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Circulating tumor cells count as a predictor of survival in lung cancer

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ABSTRACT

The presence of circulating tumor cells (CTCs) in the peripheral blood of cancer patients was first described in the second half of the 19th century, but research interest in their potential clinical utility has intensified and greatly expanded only in recent years. Herein, we summarize and critically discuss current knowledge on CTC count as a predictor of survival in lung cancer, and comment on the existing challenges and future perspectives in this field. The majority of data published to date, including the results of almost all large cohorts, are strongly supportive of the value of CTC enumeration as a predictor of survival, mainly in advanced/metastatic non-small and small cell lung cancer (NSCLC and SCLC, respectively). Nonetheless, additional research is warranted to establish the prognostic relevance of CTC count in other clinical settings, mainly encompassing earlier-stage disease as well as specific molecular subtypes of NSCLC (e.g. EGFR mutation-positive or ALK-positive cases).

1. Introduction

Lung cancer is the first cause of tumor-related death worldwide accounting for 1,6 million cancer deaths annually or 1 of every 4 cancer deaths (Ferlay et al., 2015; Siegel et al., 2016). It is currently estimated that 80% among all newly diagnosed lung cancer cases annually will ultimately succumb to their disease, although it is hoped that recent introduction of novel targeted and immunotherapeutic agents in routine oncology practice may, hopefully, alter this dire picture in the near future (Allemani et al., 2015; Herzberg et al., 2017). Considering the advanced disease stage at initial presentation and diagnosis in more than 50% of patients (Ramalingam et al., 2011), the profound clinical, histological and genomic heterogeneity of lung cancer, the challenge of obtaining adequate tissue for pathological confirmation of disease and performance of adjuvant molecular testing and the rapid and, almost universal, development of therapy resistance in the advanced/metastatic setting (Hirsch et al., 2010; Chang, 2011), these dismal survival statistics are hardly surprising, highlighting not only the aggressive nature of this malignancy but also the need to improve current treatment options.

Numerous factors may influence treatment, the most critical of which are disease stage, histological subtype of tumor and performance status of patients. Surgery (segmentectomy, lobectomy, pneumonectomy), alone or followed by chemotherapy and/or radiation therapy, offers the best chance of cure and long-term survival for localized lung cancer, while treatment options for advanced and metastatic disease include variable combinations of cytotoxic chemotherapy, radiotherapy and targeted biologic agents, often resulting in significant and additive toxicity (Wu et al., 2017). Thus, improved prediction of probability of disease recurrence and survival and treatment response are needed for a more accurate selection of lung cancer patients who might benefit the most from available treatments, with the ultimate aim to increase treatment efficacy without a parallel increase of unnecessary treatment-related side effects (Gazdar and Schiller, 2011).

Although the presence of circulating tumor cells (CTCs) in the peripheral blood of cancer patients was first described in 1869 by Ashworth (1869), followed by Stephen Paget's "seed and soil" hypothesis in 1889 (Paget, 1889), research interest in their clinical utility has expanded only in recent years, in parallel with the development of novel technology platforms for their isolation and subsequent analysis. CTCs are a subset of tumor cells with the ability to escape from the primary site, intravasate into nearby blood and/or lymphatic vessels, survive into the challenging microenvironment of bloodstream, extravasate from the vascular system into the surrounding tissue and form micrometastases in secondary organs with the potential of growth into macroscopic tumors (Joosse et al., 2015; Kang and Pantel, 2013).

The detection and subsequent enumeration of CTCs in the peripheral blood of patients with non-small cell or small cell lung cancer (NSCLC or SCLC, respectively) is increasingly investigated as a novel biomarker of tumor's growth dynamics, with the potential to offer independent prognostic information and/or predict response to treatment. The results of previously published studies on the prognostic value of CTCs in the above clinical settings are generally supportive of its role as a predictor of disease relapse and/or survival, but controversy

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remains regarding its exact clinical relevance in routine practice (Pantel and Alix-Panabières, 2010; Lianidou et al., 2015; Normanno et al., 2016).

