

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

Alfonso Fiorelli, MD, PhD, Marina Accardo, MD, Emanuele Carelli, MD, Denise Angioletti, MD, Mario Santini, MD, and Marina Di Domenico, MD

Thoracic Surgery Unit, Department of Morphopathology, and Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Naples, Italy; and Sbarro Institute for Cancer Research and Molecular Medicine and Center for Biotechnology, Temple University, Philadelphia, Pennsylvania

**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 results should be corroborated by larger future studies especially for patients having small nodules.

(Ann Thorac Surg 2015;99:1899–905)

© 2015 by The Society of Thoracic Surgeons

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.

## 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

Accepted for publication Nov 24, 2014.

Address correspondence to Dr Santini, Chirurgia Toracica - Seconda Università di Napoli Piazza Miraglia, 2, I-80138 Naples, Italy; e-mail: [mario.santini@unina2.it](mailto:mario.santini@unina2.it).

cases with 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 tumor cells 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

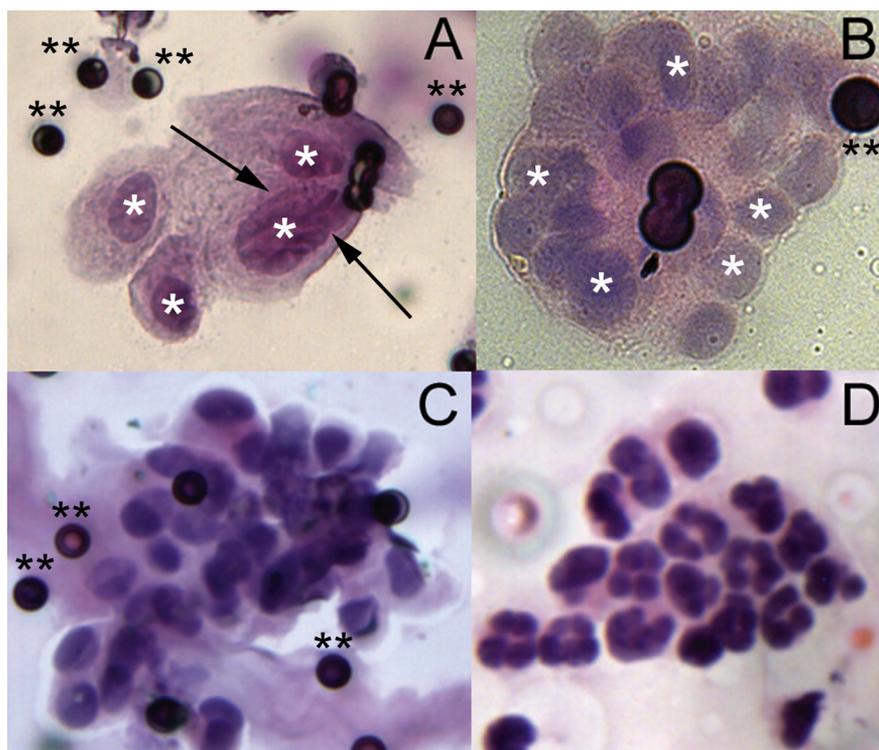
The methodology isolates intact circulating cells from blood through direct filtration without antibodies but using a polycarbonate membrane with 7.5- $\mu\text{m}$ -diameter cylindrical pores. The circulating cells including the tumor cells of even small cell lung cancer are larger (> 8  $\mu\text{m}$ ) than circulating lymphocytes or monocytes (mean diameter 7.2  $\mu\text{m}$ ). 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  $\text{NH}_3\text{-H}_2\text{O}$  (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 cytomorphic 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 7.5- $\mu\text{m}$  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 tumor cells membrane stained with hematoxylin and eosin (H&E) stain showed anisonucleosis (\*); nuclei larger than 3 times the calibrated 7.5- $\mu\text{m}$  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 7.5- $\mu\text{m}$  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 $\times$ .)



were defined as circulating tumor microemboli (CTM) (Fig 1B). The CNHCs were defined as CNHC-UMF when they had fewer than 2 criteria, but at least 1 was present (Fig 1C). The CNHCs are defined as CNHC-BF in the absence of these criteria (Fig 1D). Samples were evaluated using  $\times 20$  magnification to identify and count CTCs. For morphologic evaluation  $\times 400$  magnification was used.

