



Towards the Dreamed Biomarkers?

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Editorial

Detection and determination of tumor biomarkers are made from tissue and blood collection. During the last decade new biological sources are feasible: circulating tumor cells and circulating nucleic acids. They are composing a new field named as liquid biopsy. The availability of next-generation sequencing and Digital-PCR among other techniques, led to the possibility of getting the most out of this "circulating material" that would mirror the genetic and epigenetic features of the tumor.

It would allow the genetic profiling of a patient tumor, contributing to the personalized medicine, with a very little invasive method. Furthermore, it would provide the possibility of getting multiple serial samples. A liquid biopsy could represent a diagnostic tool as well as it would be suitable to provide prognosis and predictive information. It could also be an interesting tool to screen individuals at risk to develop certain disease.

Circulating nucleic acids and tumor cells is a very old discovery and represents a late development. The first reference to circulating tumor cells (CTCs) is from 1869. An Australian resident physician reported a case of cancer in which cells, similar to those in the tumor, were seen in the blood following the patient death [1]. It was not until 1948 when Mendel and Métais found circulating nucleic acids in blood, that light was shed again on this topic [2]. The years between 1955 and 1965 showed increased interest in this field with many reports being published [3,4]. During the past ten years, CTCs have received enormous attention as new biomarkers and the subject of basic research.

Among the immunomagnetic assays, based upon positive CTCs selection, the Cell Search method developed by Janssen Diagnostics, is already approved for clinical use in metastatic breast cancer patients [5,6]. A cut off point of 5 CTCs detected in this group of patients showed prognosis value in terms of Disease Free Survival and Overall Survival. Patients with less than 5 CTCs per 7.5 ml/blood, determined by applying the CTC-Chip, has a better prognosis [5,6]. Also among the immunomagnetic assays, but based on negative selection and close to be approved for clinical use, are the microfluidic chips named as CTC-Chip and CTC-iChip [5,6].

Circulating cell free DNA (ccfDNA) is not derived from CTCs. There is a discrepancy between the number of CTCs and the peripheral blood ccfDNA quantity. A single human cell contains 6 pg of DNA and there is a median of 17 ng of DNA per ml of plasma in advanced-stage cancers; therefore, if CTCs were the primary source of ccfDNA it would require over 2,000 cells per ml of plasma. In reality, there are on average, less than 10 CTCs per 7.5 ml of blood [1]. Everybody reading the current text has ccfDNA, it is just a matter of quantity. In average, healthy individuals could have 2-5 ng of ccfDNA increasing the concentration up to 10-1000 ng in metastatic cancer patients, having individuals that bear a chronic inflammatory disease, an intermediate concentration. As an example, Dr. Thierry's group reported a median of 24.37 ng of ccfDNA per ml of plasma in a group of 229 colorectal carcinoma patients versus a median concentration of 4.76 ng/ml in a

group of 109 healthy individuals analyzed [7]. The majority of studies that evaluate total plasma ccfDNA have been performed in solid malignant tumor patients [7-10]. Only during the last few years, studies in this regard, have been reported in onco-hematology patients with similar findings as in solid tumors [11-18]. More recently, exosomes and microvesicles have been reported as the most successful way of isolating DNA, RNA and protein simultaneously [19].

In normal physiological conditions, sources of ccfDNA are mainly the epithelial tissues with a high turnover rate such as the gastrointestinal tract, the bone marrow and the fetus in a pregnant woman. In a pathological scenario, a solid tumor, is a complex tissue comprising both malignant and non-malignant cells (the last including endothelial, stroma cells and the immune system cells) that contribute to the so called tumor derived ccfDNA. Thus, there are three types of circulating DNA origin to be considered in a cancer patient: malignant tumor cells, non-malignant tumor cells, as well as, non tumor cells (germline circulating DNA) [5-21].

Not just DNA and not only from blood. Cell free DNA can be isolated both from circulating fluids (whole blood, serum, plasma, lymph) as well as from non-circulating fluids (feces, urine, milk) and from pathological accumulated fluids such as ascites [22]. Extracellular DNA can also be isolated from cell culture supernatant.

Different types of circulating nucleic acids can be isolated: DNA, RNA, small non-coding RNA (being the microRNAs the most deeply analyzed), long non-coding RNAs and telomerase activity can be determined. Circulating nucleic acids can be found in different forms such as apoptotic bodies, nucleosomes, exosomes and protein-lipid-DNA complexes. This partially depends upon the mechanism of release that could be apoptosis, necrosis, or other cell death mechanisms [23-26]. Also a considerable DNA amount can be found in circulation attached to platelets and bound to cell plasma membranes.

