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Editorial

Liquid biopsy for cancer patients: Principles and practice



A B S T R A C T

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Liquid biopsy has the potential to provide information about cancers without invasive biopsy, using circulating biomarkers. These include proteins, RNA and DNA. They can be used in detection, diagnosis, monitoring and detection of recurrence. While protein-based tumour markers have been used in routine pathology for many years, the ability to detect mutations in circulating DNA is relatively new, and poised to enter clinical practice. A number of issues remain, and it is important that such markers are fully validated before they enter clinical practice. Evidence of clinical utility and cost effectiveness are major hurdles, but it is likely that the use of liquid biopsy in defined settings could benefit cancer patients substantially.

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1. Introduction

Blood-based biomarkers (liquid biopsy) in cancer have considerable potential for the initial detection, diagnosis and monitoring of cancer [1]. Circulating tumour cells (CTC), circulating tumour DNA (ctDNA) and microRNA (miRNA) are all options, alongside existing and new protein markers. CTCs are present in the peripheral blood of patients with most types of advanced cancer, but there is uncertainty around the sensitivity of CTCs in low volume disease in comparison with ctDNA or miRNA [2–4]. ctDNA levels reflect tumour load, and increased levels can be found in pre-malignancy [5]. ctDNA permits the detection of mutations, which can be used to guide therapy [6], and altered gene methylation. miRNA signatures are providing promising results for cancer detection, though their ability to predict response to therapy is uncertain [7].

DNA-based markers have considerable potential. We and others have shown that cfDNA appears in blood from tumours at an early stage, and is produced by benign tumours (adenomas) as well as invasive carcinomas [5]. Within ctDNA there is now a considerable literature showing that mutations, including those of relevance to treatment choice, can be detected with high sensitivity and specificity [6]. This includes fusion genes. While changes in copy number are more difficult to assess, this has been done with some success – though at considerable cost [8]. Changes in methylation occur within ctDNA and such assays may be clinically as well as technically feasible [9,10].

In contrast to DNA, RNA is often thought of as very labile and difficult to work with. While this may be true of cytokine mRNA in particular, it is less so for some other types of RNA, as follows.

1. miRNA: a number of groups have published data showing that miRNA profiles in plasma and serum have good sensitivity and specificity for cancer diagnosis [7,11,12].
2. RNA from plasma exosomes and CTCs which can be used for RNA sequencing, permitting the identification of fusion genes from RNA as well as DNA.
3. RNA from blood leukocytes permits identification of immunological reactions to cancer, which may become of considerable future importance, particularly for patients on immunotherapy (e.g. anti-CTLA4).

The classical tumour markers in clinical practice are mostly proteins. Tumour markers are particularly important in following the response of patients to treatment, and making an early diagnosis of relapse. These molecules are used in initial diagnosis (usually in combination with imaging and other diagnostics), though they are less than successful in this role. With a few exceptions, such as CA125 in ovarian cancer, these markers are not specific to a particular cancer type. They are however regarded as the standard that new biomarkers need to exceed to be clinically useful, and can act as a useful gold standard for studies.

The appearance of neoplastic cells in blood is unsurprising as it is a requirement for metastasis. Moreover, in most tumours types, the number present reflects the stage of the tumour: low stage tumours with limited metastatic ability have low numbers of circulating tumour cells (CTC), while advanced tumours may have many CTC. CTCs contain intact DNA, RNA and proteins, and arguably represent clones within the tumour with metastatic potential. They have therefore considerable diagnostic potential, at least in advanced disease.

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2. Current issues in the field

It is debatable which of these sources best identify markers of most importance, but the reality is that all have potential to replace standard biopsy in various patients, as follows:

1. Biopsy of any cancer is a surgical procedure and inherently more risky to the patient than taking a blood sample.
2. It is difficult and often impossible to take further biopsies during treatment due to risk to the patient.
3. Tumours show intra-tumoural heterogeneity and clones within the tumour may arise showing different behavior.

We would add a fourth issue: that of affordability to hard-pressed healthcare systems. A transthoracic needle biopsy under CT control may cost around £400, which is more than enough to pay for several of the assays considered above.

It is therefore no surprise that very few blood biomarkers have entered practice over the last 25 years, with the possible exceptions of serum HER2^{neu} and S100, which are not widely used. Most clinical laboratories offer a handful of tumour markers, used mainly during treatment to monitor response. Yet, over that period a recent search returned over 200,000 references in the scientific literature [13]. The majority of these are small case control studies, and provide basic analytic validation rather than the clinical validation and demonstration of utility required for implementation by bodies such as the UK National Institute for Health and Care Excellence (NICE), which has pioneered the use of health economics to justify the introduction of tests to clinical practice within its Diagnostics Assessment Programme.

NICE have been disappointed to find that many tests on the market lack robust analytical and clinical validation data, let alone evidence of cost effectiveness and clinical utility. This has made it impossible to recommend tests for uptake, and NICE have not yet looked at any new tumour biomarkers in blood. Other countries have similar concerns and the forthcoming IVD directive is widely expected to restrict the use of non-CE marked 'in house' or 'homebrew' tests to those laboratories accredited to ISO15189.