### Immunocytochemistry

The ScreenCell Cyto filters were hydrated with tris-buffered saline (TBS; pH7.4). The excess TBS was removed with absorbent paper and the filters were put on the paraffin film in a humid chamber. Each spot was incubated for 5 minutes at room temperature with 70  $\mu$ L of permeabilizing buffer. Each filter containing CNHC-MF or CNHC-UMF was incubated overnight with monoclonal antibodies reactive to p63 or thyroid transcription factor-1 (TTF-1). Filters having CNHC-BF were incubated with mouse monoclonal antibody to pan cytokeratin to assure the epithelial origin. The filters were then washed once with TBS for 1 minute and immersed in a bath containing distilled water. Each filter was then placed on paraffin film and the nuclei were slightly counterstained with Mayer's hematoxylin for 6 minutes. Finally, the filters were rinsed with running water and dried for 10 minutes at room temperature.

### Histologic Analysis of Primary Tumor

Diagnosis of the primary tumor was performed on specimen obtained from biopsy or surgical resection. Immunohistochemistry was carried out on formalin-fixed and paraffin-embedded tissues using antibodies directed against to p63 or TTF-1.

### Statistical Analysis

Data are presented as means  $\pm$  standard deviation (SD). The receiver operating characteristics curve calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CNHC-MF to diagnose malignant lesion. The CNHC-MF expression among different stages, and histologic subgroups were assessed by the analysis of variance test. The CNHC-MFs counts were correlated with size and standardized uptake value (SUV) of primary tumor using Spearman's rank correlation test. A *p* value less than 0.05 was considered statistically significant. The MedCalc statistical software (Version 12.3; Broekstraat, Mariakerke, Belgium) was used for analysis.

## Results

### Study Population

Among 81 patients enrolled, 4 patients were excluded because (1) the blood sample was spoiled during the process (*n* = 2), or (2) refused invasive diagnostic exams (*n* = 2). Thus, our study population counted 77 patients (Table 1). Sixty (78%) patients had primary lung cancer diagnosed with CT-needle aspiration biopsy (*n* = 37) or

bronchoscopic biopsy (*n* = 23). The majority of patients had early clinical stage tumor (stage I and II) and adenocarcinoma histology. Curative surgical resection was performed in 49 patients while 11 received palliative chemoradiotherapy. Seventeen patients had a benign disease diagnosed by the following: (1) invasive exams (*n* = 5); (2) thoracoscopic resection (*n* = 6); (3) clinical follow-up after appropriate therapy; ie, size reduction respect to previous exams (*n* = 6). The flow chart is reported in Figure 2.

### Detection of CTCs Expression

The CNHCs-MF type was detected in 54 of 60 (90%) cancer patients, and in 1 of 17 (5%) patients with benign lesion; CNHC-BF was found in 1 of 60 (1%) patients with cancer and in 15 of 17 (88%) without. The CNHC-UMF was found in 5 of 60 (8%) malignant and 1 of 17 (5%) benign lesions. the receiver operating characteristic curve (Fig 3A) for a CNHCs-MF count greater than 25 (area under the curve, 0.9; standard error, 0.02; 95% confidence interval [CI] 0.869% to 0.985%; *p* < 0.0001) showed a sensitivity, specificity, PPV, and NPV value of 89% (95% CI 79.2% to 96.2%), 100% (95% CI 80.5% to 100%), 100% (95% CI 92% to 100%), and 81% (95% CI 62% to 96%).