In the field of oncology, circulating DNA is bearing the genetic and the epigenetic alterations of the cells they come from. Mutations, microsatellite alterations, methylation patterns and copy number could be determined. Circulating DNA is found as small fragments (146 bp or close to a nucleosome) and it is most fragmented in cancer patients than the healthy population. This has now been confirmed by atomic microscopy.

Up to day, CTCs counting has prognosis value in metastatic breast

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cancer (evidence level I) and prostate cancer (evidence level II). The isolation of ccfDNA from exosomes and microvesicles has prognosis value in cancer patients (evidence level III). Regarding circulating cell free nucleic acids, ccfDNA has also prognosis value (II-2), circulating cell free RNA (ccfRNA) has shown to be useful in monitoring response to therapy (III) and enhanced circulating microRNA levels have also shown prognostic value in preclinical studies (See Note).

CTCs and ccfDNA isolation and characterization would certainly improve the diagnosis, treatment decisions and therapy response monitoring in oncology patients. The minimal invasive procedure necessary to perform a liquid biopsy makes it an excellent source to investigate a panel of multiple prognosis and predictive biomarkers at the patient diagnosis time and along the entire follow-up period (during therapy and post-treatment). As mentioned before, the CTCs number represents a prognosis factor in metastatic breast cancer. It remains a field of intense research the cancer stem cells phenotypic heterogeneity and plasticity between a MET-Like (Mesenchymal-Epithelial transition) and a EMT-Like (Epithelial-Mesenchymal transition) phenotypes, information of different cancer stem cell subsets and their low number in circulation

Current and future research in ccfDNA should concentrate efforts on tumor genotyping. Particularly considering that there is often no enough tissue after the pathology analysis to perform molecular studies, following a relapse when a biopsy is not feasible nor routine, keeping in mind that the primary tumor could have evolved during therapy acquiring new genetics and epigenetic alterations, differences between primary cells and metastasis mutations and drug resistance development. All these tumor biology aspects could be investigated more easily through a liquid biopsy. Other areas to concentrate on are therapy response evaluation, minimal residual disease monitoring and early tumor detection.

Although CTCs are already being used in numerous clinical trials, it is still a challenging dynamic field of translational research. Even though many issues regarding the detection and characterization of CTCs remain unknown, it continues to be a highly promising area.

The evaluation of epigenetic alterations, circulating cell free RNA and microRNA as potential biomarkers are still in their early steps. There are promising results in breast cancer and rectal carcinoma. Exosomes and microvesicles, as cell free nucleic acid source, are also at an early stage but there will be a pharmacological exploitation.

The analysis of tumor genetic alterations has traditionally been performed on tissue biopsy material. However, many tumors are difficult to biopsy, are accessible only using fine needle aspirates (obtaining a small sample of usable material), or are located either in unknown sites (We have to consider oncology patients with multiple metastasis bearing an unknown primary tumor), or in sites that are challenging and risky to access. The traditional methods for tumor assessment fail to capture the heterogeneity of the disease, especially during progression. The Liquid Biopsy can profoundly improve the way clinicians face these issues. The Liquid biopsy, with a simple blood drawn, can sample different parts of the disease, will provide a "fluid" picture of the tumor cells alterations, can be repeated as needed for effective monitoring, and samples the part of the tumor that is associated with metastatic events. As a more detailed picture of the mutations that drive oncogenesis emerges, new avenues for researchers, clinicians, and drug developers have opened up.

Note: Clinical evidence level: I: well designed, randomized and controlled. II-1: well designed, controlled and non-randomized studies.

II-2: analytical control case study, more than a single enter or more than a single research group. III: Authorities' opinion in the field based on clinical experience, reports from experts committees.