Herein, we provide a narrative review of the existing data on the prognostic role of peripheral CTCs in lung cancer and briefly comment on current challenges and future perspectives in this field. PubMed database was searched using the terms: "NSCLC" or "SCLC" AND "circulating tumor cells". Eligible studies for inclusion were those which: a) correlated CTC count with survival endpoints, e.g. overall survival (OS), disease free survival (DFS) and progression free survival (PFS), in patients with NSCLC or SCLC, and b)were written in English; studies with heterogeneous patient populations including not only NSCLC or SCLC cases but also patients with other solid tumors were excluded from our analysis. Studies investigating the prognostic value of CTCs in the central circulation only (e.g. pulmonary vein) and not in peripheral venous blood were also excluded.

2. CTC detection methods

In order to detect the infrequent isolated tumor cells among millions of normal hematopoietic cells (it is estimated that 1–10 CTCs can be found per 1 ml of whole blood, at a background of one billion normal blood cells) (Joosse et al., 2015; Nagrath et al., 2007), an enrichment step is first required. Methods for CTC enrichment are generally classified into label-dependent and label-independent techniques. The former (label-dependent) techniques are based on the biological features of tumor cells (e.g. expression of cell surface markers), while the latter (label-independent) are based on their intrinsic physical properties (most commonly cell size and density) (Joosse et al., 2015; Alix-Panabières and Pantel, 2013; Hanssen et al., 2015; Krebs et al., 2010).

Label-dependent CTC enrichment mainly employs immunomagnetic separation techniques, leading to selection of CTCs with the use of ferrofluids or magnetic beads coated with antibodies against epithelial antigens expressed on the surface of CTCs and not by the surrounding blood components (positive CTC selection) or against antigens expressed by blood cells only (negative CTC selection/depletion) (Joosse et al., 2015; Nagrath et al., 2007; Alix-Panabières and Pantel, 2013; Hanssen et al., 2015; Krebs et al., 2010; Toss et al., 2014; Mostert et al., 2009). The epithelial cell adhesion molecule (EpCAM) and leukocyte common antigen (CD45) are the most commonly used antigens for positive and negative selection of CTCs, respectively (Alix-Panabières and Pantel, 2013). In addition to the above methods, novel sophisticated diagnostic platforms for CTC enrichment have been developed, including the use of microfluidic assays (e.g. CTC-chip) or in vivo isolation techniques (e.g. GILUPI CellCollector™) (Nagrath et al., 2007; Sequist et al., 2009; Thege et al., 2014; Saucedo-Zeni et al., 2012; Gorges et al., 2016a).

With the use of label-independent techniques, CTCs are isolated according to cell size (e.g. ISET® filtration), density (e.g. Ficoll–hypaque density gradient separation, OncoQuick), deformability (e.g. atomic force microscopy), dielectric properties (e.g dielectrophoresis) or a combination of physical features (e.g. label-free microfluidic techniques) (Harouaka et al., 2013; Vona et al., 2000; Müller et al., 2005; Gertler et al., 2003; Kallergi et al., 2016; Shim et al., 2013a; Jen and Chang, 2011; Shim et al., 2013b; Moon et al., 2011).

Yet, even after an efficient enrichment step, CTCs need to be further identified and isolated from a substantial number of remaining blood cells (Joosse et al., 2015). A large variety of immunological, molecular and functional-based strategies may be used for this purpose, and are further classified into cytometric (whole cell-based) or nucleic acid-based techniques (Joosse et al., 2015; Krebs et al., 2010; Toss et al., 2014). Conventional or automated scanning microscopes and cytometers, in combination with immunocytochemistry (ICC) or immunofluorescence for the expression of various epithelial (e.g. cytokeratins), mesenchymal or tissue-specific markers − along with staining for the nuclear dye 4′, 6-doamidino-2-phenylindole (DAPI)- allow the detection and enumeration of CTCs (Joosse et al., 2015; Krebs et al., 2010; Toss et al., 2014; Millner et al., 2013). Nucleic acid-based assays target gene alterations or tumor-specific mRNA transcripts, mainly employing qualitative or quantitative reverse transcriptase-PCR (RT-PCR or qRT-PCR), while functional assays, like the EPithelial Immuno SPOT technology (EPISPOT) detect cell-secreted proteins, thus leading to isolation of only viable CTCs (Toss et al., 2014; Smith et al., 1991; Stathopoulou et al., 2003; Alix-Panabières and Pantel, 2015; Soler et al., 2017).