### Clinical-Pathologic Correlation Between CNHCs-MF and Primary Tumor

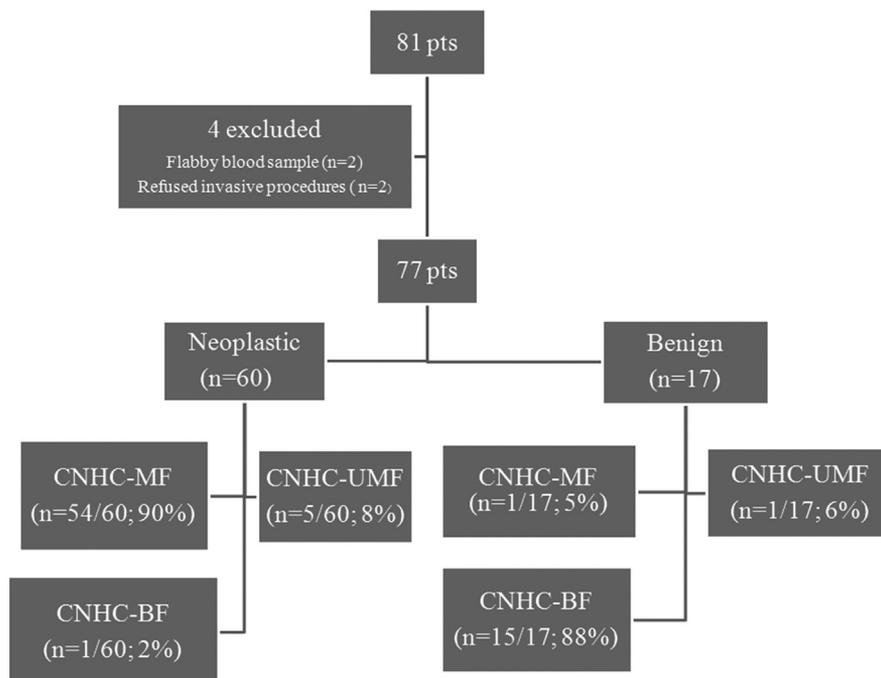
Among malignant lesions, the CNHCs-MF count was  $130 \pm 87.4$ . Stage IV patients presented a significantly

Table 1. Study Population

Variables	Malignant Lesion (n = 60)	Benign Lesion (n = 17)
Age (years)	69 $\pm$ 5.9	53 $\pm$ 8.3
Sex (M/F)	50:10	11/6
Smokers	55 (92%)	13 (76%)
Diagnosis	Adenocarcinoma: 29 (48%) Squamous cell carcinoma: 18 (30%) Large cell carcinoma: 13 (22%)	Pneumonia: 9 (53%) Tuberculoma: 4 (23%) Fibrotic nodule: 2 (12%) Active tuberculosis: 1 (6%) Amarthochondroma: 1 (6%)
Clinical stage	Stage I: 25 (42%) Stage II: 19 (31%) Stage III: 10 (17%) Stage IV: 6 (10%)	-
CNHC	• With MF 54 (90%) • With UMF 5 (8%) • With BF 1 (2%)	1 (6%) 1 (6%) 15 (88%)

BF = benign features; CNHC = circulating non-hematologic cells; MF = malignant features; UMF = uncertain malignant features.

Fig 2. Flowchart of study population. (CNHC = circulating non-hematologic Cells; CNHC-MF = CNHC with malignant features; CNHC-UMF = CNHC with uncertain malignant features; pts = patients.)

