Editorial

A preliminary study on the relationship between circulating tumor cells count and clinical features in patients with non-small cell lung cancer

Milica Kontic[1,2], Miodrag Ognjanovic[3], Dragana Jovanovic[1,2], Marko Kontic[4], Simona Ognjanovic[5]

[1]Clinic for Pulmonology, Clinical Centre of Serbia, Koste Todorovića, Belgrade, Serbia; [2]Medical Faculty, University in Belgrade, Belgrade, Serbia; [3]International Organization for Cancer Prevention and Research (IOCPR), Serbia; [4]Eye Clinic, Military Medical Academy, Belgrade, Serbia; [5]Mayo Graduate School, Mayo Clinic, Rochester, USA

Correspondence to: Milica Kontic, MD, PhD. Clinical Center of Serbia, Clinic for Pulmonology, Visegradska 26, Belgrade 11000, Serbia.
Email: milicakontic@yahoo.com.

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Non-small cell lung cancer (NSCLC) represents 80% of all lung cancers and is the leading cause of cancer death worldwide (1). A number of factors can affect survival, including patient's age, NSCLC subtype, tumor genetic characteristics, response to therapy. In addition, stage of disease at diagnosis has a profound impact on survival. According to the American Cancer Society, based on analysis of SEER registry data between 1998 and 2000, 5-year survival rates of stage IA and IB are 49% and 45% respectively, however, the rates decrease to around 30% for stage II (A&B), to 14% for IIIA and about 5% for stage IIIB, while stage IV survival rate is merely 1% (2). The vast majority of NSCLC (70%) present as advanced disease.

During the disease progression and metastasis, tumor cells shed from primary tumors or metastases and can be found in circulation (3,4). However, these cells are rare; the ratio of circulating tumor cells (CTCs) compared to blood cells is very low: approximately one CTC for every 10^8 blood cells. Despite this limitation, enumeration of CTCs was validated as an independent prognostic feature in several malignancies. Namely, CTCs have reached clinical utility for monitoring of metastatic disease in breast, colorectal and prostate cancer, by using the only FDA-approved method for CTCs isolation, CellSearch system (5-7). The system uses CTC enumeration as an aid in prognosis and determining therapeutic efficacy.

Since further validation was needed before its clinical utility in lung cancer (8), many studies employed CellSearch system to determine the cut-off number of CTCs that would provide prognostic discrimination in lung cancer. In a prospective 150-patient study, Tanaka *et al*. showed that CTCs numbers could accurately discriminate between stage I and stage IV disease and also observed that the number of CTCs in SCLC was higher compared to NSCLC (9). Muinelo-Romay used CellSearch system in a series of stage IIIB and IV NSCLC showing that the CTCs counts had prognostic value. They also confirmed findings of other studies that using this system only 30–40% of predominantly stage IV NSCLC patients would have a detectable number of intact CTCs, with only 23% of these having more than 5 CTCs per standard sample of 7.5 mL of blood used for isolation (10). While this report that patients with more than 5 CTCs had poorer PFS and OS is consistent with some previous findings (11), others using the same system have found that a cut-off of 1-2 CTCs had prognostic value (12).

In lung cancer, CellSearch system has relatively low sensitivity compared to other methods of CTC isolation, such as isolation-by size of epithelial tumor (ISET) cells (RareCell Diagnostics SAS, Paris, France), or CTC chip (13), where CTCs were detected in 80% of patients with stage IIIA to IV NSCLC. While CellSearch system isolates CTCs expressing epithelial phenotype (EpCAM expression), ISET uses filtration approach, which captures CTCs larger than 8 μm and allows blood cells to pass through. As a result, ISET has the capacity to capture not only epithelial subpopulation of CTCs, but also other subpopulations of CTCs not expressing epithelial markers.

Namely, it is postulated that a predominant mechanism for tumor cell invasion and metastasis involves epithelial to mesenchymal transition (EMT), thus many of the CTCs in circulation would not display epithelial phenotype. Indeed, a recent study demonstrated that there are several phenotypes among CTCs, including epithelial, epithelial-mesenchymal, mesenchymal and mesenchymal stem and proposed cell surface markers for characterization of each CTC subpopulation (14).

Relatively low yields of CTCs in NSCLC by using the above-described, most frequently used isolation methods in clinical studies, call for novel CTC isolation techniques. An innovative approach for CTC isolation was described by Wan *et al*. (15). The authors combined two techniques: immunomagnetic separation to deplete leukocytes and enrich CTCs in the sample, followed by quantitative PCR to quantitate CTCs. The latter utilized differential expression of folate receptor on cell surface of cell types present in the sample: unlike blood cells, lung cancer cells tend to abundantly express folate receptor on their surface. This allowed previously-established average concentration of folate receptor per tumor cell (16) to be used to design a calibrator curve and to infer from the fluorescence detected in the quantitative PCR for folate receptor CTCs (expressed as CTC unit) how many were present in each sample. Remarkably, the number of CTC units detected corresponded tightly to the stage of NSCLC.

PCR-based methods proved more sophisticated when it comes to CTC correlation with the stage of NSCLC. While Tanaka's study discriminated between stage I and stage IV disease using CellSearch (9), Lou showed that CTC number correlated with stage I/II versus stage III and stage IV disease (16). With the technique used in their study, Wan and colleagues achieved even further distinction between stages by CTC number, being able to clearly determine CTC unit cut-offs delineating even stage I from stage II, in addition to being able to distinguish stage III and IV (15).

These findings support the mounting evidence that may lead to a paradigm shift. Initially, CTCs were associated with the process of metastasis and indeed their numbers detectable in blood are the highest in the advanced disease (3,4). However, the notion that CTCs enter blood stream early in the process of carcinogenesis is supported by the detection of CTCs at earlier stages in cancer development suggesting that CTC presence in blood is an early event (9,15,16). This opens an exciting area of potential future utilization for early diagnosis of lung cancer. Although more research is needed to harness this potential, a recent study has shown that CTCs detectable in bloodstream could precede stage I cancer detected by conventional methods, such as CT scan (17). Namely, a study in patients with chronic obstructive pulmonary disease (COPD), which puts them at increased risk of lung cancer development, measured CTCs in this population. This study identified CTCs in 3% of COPD patients at baseline and these patients were followed annually for 5 years by spiral CT scan to detect lung cancer earlier in this population. CT scan detected lung nodules 1–4 years after CTC detection, demonstrating that CTCs can be detected in patients with COPD without clinically-detectable lung cancer (17).

In summary, CellSearch system, the only technique for CTC enumeration that received FDA approval in other malignancies, has low sensitivity in NSCLC, which is hampering the validation of its use in this malignancy. Another method, ISET, has a better sensitivity, when used in stage III and IV NSCLC. PCR methods for CTC detection have even better sensitivity, allowing for CTC detection at earlier stages (I and II). Wan *et al*. reported a novel PCR-based method, which could distinguish between stage I and II NSCLC based on CTC enumeration. Improving techniques for CTC detection in early NSCLC is a linchpin for furthering our understanding of the role of CTCs early in NSCLC development and expand their clinical utility. In addition to enumeration of CTCs, the focus of this discussion, characterization of CTCs isolated from blood may be invaluable, among others, in prognosis and monitoring of treatment response. Thus CTCs are rightly the focus of extensive research and hold great promise for advancing early detection and treatment of NSCLC.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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