Circulating free DNA, new dynamic marker in nonsmall cell lung cancer patients?

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cfDNA concentration might be a prognostic factor in NSCLC and could be a tool for detecting molecular alterations http://ow.ly/TF8TC

Platinum-based doublet chemotherapy prolongs survival and improves quality of life as first-line treatment for non-selected and good performance status patients with advanced nonsmall cell lung cancer (NSCLC) [1]. However, the survival benefit is limited, even if this combination is the standard of care for decades, no clinically relevant predictive biomarkers have been discovered so far. The identification of surrogates for survival is an important topic for cancer therapy selection. Indeed, overall survival is still the best criterion for predicting treatment efficacy in lung cancer but it can, of course, not be used in clinical practice. In a recent systematic review of the literature, some intermediate criteria have been shown to be potentially useful predictors such as time to progression, progression-free survival, objective response, local control after radiotherapy, downstaging in locally advanced NSCLC, complete resection and pathological TNM (tumour, node, metastases) in resected NSCLC or circulating molecular markers or cells. Retrospective studies have suggested that carcinoembryonic antigen, cytokeratin 19 fragments, pro-gastrin-releasing peptide and, to a lesser extent, neuron specific enolase, cancer antigen 125 and cancer antigen 19-9, used as single criterion to assess overall survival, could be adequate intermediate criteria for survival in lung cancer patients [2]. The use of more sophisticated molecular markers developed thanks to recent progresses in molecular biology has also so far not been successful for that purpose even if high throughput techniques are used such as transcriptomic analyses assessing multiple miRNAs and mRNAs [3].

In the study reported in the present issue of the *European Respiratory Journal*, Tissot *et al.* [4] have assessed circulating cell-free DNA (cfDNA), which comprises small fragments of nucleic acids that are released from normal cells and tumours by programmed cell death (apoptosis). cfDNA can be detected and monitored in plasma of blood in all patients [5]. Thus, cfDNA has emerged as an attractive tumour marker for its minimal invasive, convenient, and easily accepted properties.

The purpose of the study was to determine if this biomarker can be used as a prognostic tool for tumour response assessment and for survival in NSCLC patients treated by platinum-based chemotherapy. The authors report that cfDNA concentration in plasma samples collected before (baseline) platinum-based chemotherapy in 218 locally advanced or metastatic NSCLC patients had an independent prognostic value. Patients with highest cfDNA concentration showed a significantly shorter progression-free survival ($p=0.034$) and overall survival ($p=0.001$) than patients with lower cfDNA concentrations. However, changes in total cfDNA concentration during treatment were not predictive to assess the effectiveness of chemotherapy in NSCLC patients. In terms of methodology, the main advantages of the study by Tissot *et al.* [4] are its prospective character, the multivariable analysis and the relatively inexpensive and uncomplicated method of dosage, easily implementable in clinical practice. But a reproducible cut-off determination is lacking, being a necessary requirement for external validation studies. Other weak points

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of the study are the lack of *a priori* statistical considerations and the use of various chemotherapy regimens, even if all are platinum-based.

High cfDNA level has been proven to be a non-invasive biomarker of poor outcome in lung cancer patients [6–8], whereas other studies did not report such a relationship [9]. This is mostly explained by the lack of standardisation of the cfDNA test, with major differences regarding cfDNA extraction, quantification and cut-off points. Moreover, another limitation is linked to the source of cfDNA: its concentration reflects not only changes in circulating tumour DNA (ctDNA) but also reflect medical conditions or patients' characteristics that may lead to an increase in cfDNA concentration [10]. Also molecular characteristics of the tumours might have an impact in the prognostic value of cfDNA concentration [11]. Tissot *et al.* [4] reported a significant positive association between baseline cfDNA concentration and Eastern Cooperative Oncology Group performance status at diagnostic (higher cfDNA concentration in worse performance status; p=0.001), but not with stage or histological type. This suggests that patients' characteristics might influence in the basal cfDNA concentration, which could have an impact in the final prognosis.

In fact, cfDNA translates an inflammatory process. Simples scores based on routine tests have been developed to determine the prognostic and predictive role of inflammation in oncology, with many studies conducted for lung cancer [12]. The modified Glasgow score is the mostly used for clinically assessing inflammation [13]. This score includes: C-reactive protein (CRP), and albumin to define three groups of different prognosis: 0 in the absence of an elevated CRP, 1 being an increased CRP and 2 an increased CRP and a decreased albumin [14]. It has been assessed as a useful and important predictor of cancer-specific survival in NSCLC patients [15]. This score might be used as a stratifying factor when assessing new biomarkers related to inflammation such as cfDNA. Interestingly, and contrary to data obtained by Tissot *et al.* [4] with cfDNA, the modified Glasgow score also predicted response and toxicity to chemotherapy in a prospective study in patients with metastatic NSCLC [16].

Because cell-free ctDNA is a potential surrogate for the entire tumour genome, cfDNA has been extensively studied as an alternative to biopsy, leading the term "liquid biopsy" [17]. The treatment of lung cancer is increasingly guided by molecular subtyping, such as mutations in the epidermal growth factor receptor (*EGFR*) gene [18], but access to tumour tissue to perform the molecular test sometimes is limited. The detection of ctDNA allows the easy identification of druggable genomic alterations, with high concordance, specificity, and sensitivity such as demonstrated with the detection of *EGFR* mutation [19–21]. Despite the initial dramatic response rate and benefit in progression free survival to EGFR tyrosine kinase inhibitors (TKI), most of *EGFR* mutant NSCLC patients develop acquired resistance 1 year after treatment initiation [22], the T790M mutation being the main mechanism of resistance. Personalised second-line therapy in *EGFR* mutant NSCLC patients with AZD9291 [23] or CO1686 [24] requires testing the T790M status in a recent tissue biopsy, being a predictive factor for these new therapeutic strategies. Unfortunately, the location of the tumour and the risk of complications are serious limitations to re-biopsies in NSCLC [25] and not all patients are willing to perform it. Qualitative and quantitative *T790M* in plasma ctDNA provide a non-invasive and sensitive assay to predict prognosis of EGFR-TKI treatment [26] and treatment strategy. There are clear advantages to measuring ctDNA as a marker of tumour dynamics over conventional protein biomarkers or even imaging studies. For one, ctDNA has a comparatively short half-life (approximately 2 h), allowing for evaluation of tumour changes in hours rather than weeks to months [27]. Also, ctDNA may be more representative of tumour heterogeneity than a small biopsy sample and could enable the investigation of acquired resistance mechanisms and allows the investigators to monitor the evolution of resistance over the time [28].

We could conclude by Tissot *et al.* [4] study that total cfDNA concentration appears to be an independent prognostic factor in lung cancer but the most appealing application regarding cfDNA, so far, is the detection and dynamic monitoring of molecular alterations. Nevertheless, the establishment of robust and standardised protocols for blood sampling, processing, storage, DNA extraction and analysis, and cut-off points to define the clinical relevance of the findings are required before cfDNA biomarkers can be utilised in clinical practice.

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