Circulating Tumor Cells in Diagnosing Lung Cancer: Clinical and Morphologic Analysis

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Background. **The purpose of this study was to evaluate the value of circulating non-hematologic cells to differentiate benign from malignant lung lesions and their comparison with clinico-histologic features of corresponding primary lesions.**

Methods. **Circulating cells were isolated by size method from peripheral blood of 77 patients with malignant (n = 60) and benign (n = 17) lung lesions. They were morphologically classified as cells with malignant feature; cells with uncertain malignant feature; and cells with benign feature; then statistically correlated with clinico-cytopathologic characteristics of corresponding lung lesion.**

Results. **Malignant circulating cells were detected in 54 of 60 (90%) malignant patients, and in 1 of 17 (5%) benign patients; benign circulating cells in 1 of 60 (1%) malignant patients and in 15 of 17 (88%) benign patients; and circulating cells with uncertain malignant aspect in 5 of 60 (8%) malignant patients and 1 of 17 (5%) benign patients. For a malignant circulating cells count greater than 25, sensitivity and specificity were 89% and 100%, respectively. The count was significantly correlated with stage, size, and standard uptake value of primary tumor. In 39 of 54 (72%) cases, the malignant circulating cells allowed a specific histologic diagnosis of the corresponding primary tumor after immunohistochemical analysis.**

Conclusions. **Malignant circulating cells may be a valid marker in the diagnostic workup of lung lesions. However, our resuts should be corroborated by larger future studies especially for patients having small nodules.**

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Circulating tumor cells (CTCs) are cells that are shed from primary tumors and, flowing through the bloodstream, circulate in the body. Most studies have focused on their prognostic role in breast [1], prostate [2], colon-rectal [3], and lung cancer [4–6]. Presently, the standard invasive procedures for diagnosing lung lesion are bronchoscopic or computed tomography-guided tissue sampling (solid biopsy). They often yield a specific malignant diagnosis but suffer from sampling bias, which dictates additional invasive procedures including resection of the lesion. Thus the detection of CTCs, also named as liquid biopsy, might make substantial contributions in diagnostic workup of indeterminate lung lesions and avoid solid biopsy in selected cases, considering that up to 50% of resected indeterminate lung nodules are benign [7]. The present study aimed to evaluate the following: (1) the diagnostic value of CTCs to differentiate benign from malignant lung lesions; and (2) their comparison with clinicopathologic and histologic features of corresponding primary lesions.

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Material and Methods

Study Design

This is a prospective, observational unicenter study. All consecutive patients with potential lung cancer, referred to our unit from April 2011 to October 2013, were enrolled. Exclusion criteria were the following: (1) history of cancer or a recent pulmonary infection (<15 days); (2) contraindication or refusal of invasive procedures; and (3) treatment with immune-stimulating agent, anti-cancer, anti-tuberculosis, corticosteroid, or other non-steroid anti-inflammatory drugs.

Before any invasive procedure, CTCs were captured from peripheral blood samples, characterized as malignant or benign, and correlated with diagnosis and histologic features of primary lung lesion. The CTCs and tissue samples of lung lesion were reviewed by 2 different pathologists who were blinded to the results of the other. The study was approved by the Ethics Committee of our Institution and written informed consent was obtained from all patients.

Study Population

Eighty-one patients with single radiologic lung lesion were enrolled. All patients underwent positron emission tomography and computed tomography (PET/CT) scans with [18F]-fluorodeoxyglucose (FDG) and, in cases with

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positive results invasive procedures were performed for confirmation purposes. The diagnosis was obtained by bronchoscopic, CT-guided biopsy or thoracoscopic resection, and in some cases by the confirmation of instrumental exams; ie, reduction or disappearance of lesion after specific therapy. Patients with lung malignancy underwent surgical resection if indicated on the basis of standard oncologic and clinical parameters.

Circulating Cells Analysis

The CTC analysis was performed through the following sequential steps: (1) blood sample collection; (2) isolation by blood filtration on ScreenCell Cyto filtration devices (ScreenCell, Paris, France) using the isolation by size of epithelial tumor cells (ISET) methodology; (3) characterization of circulating cells with hematoxylin and eosin (H&E) staining; and (4) identification of cell origin by immunohistochemistry.

Blood Sample Collection

Peripheral blood (7.5 mL) was collected in parallel in buffered ethylenediaminetetraacetic acid, maintained at 4°C and processed within 4 hours of blood collection, as recommended by the manufacturer (ScreenCell). Briefly, blood was diluted eightfold with red blood cell lyses buffer (ScreenCell) and incubated for 10 minutes at room temperature, with gentle agitation after 3 and 6 minutes. Per patient and time point 4 filtrations, each corresponding to the processing of 2 mL of whole blood were performed using vacutainer tubes.

