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SPOTLIGHT

Liquid biopsies and molecular imaging: friends or foes?

Ignasi Carrió[1] · **Albert Flotats**[1]

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Molecular Imaging is currently central in the diagnostic field and its role seems difficult to challenge. In particular, PET/CT is offering critical molecular information in the characterisation of disease, staging and control of therapies in oncology, quantification of biologic phenomena in cardiovascular medicine, and elucidation of the pathophysiology and treatment options in neurodegenerative diseases. However, genomic and molecular characterisation of pathological processes is being rapidly adopted. Such characterisation includes liquid biopsy, an approach that has distinct advantages and may have a significant impact on the need and role of imaging in clinical medicine.

Liquid biopsy is based on the fact that several physiological and pathological conditions induce cell and tissue remodelling, leading to the rearrangement of stroma and tissue microenvironment. These events are mostly sustained by necrotic or apoptotic processes, leading to the disaggregation of tissues and the consequent dissemination of cells and cellular debris (including DNA fragments) in the intercellular space and bloodstream, where they can be detected. Independently from cell necrosis or apoptosis, cells also release cellular DNA in the extracellular space via the secretion of vesicles and exosomes.

Accordingly, liquid biopsy is the sampling and analysis of non-solid biological tissue, primarily blood, to look for different biomarkers in order to non-invasively detect or monitor diseases. There are several types of liquid biopsy depending on the condition that is being studied. In cancer patients, the most intensely investigated approaches include the analysis of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA), with some tests already approved in clinical practice [1, 2]. However, the concept is extending to other pathological conditions. For example, in cardiovascular diseases, circulating endothelial cells (CECs) can be sampled [3], or isolation of protoporphyrin IX from blood samples can be used for the assessment of atherosclerosis [4]. In prenatal diagnosis, cell-free fetal DNA (cff-DNA) extracted from maternal blood or amniotic fluid can also be analysed. The cerebrospinal fluid may be sampled and analysed in the case of neurodegenerative diseases [5].

In cancer patients, liquid biopsy may be used to help find cancer at an early stage or in the course of different therapies, where it may be used to help planning appropriate or tailored treatment [6, 7]. As it can be performed sequentially over time, it can bring information on the molecular changes that are taking place in a given tumour. Along this line, CTC analysis provides a unique opportunity to study whole cells, even at the single-cell level, allowing DNA, RNA and protein-based molecular profiling, and the prospect for functional studies on tumour heterogeneity or treatment resistance to guide personalised therapy. Detection of CTCs in increasing numbers is an indicator of active metastasis. Importantly, expression of specific markers on CTCs might enable prediction of what organ site is likely to be metastasised. However, isolation of CTCs is difficult due to the rarity of the cells, thus requiring a complex process of enrichment (e.g. using cell-specific markers), isolation and then enumeration.

On the other hand, plasma ctDNA analysis is attractive due to the ease with which plasma can be collected and analysed, requiring less blood extraction (2 mL) than CTC assays (10 mL) and no prior need to enrich and isolate a rare population of cells. Analysis of ctDNA gives information on the tumour mutational landscape. Additionally, ctDNA is more easily quantifiable than CTCs. Furthermore, the short half-life of ctDNA (spanning between 16 min and 13 h) allows immediate correlation with tumour cell status, thus offering the possibility of a continuous dynamic observation. However, ctDNA assays identify tumour activity but not tumour spread through circulation (i.e. occurring metastasis) since ctDNA can also come from necrotic and apoptotic

Ignasi Carrió and Albert Flotats contributed equally to writing and editing this paper.

✉ Ignasi Carrió
icarrio@santpau.cat

[1] Nuclear Medicine Department, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Sant Quintí 89, 08025 Barcelona, Spain

cells. Besides, organ-specific differences in the representation of ctDNA may influence the interpretation of ctDNA results. Two of the main strategies to assess ctDNA involve DNA sequencing techniques, either targeted (e.g. digital polymerase chain reaction—PCR) or non-targeted (e.g. next-generation sequencing—NGS). Non-targeted NGS provides a comprehensive view of a cancer's genetics and is appropriate for diagnosis or eventual screening. Alternatively, digital PCR offers a more targeted approach applicable to detect minimal residual disease and to monitor treatment response and disease progression in oncology [7–9].