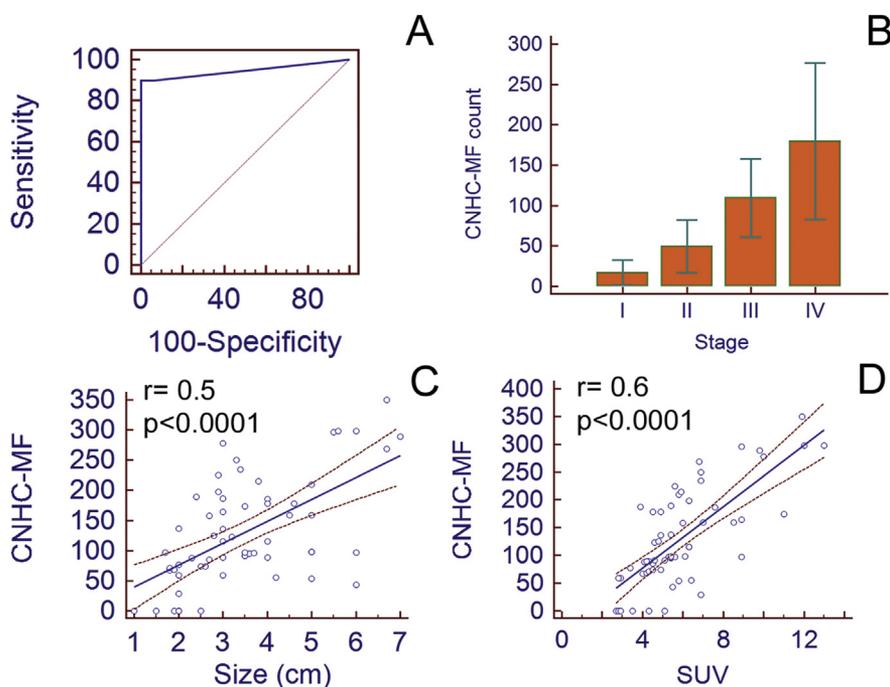


( $p < 0.05$ ) higher CNHCs-MF number ( $271 \pm 88$ ) than stage III ( $175 \pm 88$ ), stage II ( $141 \pm 44$ ), and stage I ( $70.7 \pm 56$ ) patients. A significant difference was also found between stage I versus stage II patients (Fig 3B). Patients with histology of adenocarcinoma, squamous carcinoma, and large cell carcinoma had a CNHCs-MF number of  $140 \pm 94$ ,  $101 \pm 73$ , and  $153 \pm 83$ , respectively. However,

no significant differences were found between different histologic subgroups.

Among patients with clinical stage I (T1-2aN0M0), in 13 cases the tumor size was less than 2 cm (T1a), in 9 cases greater than 2, and 3 cm or less (T1b); in 3 cases greater than 3 and 5 cm or less (T2a). In these patients, we found that CNHCs-MF cell counts were significantly correlated

Fig 3. (A) Receiver operating characteristic curve showed a sensitivity, specificity, positive predictive value, and negative predictive, of 89%; 100%; 100%, respectively; and 81% for a circulating non-hematologic cells with malignant features (CNHC-MF) count greater than 25 (area under the curve, 0.9; standard error: 0.02; 95% confidence interval 0.869% to 0.985%;  $p < 0.0001$ ). (B) A significant difference ( $p < 0.05$ ) of CNHCs-MF count was seen between the different clinical stages (analysis of variance test). (C) CNHCs-MF counts were significantly correlated with tumor size ( $r = 0.5$ ;  $p = 0.001$ ; Spearman rank correlation test) in stage I lung cancer patients and with standard uptake value (SUV) value ( $r = 0.6$ ;  $p < 0.0001$ ; Spearman rank correlation test) in all lung cancer patients (D).



with tumor size ( $r = 0.5$ ; 95% CI 0.856% to 0.918%;  $p = 0.001$ ; Fig 3C). In all malignant patients, the SUV value of primary tumor was significantly correlated with CNHCs-MF count ( $r = 0.6$ ; 95% CI 0.526% to 0.802;  $p < 0.0001$ ; Fig 3D).

#### *Cytomorphologic Comparison of CTCs With Corresponding Primary Tumor*

**CNHCs-MF CELLS.** In 39 of 54 (72%) cases, the CNHCs-MF derived from patients with adenocarcinoma ( $n = 22$ ) were distinguishable from those derived from patients with squamous cell carcinoma ( $n = 10$ ), and with large cell carcinoma ( $n = 7$ ). Adenocarcinomas were positive for TTF-1 in 21 of 22 (95%) and for TTF-1 and p63 in 1 of 22 (5%) cases. Squamous cell carcinomas were positive for p63 in 9 of 10 (90%) and for p63 and TTF-1 in 1 of 10 (10%) cases. Large cell carcinomas were positive for p63 in 4 of 7 (57%) cases, for TTF-1 in 2 of 7 (29%) cases, and for p63 and TTF-1 in 1 of 7 (14%) cases. In the remaining 15 of 54 (27%) cases, CNHCs-MF did not allow a specific histologic diagnosis. Immunohistochemistry was poorly positive in only 1 of 15 (7%) case for p63. Clusters of cells were detected in 13 of 16 (85%) cases of patients with advanced disease (stage III or IV) and in only 3 of 44 (7%) patients with early disease (stage I or II).

In comparison with tissue biopsies or pathologic specimens, the CNHCs-MF generally presented a similar morphologic aspect. However, while CTCs showed a tendency toward nuclear eccentricity, their solid counterparts were columnar in shape with presence of mitotic figures not seen in CNHCs-MF.

**CNHCs-UMF CELLS.** In 5 patients with lung cancer, the isolated CNHCs did not present typical malignant features. The immunocytochemical analysis demonstrated a poor positivity for p63 in 1 of 6 (17%) cases or in association with TTF-1 in 1 of 6 (17%) cases, while in 4 of 6 cases (66%) it was negative.

