Point-of-care routine rapid screening: the future of cancer diagnosis?

Expert Rev. Mol. Diagn. 13(2), 107–109 (2013)

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"We will experience a shift from curative medicine to predictive and highly personalized medicine."

The hallmark of point-of-care testing is that the results can be obtained in a timely manner. This will enable clinicians to make decisions that are based on solid data and to perform course corrections during the treatment of the disease [1]. There are multiple point-of-care testing methods that are currently under development. Whereas virtually any molecule (e.g., lipid spectrum) or macromolecule (e.g., proteins, DNA and RNA) of biological origin could be used as a biomarker, proteins are, to date, the most extensively studied candidates.

During the last decade, diagnostic laboratories in academia and industry have been working on the development of a multitude of different approaches, among them electrochemical analysis methods [2], immunohistochemical staining methods [3], FISH [4], ELISA [5] and the many variants of PCR [6], such as reverse transcription PCR (RT-PCR) [7] and quantitative or real-time PCR (qPCR) [8], as well as quantitative fluorescence detection/image analysis [9].

Virtually all of these methods have the potential to be miniaturized so that they can be used routinely in the physician's office, an ambulance or in the field. PCR methods for the detection of leukemia and lymphomas are already in use [10]. Electrochemical and fluorescence detection methods are especially suited to miniaturization. We expect that they will be available as implants within one or two decades. This technology will help to address the apparent disparity in the quality of healthcare that exists in the USA (Great Plains areas vs the coastal areas) and even more so when comparing the developing and the developed world. However, some of these technologies (e.g., FISH, ELISA, genomics and proteomics) will remain in high-throughput analytical laboratories for the foreseeable future because they require considerable investments and highly trained personnel.

All of these approaches combined will lead to significant changes in the way that healthcare is provided: we will experience a shift from curative medicine to predictive and highly personalized medicine. Physicians will be able to rely on frequent testing to provide the best care possible. At the same time, advances in computer networking will allow cancer patients to pursue their lives with much more personal freedom than what is possible today.

What advantages would point-of-care routine rapid screening provide in cancer diagnostics & treatment?

According to the National Cancer Institute, "Cancer is not just one disease but many diseases. There are more than 100 different types of cancer" [101]. We use statistical methods to describe the response of cancers to treatment and classify them according to the cell type of origin. The required course of action is usually determined by the stage of the disease. Whereas there are acceptable treatment options available for numerous cancers if detected in early stages, survival statistics are far less favorable for cancers

Keywords: cancer diagnosis • circulating tumor cells • electrochemical analysis methods • ELISA • FISH • immunohistochemical staining methods • PCR • point-of-care testing • protease measurements • quantitative fluorescence detection/image analysis

  ISSN 1473-7159

in late stages. One of the major obstacles in treating cancers is detecting the disease in the localized stage, where surgical excision is possible. However, since cancer usually does not show discernible symptoms in early stages, blood tests for routine screening, for example during annual check-ups, would reveal cancer significantly earlier than it is discovered today. We anticipate that this screening option will become available sometime during the next 5 years. We further anticipate that after the re-election of President Obama, the insurance industry will become interested in tests for early cancer diagnostics, because pre-existing conditions will not be an impediment to obtaining or retaining health insurance after 2014, thus creating an economic incentive for early cancer detection.

> "One of the main problems in the surgical excision of operable tumors is the definition of the tumor boundary."

Point-of-care routine rapid screening will also be most useful for cancer patients during surgery and during the course of chemotherapy. One of the main problems in the surgical excision of operable tumors is the definition of the tumor boundary. A non-negligible fraction of the mortality of breast cancer patients arises from metastases that remain in the body, because the boundary between tumor tissue and presumably healthy tissue cannot be clearly identified by means of state-of-the-art histology [11]. Rapid screening methods that could be performed during surgery would significantly improve the standard of care in cancer surgery.