It is important to note the potential impact of liquid biopsies and genomic information on the practice and volumes of medical imaging. Liquid biopsy may offer early diagnosis in the very few months of disease progression, even before any detectable change either in medical imaging or in blood protein markers levels. The ease of local blood collection, which can be sent to a central laboratory for processing and analysis, can allow more frequent disease assessment while avoiding or minimising radiation exposure. In cancer patients, for initial diagnosis and staging, it seems that impact may be limited, despite the value of liquid biopsies for screening and detection of common cancers has been recently shown. Cohen et al. [10] have recently described a blood test that can detect eight common cancer types through assessment of the levels of circulating proteins and mutations in cell-free DNA (cfDNA). They applied this test, called CancerSEEK, to 1005 patients with non-metastatic, clinically detected cancers of the ovary, liver, stomach, pancreas, oesophagus, colorectal, lung, or breast and to 812 healthy control individuals. The median sensitivity of the test among the eight cancer types evaluated was 70%, ranging from 98% in ovarian cancers to 33% in breast cancers (and 69% to 98% to detect five cancer types including ovary, liver, stomach, pancreas and oesophagus). At this sensitivity, the specificity was > 99%. The sensitivity for the detection of early tumours was rather low (highest for liver cancer—100% and lowest for oesophageal cancer—20%). Using supervised machine learning to predict the underlying cancer type in patients with positive tests, CancerSEEK was able to localise the source of the cancer to two organ sites in a median of 83% of these patients and to a single organ in a median of 63% of these patients. The accuracy of prediction varied with tumour type; it was highest for colorectal cancers and lowest for lung cancers.

During follow-up or for assessment of response to therapy, in particular in areas where an effective systemic treatment exits, a blood test will simplify management and may significantly reduce the need for additional imaging. There are proof of concept studies that target areas in which traditionally molecular imaging tests are preferred. In the case of neuroendocrine tumours (NET), Kidd et al. [11] have developed a blood-based 51 NET-specific transcript set for diagnosis and monitoring of neuroendocrine tumours and evaluated clinical performance metrics. It accurately diagnosed the tumour and reliably differentiated stable from progressive disease as determined by RECIST criteria. Blood measurements accurately diagnosed bronchopulmonary carcinoids, distinguishing stable from progressive disease, and may have clinical utility as a diagnostic liquid biopsy may be able to define disease activity and progression in real-time.

Hong et al. [12] have recently studied a subset of patients with metastatic melanoma following treatment with immune checkpoint inhibitors. They developed a molecular signature of melanoma CTCs to quantify early tumour response using blood-based monitoring with a quantitative 19-gene digital RNA signature (CTC score). A decrease in CTC score within 7 weeks of therapy correlates with marked improvement in progression-free survival [hazard ratio (HR), 0.17] and overall survival (HR, 0.12). Thus, digital quantitation of melanoma CTC-derived transcripts enables serial non-invasive monitoring of tumour burden, supporting the rational application of immune checkpoint inhibition therapies and at the same time offering a means to appropriate evaluation of tumour response.

As an example of the value of in vivo genomics in cardiovascular diseases, Miao et al. [13] have investigated the pathways and genes involved in coronary artery disease (CAD) and the associated mechanisms. Two array data sets of GSE19339 and GSE56885 were downloaded. They analysed the differentially expressed genes (DEGs) in normal and CAD specimens and identified 413 DEGs (291 up-regulated and 122 down-regulated). The relative expression of IL1B, ICAM1 and CCL2 was higher in CAD than in normal controls.

Genomic and molecular characterisation of tumours including liquid biopsy measurements is now clinically applied. A recent ESMO report describes the "multidisciplinary molecular tumour board" as a tool to improve clinical practice and selection accrual for clinical trials in patients with cancer [14]. This report indicates that the creation of molecular tumour boards for patient selection and assessment of treatment options is a result of the complexity of delivering precision medicine to cancer patients. Such boards in hospital environments allow patients with refractory cancer to be included in clinical trials and improve the precision of clinical decisions compared with a standardised set of mutation-driven recommendations.

