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Editorial Open Access

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 cctDNA 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 immflamatory 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 coloretal 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 grastrointestinal 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, lympha) 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.

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