

COMMENTARY

Bio-marker detection redundancy improves sensitivity

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Abstract

Liquid biopsy for biomarker detection has the potential to revolutionise the management of cancer by reducing the time that tumours grow and metastasize undetected and untreated. As a relatively less invasive test, the frequency of testing is constrained more by the cost and ease of analysis, which are connected to the specificity and sensitivity of the method of detection. A recent study by Zhao et al. in *Clinical and Translational Medicine* indicates that multiple forms of detection from the liquid biopsy sample may improve sensitivity and demonstrates that seemingly incremental advances in clinical practice can significantly impact patient outcomes.

KEYWORDS

biomarker, liquid biopsy, sarcoma, tumour

More than 150 years ago, Thomas Ashworth described circulating tumour cells (CTCs) in the blood of a deceased patient while working in Melbourne Hospital.¹ Liquid biopsies now permit the detection of CTC, tumour exosomes, tumour-educated platelets and tumour-derived metabolites or polynucleotides (DNA/RNA). In place of Ashworth's microscope and hand-drawn pictures, we now have flow cytometry, DNA/RNA sequencing, automated image analysis and multiple forms of polymerase chain reaction (PCR) to aid the detection of cancer incidence or relapse prior to symptoms or radiological imaging.

Much research and discussion of liquid biopsies has focussed on comparisons between circulating biomarker detection assays, with the two most studied biomarkers being CTC and circulating tumour DNA (ctDNA).^{2,3} The rationale for the test will have some bearing on the applicability of different methods. Population screening for a range of common cancers (e.g., breast, prostate and liver) will have different requirements to prognostic assays for a particular form of cancer, which will again differ for a

customised assay for early detection of relapse of a particular individual's cancer, or a rare cancer with well-characterized variants (e.g., Ewing sarcoma). One practical advantage liquid biopsies have is that they are minimally invasive and usually low cost at the point of collection; for most people, this permits increased frequency of assessment and a greater likelihood of early detection. Regardless of the rationale, the test should be able to detect the tumour at a time when intervention is able to improve clinical outcomes, at a cost that is justifiable versus competing needs.

Current methods have varying degrees of cost, speed and quality (Figure 1A). Commercially available methods for liquid biopsy for common cancers include enrichment and immuno-fluorescent detection of CTC or sequencing of ctDNA, which require significant investment in equipment or are expensive to use. CTCs are detectable by immunocytochemistry or flow cytometry based upon the expression of tumour-associated cell surface makers, and the absence of leucocyte marker CD45; these properties

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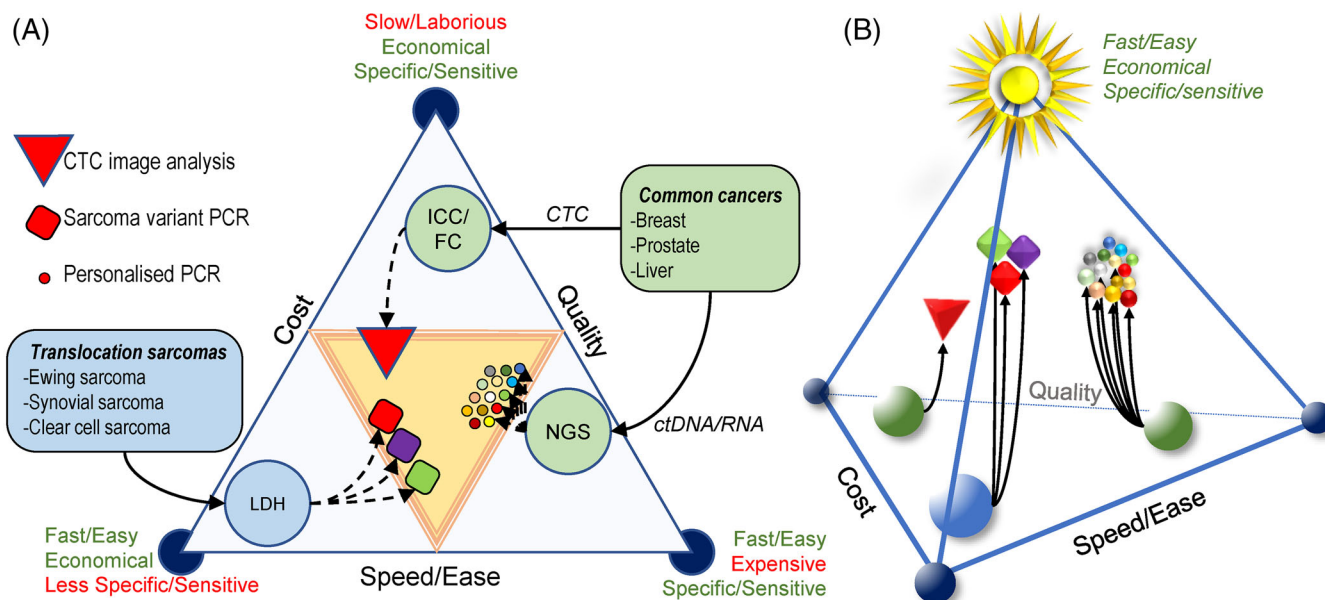