Automation provides affordability for pathology laboratories and is key to the uptake of these methods in blood sciences. In addition to large analysers, lab-on-chip technologies are coming of age and are particularly promising in CTC analysis. Several EU programmes have addressed the development of microfluidic devices for CTCs.

The question is whether any of the blood-based biomarkers have sufficient evidence to enter clinical practice in anything other than a research setting. Samples from the patient pathway, from diagnosis to recurrence allow a large number of different diagnostic assays. It is clear that liquid biopsy has a place, but it must be embedded within patient pathways and cross-validation with existing tests is a prerequisite for implementation.

3. Current technologies available

3.1. DNA

The presence of cfDNA in blood from cancer patients has been known since the 1970s [14], but methods for its detection and analysis have now advanced to the point where clinical trials are showing its utility for the detection of actionable mutations as companion diagnostics, and as a means of monitoring response to treatment. Extraction of DNA from plasma is preferred over serum, and can be accomplished using manual or automated methods (e.g. Qiagen, Siemens, Promega). For routine laboratory use, automated methods have much to recommend them in terms of reproducibility and cost effectiveness.

There are a number of PCR-based kits on the market, which can detect cfDNA generically, using LINE1 or ALU repeats to determine the fragment size [5,15]. A commercial version of this is under development. To determine that the cfDNA is actually tumour-derived (ctDNA) requires the demonstration of mutations present in the tumour, or methylation of key tumour genes (e.g. RASSF1A) [16]. These are feasible using PCR methods, and commercially available PCR-based mutation detection systems will detect a proportion of patients known to have mutations in their tumours. Improved methods under development by a number of laboratories have taken the sensitivity to 0.01% mutant in wild-type DNA and are being tested for use in clinical practice, with early clinical validation results showing excellent tumour-plasma concordance [17]. Intriguingly, it is also possible to use ELISA methods to detect methylated DNA (Volition SA, Belgium).

3.2. RNA

PCR-based methods are used to look for miRNA in plasma and several reports suggest that this is robust method for the detection of relapse, even early cancer detection [7,11,12]. A recent study demonstrated that Droplet Digital PCR technology improved day-to-day reproducibility of quantifying miRNA in serum from patients seven-fold relative to qPCR [18]. However, it is worth noting that several groups have had less success, and there may be a need for non-standard handling of blood samples for miRNA, which may make routine clinical use of this promising technology difficult.

3.3. Proteins

The measurement of protein tumour markers in serum is standard in most hospitals to monitor patient progress, and represents the gold standard for this against which other methods must be compared [19]. Most clinical centres will have the following tests available: AFP, CA125, CEA, CA15-3, CA19-9 and PSA. Some are likely to have CA72-4, HE-4, CYFRA21-1, S100, NSE, SCCA, sHER2, and ProGRP, all of which are available as ELISAs. There is evidence that thymidine kinase, circulating nucleosomes and the immunogenic cell death markers, high-mobility group box 1 (HMGB1), soluble receptors of advanced glycation end products (sRAGE) and DNase activity are also useful [20]. The advantage of such methods is that they are simple and relatively inexpensive. However, to be truly useful, there is a need for multiplex measurement and analysis, which can be difficult.

3.4. Cells

A simple PubMed search for ‘circulating tumor cells blood’ shows 9179 references. Several recent reviews have been published, including a few systematic reviews, and many methods are available [21–23]. These are based on the physical properties (e.g. size or shape) of the cells, or their biology (e.g. adhesion molecule expression). Methods for isolation include CTC microchips, filtrations systems, and bead-based capture, while a variety of PCR and cytological methods are used for analysis. Veridex (now part of Janssen) is the leader in the field and has a well established technology, CellSearch™, which is widely used in research studies (245 references) and some clinical trials, and a recent meta-analysis in metastatic breast cancer [24,25]. It is FDA approved for use in breast cancer to monitor treatment (www.cellsearchctc.com). It has limitations since living cells cannot be obtained and it is difficult to undertake molecular studies. Numbers of CTC isolated by CellSearch tend to be fewer than those found with other technologies, but there is concern that some of these confuse neoplastic cells with other cells, such as macrophages, which may carry similar markers. Recent studies suggest that CTCs show considerable molecular heterogeneity, and capture of cells that have an altered phenotype is challenging, as is knowing which are clinically relevant. Attempts to increase sensitivity and therefore utility in low volume disease run the risk of decreasing specificity for CTCs. A wide range of other methods are available or under development, and many are being commercialised, though only for research use [26]. The paper by Hong & Zu [26] considers a wide range of methods (15 are listed) and shows a lack of clinical validation data for seven of these. Different consortia are developing automated microfluidic cell isolation and biomarker (mostly molecular, multiplexed) analysis in one system, but are hampered by the fact that there is little clinical consensus on the biomarker set to be automated. There are few comparative studies and systematic reviews show that most studies are too small to be considered to provide sufficient validation to permit clinical implementation [23].

4. Conclusion

Liquid biopsy is a rapidly advancing field and the subject of intense interest from both academia and industry. Implementation within pathology is likely and the ability to improve diagnosis from less invasive blood tests has the potential to improve the management of many cancer patients.

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Conflict of interest statement

The author has no other relevant disclosures.

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