

## Histological diagnosis of lung cancer micrometastases

O diagnóstico histológico de micrometástases de tumores de pulmão

Simone Gusmão Ramos

Currently, lung cancer causes more deaths than do prostate, lymph node, breast and colon cancer combined. Lung cancer is the leading cause of cancer death in the United States and in Europe. In Brazil, this estimate has been increasing in recent years. According to the Brazilian National Cancer Institute, the number of new cases of lung cancer in 2008 is estimated at 17,810 among males and 9,460 among females, corresponding to an estimated risk of 19/100,000 men and 10/100,000 women.<sup>(1)</sup>

Dissemination to locoregional lymph nodes is a determining factor in the prognosis of lung cancer. It is known that patients with pathological stage I non-small cell lung cancer (NSCLC) and undetected lymph node metastases can develop early recurrence, even after the complete surgical resection of the tumor. In such patients, the estimated 5-year survival rate ranges from 64 to 75%,<sup>(2)</sup> suggesting that the pathological staging, routinely carried out using histological sections stained with hematoxylin and eosin (H&E), is underestimated, and that occult metastases might go undetected. Therefore, the accurate evaluation of the presence/absence of tumor cells in regional lymph nodes is fundamental in the staging, treatment and prognosis of patients with lung cancer.

The greatest problem, even for experienced pathologists, is to identify isolated malignant cells or even minimal clusters of these cells in histological sections routinely stained with H&E. These cells may go unnoticed among lymphocytes and sinusoidal histiocytes, which can lead to false-negative results. In an attempt to improve this situation, immunohistochemical techniques involving the use of various antibodies, such as Ber-Ep4, CAM-5.2, MNF116, and AE1/AE3,<sup>(3,4)</sup> as well as molecular biology techniques,<sup>(5)</sup> have been used in the identification of occult micrometastases in regional lymph nodes in NSCLC. However, the significance of these findings remains controversial.

In the study conducted by Franco et al. and published in this issue of the Brazilian Journal of Pulmonology,<sup>(6)</sup> an alternative method for the detection of micrometastases in lymph nodes previously considered negative using the routine technique (H&E) in NSCLC is proposed. A more sophisticated molecular biology technique, tissue microarray (TMA), was combined with immunohistochemistry, which is a technique that has been previously used with this

aim. The use of TMA blocks have shown various advantages compared to that of traditional sections: savings of time and reagents required to perform the reactions; standardization of reactions; facility in the comparative interpretation of cases; possibility of repeating the reactions at multiple levels of the block; and simplification of work. In addition, the total cost of reactions is reduced by 85%, and the surface of the analyzed area is multiplied by 12, increasing the possibility of finding occult metastases. The collected material does not jeopardize the analysis of the original block, and more elaborate genetic studies can also be performed. The most important factor is that, considering the group of antibodies used in this technique, micrometastases were detected in 50% of the analyzed lymph nodes that had previously been considered negative. An interesting finding in the study was the detection of positive neuroendocrine cells in approximately 13% of the patients with NSCLC. This finding was associated with a worse prognosis. Although the authors do not discuss the significance of this finding in their study, it is interesting to investigate the heterogeneity of these tumors, which might imply the need for a revision of the final classification of the most common types of lung tumors. Most of the previous studies using immunohistochemistry alone showed an increase (4% and 27.8%).<sup>(7)</sup> Therefore, combining these two techniques was truly more efficient.

Unfortunately, these data are not easily comparable. There are many variables, including the initial staging of the tumor, the histology and the presence/absence of lymph node metastases. Without the explicit recommendation by the American Joint Committee on Cancer for the use of the term micrometastases in NSCLC,<sup>(8)</sup> most authors did not differentiate between isolated tumor cells (single tumor cells or small clusters of tumor cells smaller than 0.2 mm at their largest diameter) and micrometastases (clusters of tumor cells from 0.2 to 2 mm at their largest diameter), generalizing the term micrometastases. Undoubtedly, the presence of malignant cells in regional lymph nodes in NSCLC alters the staging of the patient and, consequently, the prognosis. However, the difference between these two findings (isolated tumor cells and micrometastases) and their association with other clinical factors, such as age and the histological type of the tumor, are not well defined. Some authors found

no significant differences regarding the prognosis of patients with negative lymph nodes and isolated tumor cells.<sup>9,10</sup> The only element of concordance is that, when the method is more sophisticated, the number of patients with occult metastases in the regional lymph nodes is higher. Consequently, the simultaneous use of more than one technique can increase the detection of positive lymph nodes considerably. Therefore, until we discover the true importance of isolated malignant cells or micrometastases in regional lymph nodes, it is fundamental that new techniques be developed in order to improve detection. Only with larger samples, similar histopathological criteria, and analyses of possible associations regarding prognosis will we be able to discern the true importance of these findings. This should occur in the near future.

**Simone Gusmão Ramos**  
**PhD Professor**  
**Department of Pathology**  
**Ribeirão Preto School of Medicine**  
**University of São Paulo**

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