References

- Ashworth TR (1869) A case of cancer in which cells similar to those in the tumours were seen in the blood after death. *The Medical Journal of Australia*, 14: 46-147.
- Crowley E, Nicolantonio Di, Loupakis F, Bardelli A (2013) Liquid biopsy: monitoring cancer-genetics in the blood. *Nature Reviews Clinical Oncology* 10: 472-484.
- Engell HC (1955) Cancer cells in the circulating blood; a clinical study on the occurrence of cancer cells in the peripheral blood an in venous blood draining the tumor area at operation. *Acta Chir Scand Suppl* 201: 1-70.
- Christopherson W (1965) Cancer cells in the peripheral blood: a second look. *Acta Cytol* 9: 169-174.
- Karaback NM, Spuhler PS, Fachin F, Lim EJ, Pai V (2014) Microfluidic, marker-free isolation of circulating tumor cells from blood samples *Nat Protoc* 9: 649-710.
- Yap TA, Lorente D, Omlin A, Olmos D, de Bono JS (2014) Circulating tumor cells: a multifunctional biomarker. *Clin Cancer Res* 20: 2553-2567.
- Mouliere F, El Messaoudi S, Pang D, Dritschilo A, Thierry AR (2014) Multi-marker analysis of circulating cell-free DNA toward personalized medicine for colorectal cancer. *Mol Oncol* 8: 927-941.
- Gorges TM, Pantel K (2013) Circulating tumor cells as therapy-related biomarkers in cancer patients. *Cancer Immunol Immunother* 62: 931-939.
- Lianidou ES, Strati A, Markou A (2014) Circulating tumor cells as promising novel biomarkers in solid cancers. *Critical reviews in clinical laboratory sciences* 51: 160-171.
- Bettegowda Ch, Sausen M, Leary RJ, Kinde I, Wang Y, et al. (2014) Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. *Sci Transl Med* 6
- Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, et al. (2009) Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. *Ann Oncol* 20: 1408-1413.
- García V, García JM, Silva J, Martín P, Peña C (2009) Extracellular tumor-related mRNA in plasma of lymphoma patients and survival implications. *Plos One* 4: 1-7.
- Armand P, Oki Y, Neuberger DS, Faham M, Cummings C, et al. (2013) Detection of circulating tumour DNA in patients with aggressive B-cell non-Hodgkin Lymphoma. *Br J Haematol*. 163: 123-144.
- Mussolin L, Burnelli R, Pillon M, Carraro E, Farruggia P, et al. (2013) Plasma cell-free DNA in paediatric lymphomas. *J Cancer* 4: 323-329.
- Jones K, Nourse JP, Keane C, Bhatnagar A, Gandhi MK (2013) Plasma MicroRNA are disease response biomarkers in classical Hodgkin Lymphoma. *Clin Cancer Res* 20: 253-264.
- Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, et al. (2015) Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *The Lancet Oncology* 16: 541-549.
- Bothers E, Viailly PJ, Dubois S, Bertrand P, Maingonnat C, et al. (2015) Somatic mutations of cell-free circulating DNA detected by next generation sequencing reflect the genetic changes in both germinal center B-cell-like and activated B-cell like diffuse large B-cell lymphoma at the time of diagnosis. *Haematologica*.
- Kurtz DM, Green MR, Bratman SV, Scherer F, Liu ChL, et al. (2015) Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood* 125: 3679-3687.
- Minciacchi VR, Freeman MR, Di Vizio D (2015) Extracellular vesicles in cancer: Exosomes, microvesicles and the emerging role of large oncosomes. *Semin Cell Dev Bio* 40: 41-51.
- Heitzer E, Auer M, Ulz P, Geigl JB, Speicher MR (2013) Circulating tumor cells and DNA as liquid biopsies. *BioMed Central* 5: 73.

21. Pantel K, Speicher MR (2016) The biology of circulating tumor cells. *Oncogene* 35: 1216-1224.
22. Chan AKC, Chiu RWK, Lo YMD (2003) Clinical Sciences Reviewers Committee of the Association of Clinical Biochemists, Cell-free nucleic acids in plasma, serum and urine: a new tool in molecular diagnosis. *Ann Clin Biochem* 40: 122-130.
23. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, et al. (2001) DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res* 61: 1659-1665.
24. Diehl F, Li M, Dressman D, He Y, Shen D, et al. (2005) Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc Natl Acad Sci USA* 102: 16368-16373.
25. Sikora K, Bedin C, Vicentini C, Malpeli G, D'Angelo E, et al. (2014) Evaluation of cell-free DNA as a biomarker for pancreatic malignancies. *Int J Biol Markers* 30: e136-141.
26. Heitzer E, Ulz P, Geigl JB (2015) Circulating tumor DNA as a liquid biopsy for cancer. *Clinical Chemistry* 61: 112-123.

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