FIGURE 1 Current constraints on the development of liquid biopsies. (A) The cost of equipment, technicians, reagents and licensing must be weighed against the quality of the assay. The quality of the assay is a measure of sensitivity, specificity and scope. The speed and ease of the assay will be determined by such factors as the difficulty of acquiring and processing the specimen and the analysis of results. (B) A liquid biopsy without compromise is easy to perform, low cost and has high sensitivity/specificity for each test. CTC, circulating tumour cell; FC, flow cytometry; ICC, immunocytochemistry; LDH, lactate dehydrogenase; NGS, next generation sequencing

have been refined into a less laborious process using the US Food and Drug Administration-approved CellSearch (Veridex). For monitoring cancer patients, whole genome next generation sequencing (NGS) of ctDNA has been refined to panels of known oncogenes (e.g., AmpliSeq Focus panel (Illumina)). Based upon an initial sequencing of the tumour genome, several studies have demonstrated the utility of PCR assays customised for each patient, despite the cost of creating personalised tests.⁴ In contrast to the specificity and scope of these examples, a blood test for lactate dehydrogenase (LDH) is significantly less expensive and does not require highly specialised equipment or expertise. While LDH at unelevated levels is reasonably specific for the absence of disease, the presence of elevated LDH is not uniformly specific or sensitive for the presence or prognosis of cancer.⁵ For rare sarcomas caused by chromosomal translocation, the use of LDH to detect relapse may be superseded by PCR for the fusion genes (e.g., *EWSR-FLII*, *EWSR-ERG*) either in ctRNA or CTC.⁶

These varying methods have been improved by (1) refining the scope of a liquid biopsy, such as using personalised ctDNA PCR assays based upon tumour DNA sequencing, (2) improvements to the method of detection by means of less laborious technology (e.g., flow cytometry vs. automated enrichment of CTC and image analysis) or (3) the application of a new technique of greater specificity and sensitivity. As well improving liquid biopsies to be inexpensive, expeditious and of greater quality (Figure 1B), another

strategy is to use two different but complementary methods to improve detection. Recently, Withrow et al.⁷ combined analysis of common diagnostic tests with a faecal immunochemical test (FIT) to improve detection of colorectal cancer, as 1 in 10 colorectal cancers are not detected using FIT alone; ultimately, stratifying patient risk using FIT could not be improved upon by other common blood tests. When assessing two forms of biomarker that are both derived from the tumour, Cohen et al.⁸ had greater success with a combination of detecting *KRAS* mutations (by sequencing) and a series of protein biomarkers associated with disease, but lacking the sensitivity for screening, which was an improvement on either method alone for the detection of pancreatic cancer.

In a recent study, Zhao and colleagues⁹ combined two methods of liquid biopsy to monitor minimal residual disease in hepatocellular carcinoma. From blood collected pre- and post-surgery (hepatectomy or transplant), the researchers isolated CTC based upon expression of asialoglycoprotein receptor using a microfluidics system, then stained the captured cells with antibodies for cytokeratin and CD45 to measure CTC. DNA from each patient's plasma was extracted and analysed by NGS, by a universal panel of targeted sequencing or a personalised panel for mutations identified from whole exome sequencing of each tumour. The combined assays improved sensitivity for predicting recurrence and resulted in a median time from detection to relapse of greater than 3 months;

this combination of liquid biopsies builds upon similar approaches in breast³ and prostate cancer.¹⁰

In conclusion, the time gained for intervention by early detection can significantly reduce mortality, and the most appropriate test or combination of tests will differ according to clinical need, while the development of less expensive, faster and better liquid biopsies continues.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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