**CNHCs-BF CELLS.** The CNHCs-BF cells were found in 15 patients with benign lesions and in 1 with cancer. The cells did not present signs of malignity but the morphologic analysis did not allow a definitive diagnosis. The immunocytochemical staining was negative for p63 and TTF-1. However, 14 of 15 (93%) cases were positive for pan-cytokeratin confirming the epithelial origin of the filtered cells.

#### **Comment**

The concept of CTCs was introduced by Stephen Paget in 1889. Circulating tumor cells offer potential utility as a prognostic, predictive, or pharmacodynamic biomarker. The value of CTCs in diagnosing lung cancer is underestimated despite that “liquid biopsy” might facilitate the standard clinical workup and provide additional information on staging and prognosis not currently available with standard systems. The CTCs can be detected using indirect or direct approaches. Indirect methods depend on cell enrichment, differential cell centrifugation, or immunomagnetic separation, and then immunofluorescent

or immunocytochemical labeling with epithelial specific antigens. CellSearch (Veridex) is the most sensitive indirect method for CTCs detection using immunomagnetic purification with antibodies against epithelial cell adhesion molecule (EpCAM). Despite its high sensitivity, CellSearch is unable to detect CTCs without EpCAM expression and the cytologic details of the selected cells are not discernible.

Direct methods, though less developed, have the advantage of allowing cytomorphologic analysis of captured CTCs (ScreenCell, ISET), similar to that performed in exfoliative cytology and in fine needle aspiration cytology. Size of tumor cells technologies are direct methods first applied in patients with liver and breast cancers [10], and recently in lung cancer patients [8, 9]. ScreenCell allows the separation of all CTCs present in blood samples based on cell size, irrespective of surface markers, and the possibility of their cytopathologic analysis. After erythrocyte lysis, blood cells are filtered using filters with pores of 7.5  $\mu\text{m}$  in diameter. Cells larger than pores size, including tumor cells, are retained.

First we found that with a CNHC-MF count greater than 25, the sensitivity and specificity to identify a malignant lesion was 89% and 100%, respectively. Tanaka and colleagues [11] reported lower value of sensitivity and NPV but higher specificity and PPV. To detect CTC cells, the authors [11] used the CellSearch method, thus the presence of CTCs in patients with benign lesions may be due to false positive staining of contaminating non-epithelial cells. Second, in our study CNHC-MF count increased with clinical stage, size of primary lesion, and SUV.

The correlation of CTC with clinical stage is not surprising, as previously reported [6, 9, 11]. Metastasis is a complex mechanism that includes several phases: tumor growth and angiogenesis; local invasion and epithelial-mesenchymal transition, intravasation, dissemination, arrest in organs, extravasation, proliferation, and formation of metastases [12]. Occasionally, tumor cells can enter the circulation as multicellular aggregates or clusters of epithelial-like cells also known as CTM having a high metastatic potential. A CTM was found in about 80% of our patients with advanced disease, confirming such theory. Interesting data were the high numbers of CNHC-MF (>185) in 3 patients with early stage, for whom we would expect a very low number of CNHC-MF. The CTCs, although necessary, are not sufficient for metastatic formation. The majority of CTCs dies; it has been estimated that less than 0.01% of CTCs will implant and form metastasis and most CTCs are cleared from the circulation within 24 hours [13]. Metastases do not form randomly but can form only when the seed (CTC) and the soil (target tissue) are compatible (“seed and soil” theory) [14]. Thus, in our cases such CNHC-MF may correspond to passively shed cells that remain dormant for several years and in the future may grow into macrometastases. It is supported by the absence of mitotic future in our filtered CNHC-MF. Thus, such patients could have higher risk of recurrence after surgery than those with low numbers of CNHC-MF or without. Hofman and

colleagues [9] found that CNHC count 50 or greater was correlated with poor outcome independent from pathologic TNM staging. Thus, it will be of great interest to follow up this subgroup of patients and compare their outcome with patients having similar stage but with lower or without CNHC-MF.