Isolation

The ISET methodology isolates intact circulating cells from blood through direct filtration without antibodies but using a polycarbonate membrane with 8-μm-diameter cylindrical pores. The circulating cells including the tumor cells of even small cell lung cancer are larger (> 8 μm) than circulating lymphocytes or monocytes (mean diameter 7.2 μm). Thus, they were differentiated by circulating blood cells and were generically defined as circulating non-hematologic cells (CNHC). Thereafter the filters were rinsed with 2 mL of sterile phosphate-buffered saline (pH 7.4) and collected from the device.

Characterization

The filters were counterstained with hematoxylin (Merck, Darmstadt, Germany) for 5 minutes and eosin for a few seconds with NH_3-H_2O (ammonia + water; 0.06% m/V), washed in distilled water, air dried, and mounted on a glass slide for evaluation by light microscopy. Using the cytomorphologic criteria validated by Hofman and colleagues [8, 9], CNHCs were divided into 3 subgroups: CNHC with malignant features (CNHC-MF), CNHC with uncertain malignant features (CNHC-UMF), and CNHC with benign features (CNHC-BF).

The CNHC-MF were defined by the presence of at least 4 of the following criteria: (1) anisonucleosis (ratio > 0.5); (2) nuclei larger than 3 times the calibrated 8-μm pore size of the membrane; (3) irregular nuclear outline; (4) presence of tridimensional cellular sheets; and (5) high nuclear to cytoplasm ratio (Fig 1A). Groups or clusters of CNHC-MF containing 3 or more distinct nuclei

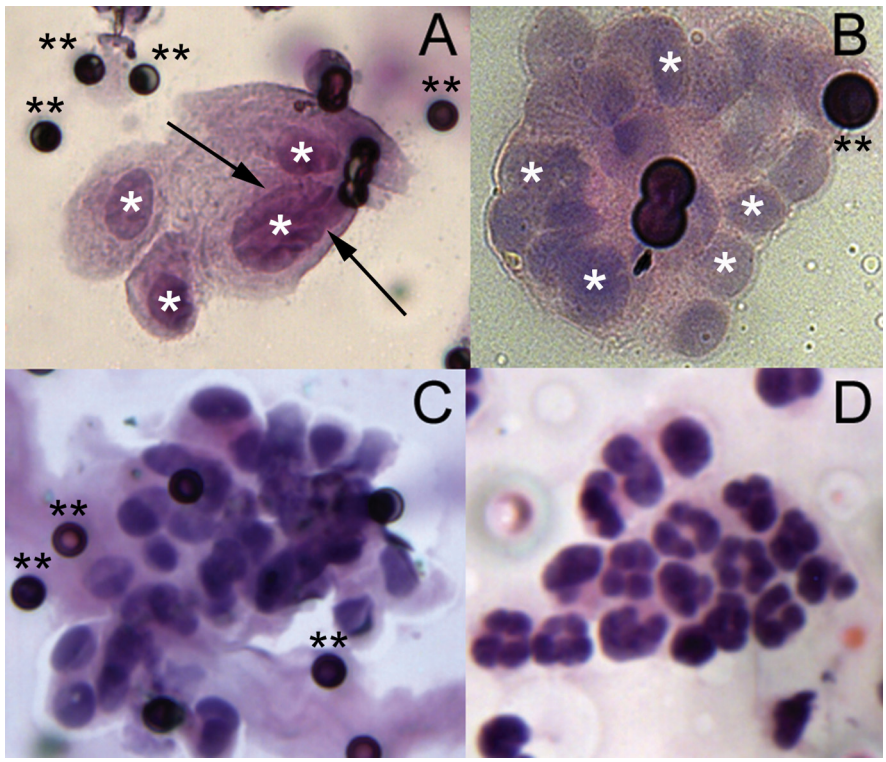

Fig 1. (A) CNHC-MF isolated from patient with squamous carcinoma (clinical stage II). Cells on isolation by size of epithelial tumor cells membrane stained with hematoxylin and eosin (H&E) stain showed anisonucleosis (*); nuclei larger than 3 times the calibrated 8-μm pore (**) size of the membrane; irregular nuclear outline (black arrow); and high nuclear to cytoplasm ratio. (B) Clusters of circulating non-hematologic cells (CNHC) with malignant features (CNHC-MF), named as circulating tumor microemboli, isolated from patient with adenocarcinoma (clinical stage IV); H&E showed 3 or more distinct nuclei (*). (C) CNHC with uncertain malignant features (CNHC-UMF) isolated from patient with squamous carcinoma (clinical stage I); the H&E showed the tridimensional cellular sheets. The presence of this criteria did not allow a definitive diagnosis of malignancy. (**) The calibrated 8-μm pore size of the membrane. (D) CNHC with benign features (CNHC-BF) from a patient with fibrotic lesion; H&E showed no malignant feature. (Magnification for images A, B, C, D is 400×.)