Assays for CTC isolation and analysis, combining enrichment and detection steps (e.g. CellSearch, AdnaTest, and the above described techniques ISET and EPISPOT), are also commercially available (Vona et al., 2000; Soler et al., 2017; Allard et al., 2004; Allard and Terstappen, 2015; Andreopoulou et al., 2012). The CellSearch® system is the only U.S. Food and Drug Administration (FDA) approved test for the enumeration of CTCs of epithelial origin in patients with metastatic breast, prostate and colorectal cancer (Gorges et al., 2016b). This widely used cytometric platform employs immunomagnetic separation for CTC enrichment using EpCAM-coated ferrofluids, followed by immunofluorescent staining for cytokeratins (CK8, 18 and 19), CD45 and DAPI (Allard et al., 2004).

Although a comparative analysis of the pros and cons of CTC enrichment and detection methods is outside the scope of the present review, a main limitation of label-dependent techniques must be emphasized, i.e. their inability to efficiently capture CTCs which have switched to a mesenchymal phenotype via epithelial to mesenchymal transition (EMT). (Joosse et al., 2015; Alix-Panabières and Pantel, 2013; Grover et al., 2014). EMT is a key process for the formation of metastases, aiming to confer improved invasive and survival traits to tumor cells and thus facilitate their detachment from the primary site and metastatic spread to secondary locations; furthermore, it may be partial or complete, leading to highly heterogeneous subpopulations of CTCs with hybrid/mixed epithelial-mesenchymal or purely mesenchymal phenotypes, respectively, (Garg, 2017; Jolly et al., 2016; Joosse and Pantel, 2013; Lecharpentier et al., 2011). CTCs that have undergone any degree of EMT may be difficult to discern from normal hematopoietic cells on the basis of their immunological properties alone, while their increased levels in the bloodstream may carry significant prognostic implications as an indicator of a more aggressive disease course, higher metastatic potential and drug resistance (Garg, 2017; Jolly et al., 2016; Joosse and Pantel, 2013; Lecharpentier et al., 2011).

3. Prognostic significance of CTCs in lung cancer

The potential association between CTC count and prognosis has been previously investigated in several observational studies or exploratory analyses of clinical trial data. A summary of these studies is shown in Tables 1 and 2 (including NSCLC and SCLC cohorts, respectively) (Hofman et al., 2011a; Hofman et al., 2011b; Krebs et al., 2011; Nieva et al., 2012; Isobe et al., 2012; Punnoose et al., 2012; Hirose et al., 2012; Muinelo-Romay et al., 2014; Juan et al., 2014; Zhang et al., 2016; Bayarri-Lara et al., 2016; Crosbie et al., 2016; He et al., 2016; Qi and Wang, 2017; Chudasama et al., 2017; Zhou et al., 2017; Yang et al., 2017; Coco et al., 2017; Lindsay et al., 2017; Li et al., 2017; Hou et al., 2009; Hou et al., 2012; Naito et al., 2012; Hiltermann et al., 2012; Igawa et al., 2014; Normanno et al., 2014; Huang et al., 2014; Cheng et al., 2016; Messaritakis et al., 2017a; Shen et al., 2017; Salgia et al., 2017; Messaritakis et al., 2017b), while their results are analyzed in detail below.

3.1. Non-small cell lung cancer (NSCLC)

3.1.1. Resectable or locally advanced NSCLC (stages I–III)

Bayarri-Lara et al. (2016) prospectively assessed the prognostic value of CTCs in 56 patients with resectable (stage I-IIIA) NSCLC, using immunocytochemistry methods for CTC detection. Positive