are of the opinion that the presence CTC in BM and LN is bad prognostic factor even on early stage of cancer /8, 9, 10/.

MATERIALS AND METHODS

The aim of the given study was improving diagnosis of LC extension to LN and BM by using of immune cytochemical and immune histochemical methods as well studying of influence of these factors on disease prognosis. For this purpose 83 patients with non-small cell LC without affection of regional lymph nodes, which had been radically operated (pneumonectomy – 29 and lobectomy – 54), were examined with mandatory ipsilateral mediastinal lymphadenectomy. Among examined were 66 men and 17 women. 41 patients had disease stage T₂N₀M₀, 42 – T₃N₀M₀. After treatment 3 years survival rate of patients was studied.

Before the operation patients with LC were subjected to sternal puncture and some smears were placed on glass slides. Some of them were stained with hematoxylin and eosin and light microscopy was performed to find cancer cells. For those patients in whom no cancer cells were found, smears of BM were studied with MAB to cytokeratins. In those areas where the reaction with MAB occurred, pink coloration was observed, which was regarded as the presence of cancer cells, because there are not epithelial cells among cell elements of BM.



Deleted during the operation LN were marked and sent to the laboratory, where, after treatment with reagents, paraffin blocks were performed and usual studies with light microscope were carried out to find cancer metastasis. In those cases where in LN cancer cells were not found, 4-5µm paraffin sections of LN were performed, attached on glass slides and after appropriate preparation treated with MAB to cytokeratin. In the sites of the antigen localization, which is detected by MAB, a brown granular staining arises, which is regarded as the presence of cancer cells.

RESULTS AND DISCUSSION

Using light microscopy not only searching of cancer cells in BM was carried out, but status of surrounding cells was studied. BM in 48 (57.8%) patients was cellular and active. Ratio of lymphoid cellular elements to erythron was 3:1, cells, which are non relevant to normal bone marrow hematopoiesis, were not found.

In 19 (22.9%) patients against the background of depressed BM cellularity, cells, which are increased in size, round shape, multinucleated with hyper basophylic vacuolated cytoplasm, were detected (fig. 1). These cells were considered as malignant, which are metastasizing in BM. Complexes on several cancer cell were found most often. It should be noted that the detection of isolated cancer cells in smears of BM punctates only by cytological characteristics is associated with many difficulties. This is due to the presence of cells, which have certain similarities with tumor cells, in the BM. This is concerned to the reticular cells, immature cell elements of megakaryocytic series. In addition, tumor cells, getting to another microenvironment may lose some of their characteristic cytomorphological features and thus mask as reactively changed or young cellular elements.

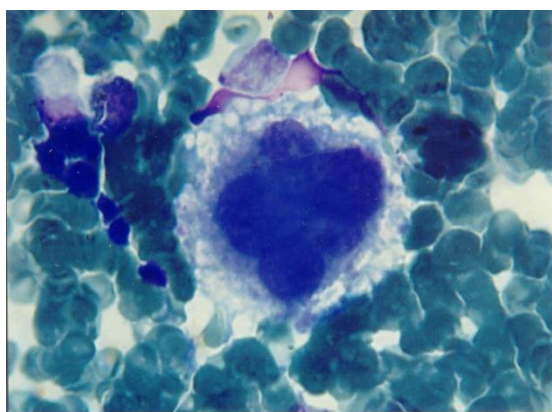


Figure1: Micrograph of bone marrow punctate of patient with lung carcinoma.

Multi nucleated cancer cell. Pappenheim staining x 400.

The appearance of tumor cells in BM causes reactive changes of stroma, namely, hyperplasia of reticular cells, fibroblasts, endothelium of sinuses and capillaries, increase in the number of osteoblasts and osteoclasts. The result of immunologic changes was raising the amount of T-lymphocytes in BM and the formation of so-

called plasma cell islets. Such formations consisting of macrophages and plasma cells surrounding them were seen in our study in 26.6% of patients, in which the tumor cells were found in BM. They were also made observations on the reactions of hematopoiesis in patients with LC, when the tumor cells were detected in BM.

Smears of BM of patients with LC, in which cancer cells were not found by usual cytological test, were studied using immuno cytochemical method with MABs to cytokeratins. After reaction with the MAB, isolated cancer cells, which had a pink coloration, were found in 6 patients in addition. (Fig.2).

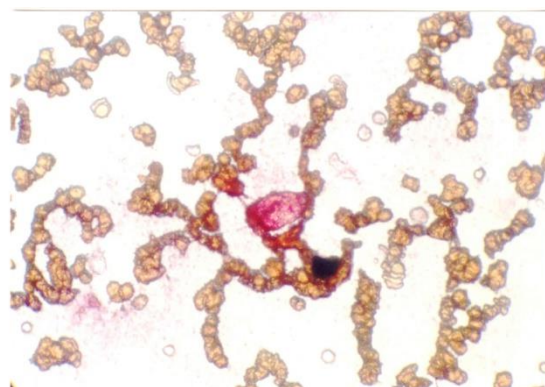


Figure 2: Micrograph of bone marrow punctate of patient with lung carcinoma.

An isolated cancer cell in BM has pink coloration. Immuno cytochemical reaction with MAB to cytokeratins x 400.

The total number of patients with the presence of tumor cells in BM was 25 (30.1%). Thus, the similarity of cancer cells by cytomorphological features with the parental BM cells, and their known polymorphism allows to understand why, in a routine cytological test we obtain clearly underestimated data of the rate of metastasis in BM.

Significance of detection of isolated cancer cells or clustrs in BM is not completely known, but there are studies that confirm the correlation between the presence of cancer cells in BM and the appearance of distant metastases in 6-9 months / 10 /.