Certainly such molecular tumour boards will become important in clinical decision making and selecting patients for new therapies in oncology. Although molecular and genomic characterisation of tumours is mainly based on the analysis of specimens and blood samples, molecular imaging, and in particular PET/CT, can play an important role if appropriate combination of the information coming from liquid biopsies from peripheral blood, molecular

characterisation from tumour specimens and in vivo molecular imaging technologies are applied together.

Examples of such combination include recent work in lymphomas, where the merging of image parameters such as metabolic tumour volumes and genomic phenotypes may provide better stratification of patients. Toledano et al. [15] evaluated the predictive significance of total metabolic tumour volume (TMTV) measured on baseline 18F-fluorodeoxyglucose (18F-FDG) PET/CT and its value in addition to gene expression profiling using a new method of gene analysis of rapid reverse transcriptase multiplex ligation-dependent probe amplification assay, in 114 patients with diffuse large B cell lymphoma treated with R-CHOP or R-CHOP-like chemotherapies. TMTV or gene analysis identified two risk groups, respectively. TMTV combined with molecular data identified three groups with very different outcomes: patients with a low TMTV whatever their phenotype, patients with a high TMTV and GCB phenotype and patients with a high TMTV and ABC phenotype, with respective 5-year progression-free survival (PFS) rates of 72%, 51% and 17% ($p < 0.0001$), and 5-year overall survival (OS) rates of 83%, 55% and 17% ($p < 0.0001$).

A single-centre prospective study in patients with untreated stage I–IIIA non-small cell lung cancer (NSCLC), who underwent radical resection, detected CTCs in 39.2% of patients before (CTCs1) and in 27.5% 1 month after the surgery (CTCs2) [16]. The presence of CTCs2 was significantly correlated with preoperative tumour maximum standardised uptake value (SUV_{max}) on 18F-FDG PET/CT, pathological stage and surgical approach (thoracotomy or video-assisted thoracic surgery), and it was associated with recurrence-free survival (RFS) on multivariate analysis, independently of disease staging. CTCs2 were significantly correlated with a shorter RFS, and only SUV_{max} was an independent predictor for the presence of CTCs2 [16].

In NET it has been shown that the combination of the genomic signature of the tumour in blood together with imaging signals offers improved prognostication. Bodei et al. [17] have shown in a multicentre study that NET transcript expression in blood integrated with tumour grade and with imaging parameters provides a peptide receptor radionuclide therapy (PRRT) predictive quotient (PPQ) that effectively stratifies PRRT "responders" from "non-responders".

In breast cancer, Groheux et al. [18] have shown that the combination of genomic tumour grades and 18F-FDG PET/CT, both determined before and after 2 cycles of neoadjuvant chemotherapy (NAC), better predicts pathologic complete response (pCR) in triple negative breast cancer (TNBC) patients. 18F-FDG PET/CT and the genomic grade index (GGI), each separately, showed good potential to predict pCR. Results in 55 patients showed that the early tumour metabolic change during NAC is a powerful parameter to predict pCR and outcome in TNBC patients. The GGI, determined on pre-treatment biopsy, is also predictive of pCR but the combination GGI and baseline SUV_{max} improves such prediction.

In patients with multiple myeloma (MM), Abe et al. [19] assessed the value of 18F-FDG PET/CT coupled with clonal circulating plasma cell (CPC) quantification for MM prognostication. PET-CPC staging system predicted survival outcomes independently of established risk factors in 163 consecutive patients with newly diagnosed, symptomatic MM receiving novel agents during induction therapies. The study shows that pre-treatment 18F-FDG PET/CT assessment combined with CPC quantification improves the prognostication of MM and facilitates the development of novel risk-adapted approaches for such patients.

All these studies together indicate that the combination of genomic/molecular information taken from liquid biopsies or tumour specimens and imaging parameters derived from PET/CT has incremental value over both technologies used separately. Such combination has the potential to facilitate the development of new theragnostic models, and new treatment strategies based on molecular targeting of the disease. It is important for the molecular imaging community to explore and demonstrate the value of integrating biomarkers offered by imaging with those offered by genomic/molecular characterisation in cancer patients and other major medical conditions.

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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