To evaluate if the CNHC-MF cells seeded in the blood stream are correlated with the size of the primary tumor, we selected only stage I (T1-2aN0M0) lung cancer patients in order to reduce as much as possible the interference of other variables as lymph node involvement (N status) and distant metastases (M status). Our analysis showed that tumor size was significantly correlated with CNHC-MF count in agreement with other studies in primary liver [15], gastric [16], and adrenocortical carcinoma [17]. Thus, if the number of CNHC-MF depends on tumor size, in theory a small tumor may seed in circulation a very limited number CTCs that may be difficult to detect. Previous studies [5, 11] showed a low sensitivity of CTC analysis in diagnosing early lung cancer ranging from 19% to 39% by CellSearch analysis, and from 36% to 50% by the ISET approach. In advanced stage, CTC counts were generally higher and investigations found 32% to 78% positivity by CellSearch analysis, and up to 80% by the ISET approach. Thus, in patients with small nodules the lack of CNHC-MF detection may not exclude the malignant nature of the lesion and standard procedures are still necessary for a differential diagnosis. On the other hand, the presence of CNHC-MF may be a sign of high aggressive lesion with potential poor prognosis despite the small tumor size.

At the time of the present work, in the literature no other papers, to our knowledge, investigated the correlation between the SUV of primary tumor and CTCs expression. Multiple studies have observed that patients with high-FDG avid lung cancers tend to have a more aggressive clinical course than those with a low FDG uptake [18]. Thus, in theory large tumors with high FDG uptake have the potential of shedding a high number of CNHCs-MF in the blood, resulting in a high risk of metastasis. We did not observe any difference of CNHC-MF in different histologic subtypes in agreement with the data of Hofman and colleagues [9].

Third, CNHCs-MF had cytomorphologic features similar to malignant cells obtained by preoperative diagnostic exam or surgical specimen and they were diagnostic for the histologic subtype of the corresponding primary tumor in 72% of cases. Hofman and colleagues [9] found no correlation between CNHCs and primary tumors. Probably, in most of our cases the isolated CNHCs-MF were not completely de-differentiated and this allowed a specific histologic diagnosis. Our results seem to contradict the current idea that only poorly differentiated tumor cells can cross the endothelial barrier and, migrating into the blood stream, concur to the metastatic process [12]. Similarly, Marrinucci and colleagues [19] in patients having primary lung adenocarcinoma, and Murray and colleagues [13] in patients having primary colon cancer, found similar cytomorphologic features between primary tumor and CTCs. These data

support the hypothesis that CTCs represent a random sampling of the many phenotypic cell types present in primary and metastatic disease and the presence of CTCs with similar morphology, as signet cells argue against the fact that only particular subsets of tumor cells (such as very poorly differentiated “stem cell like” tumor cells, or only visibly dead or apoptotic tumor cells) enter the circulation. Conversely, in the remaining 28% of cases, it was not possible to have a definite histologic diagnosis probably because the CNHCs-MF were poorly differentiated. In theory, these cells by changes of epithelial-mesenchymal transition were able to migrate through the stroma, cross the endothelial barrier and circulate in the bloodstream but lost the characteristic signs that allowed a specific histologic diagnosis.

The same theory may explain why in 5 patients with lung cancer we found CNHCs-UMF. Finally, the CNHCs-BF found in malignant patients and in benign patients are epithelial cells without signs of malignancy that are detached from a benign lesion or from other sites subjected to tissue micro-trauma leading to vascular barrier disruption. The epithelial origin of these cells was confirmed by the positive staining for pan-cytokeratin.

Our results may facilitate the diagnostic workup of lung lesions. In patients with high suspect of having lung cancer according to the clinical and radiologic findings, CNHC-MF count greater than 25 might increase the risk that the lesion is malignant in the light of high PPV (100%). Thus, a rational approach might be to directly perform surgical resection (if indicated), reducing the morbidity from invasive diagnostic procedures. Conversely, in patients with the presence of CNHC-BF and a low clinical suspicion of malignancy (ie, low value of SUV) a conservative management could be applied with radiologic follow-up rather than invasive risky procedures considering the NPV value (81%) of CNHC-BF. In conclusion, in patients with CNHC-UMF, before proceeding to surgical resection further invasive diagnostic strategies appear to be required to avoid unnecessary resection.

#### *Study Limitations*

First, the diagnostic accuracy of CTCs may be affected by the selection of the patients; namely, by the high prevalence of malignant lesions. Second, our study includes also patients with advanced disease and a limited number with small malignant nodules. Thus, we were unable to draw definitive conclusions regarding the role of liquid biopsy in differential diagnosis of small nodules. Third, the small sample size and the lack of other histologic types, such as small cell lung cancer, may affect our results.