Similarly, there is a need for rapid screening methods during chemotherapy. Whereas immunoassays (e.g., HER2) [12] and, increasingly, testing for genetic subtypes will be very useful in selecting the most promising course of action in chemotherapy, these methods are not capable of detecting whether the selected drugs are actually working. The evaluation of a chemotherapy's success or failure is traditionally performed based on the comparison of tumor sizes before and after therapy. However, it is usually the metastases that kill the patient, not the primary tumor, and the former are not easily quantifiable using state-of-the art imaging procedures. Most importantly, this evaluation is performed much too late in the game, due to the severe collateral damages inflicted by virtually all chemotherapeutic drugs. Point-of-care routine rapid screening methods will have to be developed that are capable of measuring the 'rate of killing' tumor cells 24 h after chemotherapy has begun. Assessing the rate of cell damage of noncancerous cells at the same time is of equal importance. This would permit the adjustment of the doses of chemotherapeutic drugs and/or to change the cocktail that is administered if necessary.

The fourth group of cancer patients who could benefit from routine rapid screening is patients in remission and patients who have entered a chronic phase of the disease due to chemotherapy blocking the progress of metastases [13]. It is of vital importance to recognize a recurring cancer as early as possible. Otherwise, there are practically no viable treatment options available, because recurrent cancer is often highly invasive and drug resistant.

How do we envision point-of-care routine rapid screening methods in 2025?

Cancer medicine will be highly personalized: testing of genetic subtypes will be routinely employed to predict whether chemotherapy and/or signaling pathway blockade should be used and what kind of therapy is most promising in treating the particular cancer. Epigenetics (DNA methylation and expression of miRNA) is one promising approach to determining whether chemotherapy actually works. Another approach would be monitoring the protease signature of the patient. There is a wealth of literature available that serine proteases (urokinase plasminogen activator), numerous matrix metalloproteinases and many cathepsins are overexpressed by solid tumors [14]. These enzymes can be measured in tissue and blood by using immunoassays [15] or fluorescence detection methods relying on protease-selective consensus sequences [16]. The latter have the significant advantage of being up to four orders of magnitude more sensitive (~10^{-16} vs 10^{-12} M). Furthermore, many proteases are secreted as inactive zymogens, which are detected by immunoassays, but not by fluorescence detection methods, which only quantify protease activities. Epigenetics and protease monitoring are also most promising in the early detection of cancer and monitoring chronic cancer patients and patients in remission.

> "...we are less enthusiastic with regard to the potential of circulating tumor cells as prognostic markers."

During the last decade, there has been considerable discussion about monitoring circulating tumor cells (CTCs) for the purpose of early cancer detection and prediction of treatment outcome [15]. Several methods for capturing CTCs from peripheral blood have been developed. Although we regard the discovery of CTCs as a milestone in cancer research that is shedding light on the mechanisms of cancer progression, we are less enthusiastic with regard to the potential of CTCs as prognostic markers. First, CTCs represent only a very small fraction of normal cells in peripheral blood to be isolated and counted. Second, there is a paucity of tumor markers applicable for CTC screening, and third, CTCs exhibit considerable heterogeneity and plasticity with subpopulations that have lost epithelial features [17]. Metabolic markers, such as the levels of matrix metalloproteinase expression, are mandatory to avoid mischaracterizations. Third, we may look at a minor fraction of the CTCs due to their favorable size and relative ease of identification via surface markers. What alternatives do we have to CTCs? Instead of counting and interrogating a very minor fraction of cells in peripheral blood, neutrophils and monocytes may provide a much better insight into the characterization of the tumor-bearing host cells. Some early tumors, for example gliomas, are capable of changing the type of neutrophils from N1 (active) to N2 (inactive) [18]. These two types can be distinguished by means of their morphology and by measuring their protease expression levels of MMP8 and MMP9 (N1) and arginase (N2). This method would have the advantage of counting

and characterizing a major fraction of blood cells, leading to much smaller detection errors.

In conclusion, developing point-of-care routine rapid screening methods for cancer diagnostics and therapy holds great promise for individual patients and for the healthcare system as a whole. Advances in this field will be truly transformative, but they require synergy between public resources and private investments.

Financial & competing interests disclosure

SH Bossmann and DL Troyer are coauthors of two patents (Bossmann SH, Troyer DL, Basel MT: WO2009111470A2 [2009]; and Bossmann SH, Troyer D, Basel MT et al.*: WO2011028698A2 [2011]). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.*

No writing assistance was utilized in the production of this manuscript.