At routine histological examination of distant LN in the examined patients with LC, it has not been detected the presence of metastases in LN. Light microscopy method has disadvantages that are inherent in conventional pathological tests in which to be viewing only a small part of LN, and most remain outside the field of view of morphologist. Furthermore, by usual dyeing methods are difficult to detect isolated tumor cells or their clusters in the LN.

After routine histological study had been conducted in addition we investigated LN in 83 patients with NO nodal status. An immune histochemical study of intact LN with MAB to cytokeratins has been carried out. In 17 patients small clusters of tumor cells with a brown coloration were

detected in LN, which correspond to cytokeratin - positive cells (Fig. 3) and make up 20.5% of total patients.

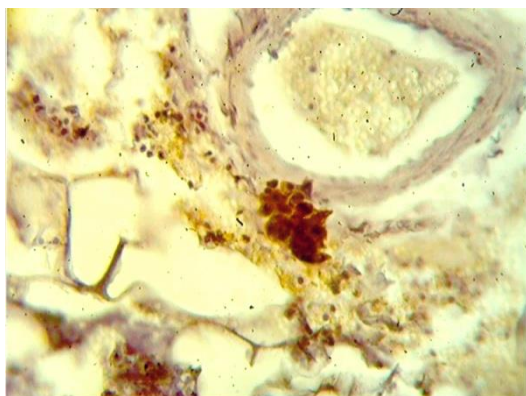


Figure 3: Micrograph of lymph node of patient with lung carcinoma.

A single cluster of cytokeratin - positive tumor cells has brown coloration. x 400.

The simultaneous presence of CTC in BM and LN was observed in 15 patients (18.1%). Patients with CTC in BM prevailed quantitatively (30.1%). This fact may indicate that the affection of tumor cells in BM and LN is not sequentially but simultaneously.

Studying of long-term results of treatment, in the form of 3-year survival rate of patients, showed that in patients with simultaneous affection of BM and LN survival rate was lower and amounted to $43.3 \pm 3.1\%$ as compared with patients without affection of BM and LN – $78.3 \pm 2.6\%$. The death of these patients, as a rule, came from the distant metastasis or intrathoracic recurrence of disease.

The presence of CTC only in LN or only in BM in patients with LC effects on 3-year survival rate. It was also lower in comparison with patients without affected BM and LN. However, these differences were not statistically significant.

Submitted data convincingly testify the diagnostic value of immune histochemical study of LN, and indicate that the intraoperative visual and manual investigation of LN, as well as post-operative routine morphological examination failed to detect metastases of LC in LN. This confirms the appropriateness of performing of ipsilateral mediastinal lymphadenectomy in radical surgery for LC in all cases and carrying out additional immunohistochemical study of LN with MABs to cytokeratins.

CONCLUSIONS

The presence of CTC in BM in 30.1% operable patients with lung carcinoma with NO nodal status indicates possibility of independent hematogenous metastasis.

The negative impact of the presence of CTC in BM and LN on survival of patients with LC gives the opportunity to consider their presence as an additional negative prognostic factor.

Given data make possible to consider that the immune cytochemical study of BM and the immunohistochemical study of LN with MABs to cytokeratins can provide real help for the diagnosis and choice of treatment tactics of patients with LC, predicting the future clinical course of the disease and results of treatment.

Identification of isolated cancer cells in BM in patients with LC can be considered as a new generation of methods of screening and monitoring of tumors.

REFERENCES

1. Leong S.P., Tseng W.W. Micrometastatic cancer cells in lymph nodes, bone marrow, and blood: Clinical significance and biologic implications. *Cancer Clin.* 64(3), 2014, 195 -206.
- a. Ryan P., Furlong H., Murpfiy C.G. Prognostic significance of prospectively detected bone marrow micro metastases in esophagogastric cancer: 10-year follow up confirms prognostic significance, *Cancer Med.* 4(8), 2015, 1281 -8.
2. Märkl B., Wilhelms N., Anthuber M. Circulating cytokeratin – positive cells and tumor budding in colorectal cancer. *World J. Clin. Oncol.* 107 (6), 2016, 433 -440.
3. Sinnestvedt M., Borgen E., Schlichting E. Dissiminated tumour cells in the bone marrow in early breast cancer: morphological categories of immunocytochemically positive cells have different impact on clinical outcome. *Breast cancer Res Treat.* 138(2), 2013, 485 -97.
- a. Deng X.F., Liu Q.X., Zhou D. Bone marrow micrometastasis is associated with both disease recurrence and poor survival in surgical patients with node negative non small cell lung cancer a meta analysis. *Cardiovasc. Thorac. Surg.* 21(1), 2015, 21 -27.
4. Osaki T., Yasumoto K. Clinical significance of micrometastases in the limphnodes of patients with resectable lung cancer. *Nihon – Geka Gakkai Zasshi* 114 (1), 2013, 28 -33.
5. He Z., Xia Y., Tang S. Detection of occult tumor cells in regional lymph nodes is associated with poor survival in p No non small cell lung cancer: a meta analysis. *J. Thorac. Dis.* 8 (3), 2016, P. 375-85.
6. Lei Y., Wu Y. The prognostic value of micrometastasis in non small cell lang cancer. *Zhangguo Fei Ai Za Zhi.* 16 (9), 2013, 492-8.
- a. Houvenaeghel G., Cohen M., Bannier M. Micrometastasis: biological entity and clinical impact? *Bull. Cancer.* 100 (4), 2013, 351 -6.
7. Fehm T., Banys M., Rack B. Pooled analysis of the prognostic relevance of disseminated tumor cells in the bone marrow of patients with ovarian cancer *Int. J. Gynecol. Cancer.* 23 (5), 2013, 839 -45.

Source of Support: Nil, Conflict of Interest: None.