#### *Conclusions*

This small study is an example of what is considered to be one of the most exciting clinical applications of liquid biopsy as a diagnostic procedure that can be complementary to painful and often unfeasible biopsies of a primary tumor. The study shows that CNHC-MF counts were significantly correlated with size, SUV value, and stage of primary tumor and allowed a

definitive histologic diagnosis in 72% of cases. Thus, it may be the basis for future larger works in such settings, especially in patients having small nodules found during cancer screening.

---

The authors thank Anna Rota for blood sample collection.

---

## References

1. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 2006;12(14 Pt 1):4218–24.
2. Cohen SJ, Punt CJ, Iannotti N, et al. Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 2009;20:1223–9.
3. Ma X, Xiao Z, Li X, et al. Prognostic role of circulating tumor cells and disseminated tumor cells in patients with prostate cancer: a systematic review and meta-analysis. *Tumour Biol* 2014;35:5551–60.
4. Igawa S, Gohda K, Fukui T, et al. Circulating tumor cells as a prognostic factor in patients with small cell lung cancer. *Oncol Lett* 2014;7:1469–73.
5. Hofman V, Ilie M, Long E, et al. Detection of circulating tumor cells from lung cancer patients in the era of targeted therapy: promises, drawbacks and pitfalls. *Curr Mol Med* 2014;14:440–56.
6. Krebs MG, Hou JM, Sloane R, et al. Analysis of circulating tumor cells in patients with non-small cell lung cancer using epithelial marker-dependent and -independent approaches. *J Thorac Oncol* 2012;7:306–15.
7. Jeong YJ, Yi CA, Lee KS. Solitary pulmonary nodules: detection, characterization, and guidance for further diagnostic workup and treatment. *AJR Am J Roentgenol* 2007;188:57–68.
8. Hofman V, Long E, Ilie M, et al. Morphological analysis of circulating tumour cells in patients undergoing surgery for non-small cell lung carcinoma using the isolation by size of epithelial tumour cell (ISET) method. *Cytopathology* 2012;23:30–8.
9. Hofman V, Bonnetaud C, Ilie MI, et al. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. *Clin Cancer Res* 2011;17:827–35.
10. Vona G, Sabile A, Louha M, et al. Isolation by size of epithelial tumor cells : a new method for the immunomorphological and molecular characterization of circulating tumor cells. *Am J Pathol* 2000;156:57–63.
11. Tanaka F, Yoneda K, Kondo N, et al. Circulating tumor cell as a diagnostic marker in primary lung cancer. *Clin Cancer Res* 2009;15:6980–6.
12. Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 2009;9:274–84.
13. Murray NP, Albarran R-C, Pérez OG, Ruiz MA, Porcell EJ, Castillo AM. Cytomorphology of circulating tumor cells and cell clusters in Chilean patients with colon-rectal cancer. *Int J Morphol* 2012;30:834–9.
14. Jacob K, Sollier C, Jabado N. Circulating tumor cells: detection, molecular profiling and future prospects. *Expert Rev Proteomics* 2007;4:741–56.
15. Xu W, Cao L, Chen L, et al. Isolation of circulating tumor cells in patients with hepatocellular carcinoma using a novel cell separation strategy. *Clin Cancer Res* 2011;17:3783–93.
16. Wu CH, Lin SR, Hsieh JS, et al. Molecular detection of disseminated tumor cells in the peripheral blood of patients with gastric cancer: evaluation of their prognostic significance. *Dis Markers* 2006;22:103–9.
17. Pinzani P, Scatena C, Salvianti F, et al. Detection of circulating tumor cells in patients with adrenocortical carcinoma: a monocentric preliminary study. *J Clin Endocrinol Metab* 2013;98:3731–8.
18. Fiorelli A, Rizzo A, Messina G, et al. Correlation between matrix metalloproteinase 9 and 18F-2-fluoro-2-deoxyglucose-positron emission tomography as diagnostic markers of lung cancer. *Eur J Cardiothorac Surg* 2012;41:852–60.
19. Marrinucci D, Bethel K, Bruce RH, et al. Case study of the morphologic variation of circulating tumor cells. *Hum Pathol* 2007;38:514–9.