References

1 D'Orazio P. Biosensors in clinical chemistry – 2011 update. *Clin. Chim. Acta* 412(19–20), 1749–1761 (2011).

2 Chikkaveeraiah BV, Bhirde AA, Morgan NY, Eden HS, Chen X. Electrochemical immunosensors for detection of cancer protein biomarkers. *ACS Nano* 6(8), 6546–6561 (2012).

3 Idikio HA. Immunohistochemistry in diagnostic surgical pathology: contributions of protein life-cycle, use of evidence-based methods and data normalization on interpretation of immunohistochemical stains. *Int. J. Clin. Exp. Pathol.* 3(2), 169–176 (2009).

4 Bishop R. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. *Biosci. Horiz.* 3(1), 85–95 (2010).

5 Tjalsma H, Schaeps RM, Swinkels DW. Immunoproteomics: from biomarker discovery to diagnostic applications. *Proteomics. Clin. Appl.* 2(2), 167–180 (2008).

6 Baehner FL, Lee M, Demeure MJ, Bussey KJ, Kiefer JA, Barrett MT. Genomic signatures of cancer: basis for individualized risk assessment, selective staging and therapy. *J. Surg. Oncol.* 103(6), 563–573 (2011).

7 Sánchez-Navarro I, Gámez-Pozo A, González-Barón M *et al.* Comparison of gene expression profiling by reverse transcription quantitative PCR between fresh frozen and formalin-fixed, paraffin-embedded breast cancer tissues. *BioTechniques* 48(5), 389–397 (2010).

8 Ståhlberg A, Zoric N, Aman P, Kubista M. Quantitative real-time PCR for cancer detection: the lymphoma case. *Expert Rev. Mol. Diagn.* 5(2), 221–230 (2005).

9 Wagner MK, Li F, Li J, Li XF, Le XC. Use of quantum dots in the development of assays for cancer biomarkers. *Anal. Bioanal. Chem.* 397(8), 3213–3224 (2010).

10 Kroenlein H, Schwartz S, Reinhardt R *et al.* Molecular analysis of the t(2;8)/MYC-IGK translocation in high-grade lymphoma/leukemia by long-distance inverse PCR. *Genes. Chromosomes Cancer* 51(3), 290–299 (2012).

11 Udukala DN, Wang H, Bossmann LK *et al.* A nanoplatform-based approach for detecting the tumor boundary. Presented at: *47th Midwest Regional Meeting of the American Chemical Society.* Omaha, NE, USA, 24–27 October 2012.

12 Wu Y, Shang X, Sarkissyan M *et al.* FOXO1 is a target for HER2 overexpressing breast tumors. *Cancer Res.* 70(13), 327–358 (2010).

13 Stafford LJ, Vaidya KS, Welch DR. Metastasis suppressors genes in cancer. *Int. J. Biochem. Cell Biol.* 40(5), 874–891 (2008).

14 Bogenrieder T, Herlyn M. Axis of evil: molecular mechanisms of cancer metastasis. *Oncogene* 22(42), 6524–6536 (2003).

15 Raimondi C, Naso G, Gradilone A, Gianni W, Cortesi E, Gazzaniga P. Circulating tumor cells in cancer therapy: are we off target? *Curr. Cancer Drug Targets* 10(5), 509–518 (2010).

16 Funovics M, Weissleder R, Tung CH. Protease sensors for bioimaging. *Anal. Bioanal. Chem.* 377(6), 956–963 (2003).

17 Riethdorf S, Pantel K. Advancing personalized cancer therapy by detection and characterization of circulating carcinoma cells. *Ann. NY Acad. Sci.* 1210, 66–77 (2010).

18 Fridlender ZG, Sun J, Kim S *et al.* Polarization of tumor-associated neutrophil phenotype by TGF-β: 'N1' versus 'N2' TAN. *Cancer Cell* 16(3), 183–194 (2009).

Website

101 National Cancer Institute. What is cancer? www.cancer.gov/cancertopics/cancerlibrary/what-is-cancer