Liquid biopsy in clinical practice

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In current clinical practice in Slovakia, histological examination of tumor tissue is used for the analysis of cancer diseases. However, this approach is not a perfect solution because it requires a very invasive procedure, nevertheless the obtained information is not always sufficiently representative. Appropriate solution is a liquid biopsy, which appears to be a representative and less invasive method. Blood plasma analysis get attention, since free circulating nucleic acids, proteins, cells and extracellular vesicles from the whole body are delivered to the bloodstream. Previous results suggest that introducing this approach to routine clinical practice would be a tremendous contribution to improving and personalizing anticancer therapy.
Keywords: cancer, liquid biopsy, blood plasma, personalization, therapy

Tekutá biopsia v klinickej praxi
V súčasnej klinickej praxi sa pre analýzu nádorových ochorení na Slovensku využíva histologické vyšetrenie tumorového tkaniva. Tento prístup však nie je ideálnym riešením, pretože vyžaduje veľmi invazívny zákrok a aj napriek tomu nie je získaná informácia vždy dostatočne reprezentatívna. Vhodným riešením je tekutá biopsia, ktorá sa javí ako reprezentatívna a oveľa menej invazívna metóda. Veľkú pozornosť púta najmä analýza krvnej plazmy, pretože do krvného riečiska sa dostávajú voľné cirkulujúce nukleové kyseliny, proteíny, bunky a extracelulárne vezikuly, ktoré pochádzajú z celého organizmu. Doterajšie výsledky naznačujú, že zavedenie tohto prístupu do rutinnej klinickej praxe by bolo obrovským prínosom pre zlepšenie a perzonalizáciu protinádorovej terapie.
Kľúčové slová: nádorové ochorenie, tekutá biopsia, krvná plazma, personalizácia, terapia

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Introduction

Cancer is among the main causes of morbidity and mortality in the Slovak Republic. From all countries of the European Union, Slovakia is one of the most affected countries by this disease with a death rate 324.05 people per 100,000 residents[1] ***(Table 1)***. Cancer affects patients and their relatives as well, therefore it leads to serious health and social problems. In addition, it also has an impact on the financing of health care and the national economy of the country.

Due to the asymptomatic onset of cancer, most oncological patients are identified at advanced stage of disease and many times only by random diagnoses[2]. This significantly worse the outcome of antitumor therapy, which already varies between patients. In addition, during tumor therapy, tumor cells are under strong selection pressure, therefore their genome is dynamically evolving, and within one tumor, different cell populations can be formed that can be resistant to the selected therapy[3]. Due to the heterogeneity between various parts of the tumor, information obtained through tissue biopsy is often misrepresenting. Therefore, it would be necessary to obtain a tissue sample from multiple parts of the tumor and from all tumors that are present in the organism. This requires a very invasive procedure that leads to physical and mental burden on patients[4]. These findings suggest that key factor for successful cancer treatment is early diagnosis of patient before the onset of the aggressive phenotype of disease. Consequently, it is important to determine the appropriate treatment for each patient to minimize the side effects and it is also necessary to continuously and effectively monitor the progression of the disease in order to respond as quickly as possible to the developing cells.

These are reasons why there is a rising need for biomarkers to diagnose cancer, to monitor cancer progression and to predict patient response to therapy[5]. Appropriate solution are biomarkers usable for a liquid biopsy, which appears to be sufficiently representative, and much less invasive method compared to tissue biopsy. Liquid biopsy consists of the analysis of body fluids such as blood, urine, saliva, or cerebrospinal fluid that contain genetic information derived from tumor tissue. The most attention is currently attributed to the analysis of blood plasma because nucleic acids, proteins, cells and extracellular vesicles (EVs) gets to the bloodstream from all parts of the human body and in a case of oncological patients also from the tumor tissue[6] ***(Figure 1)***. In the future, this area of medicine could be called "Liquid Dynamic Medicine". This concept represents this methodological approach because it describes the dynamic changes in tumor development that we must take into account in tumor therapy, whereas information about these changes is obtained from the body fluids of patient[3].

Cell-free DNA

Cell free DNA (cfDNA) represent 150-180 bp long fragments which are released from cells into the bloodstream through apoptosis and necrosis. It has been shown that higher levels of cfDNA are found in the blood of oncological patients than in healthy subjects[7]. cfDNA that is released from tumor cells (ctDNA) contain the molecular characteristics of primary tumors and therefore their analysis could have a potential for the diagnosis and monitoring of cancer by liquid biopsy[8]. Experimental studies have confirmed that tumor-specific mutations can be detected with high sensitivity

Table 1. Standardized rate of mortality in the European Union countries in 2014. Table describe the total number of deaths; the number of deaths caused by cancer and the ratio of deaths due to cancer. (Data is standardized per 100,000 residents)

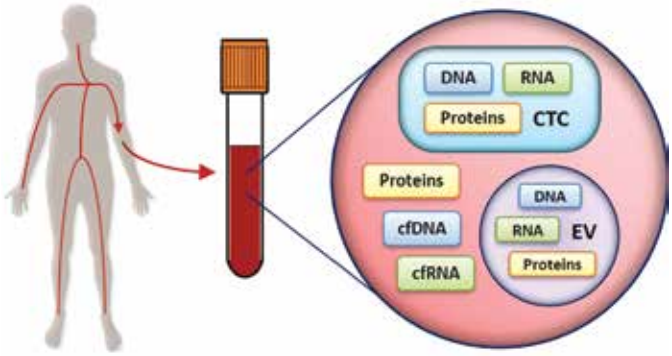
	Deaths per 100 000 residents		
	Total	Cancer	Ratio
European Union	1 003.06	261.5	26%
Belgium	970.63	252.61	26%
Bulgaria	1 646.45	242.41	15%
Czech Republic	1 237.32	284.55	23%
Denmark	1 028.33	300.61	29%
Germany	1 017.07	253.23	25%
Estonia	1 269.27	299.41	24%
Ireland	981.07	288.29	29%
Greece	966.64	249.32	26%
Spain	837.46	232.7	28%
France	829.86	245.41	30%
Croatia	1 355.92	336.39	25%
Italy	854.06	246.55	29%
Cyprus	980.1	200.39	20%
Latvia	1 502.96	299.33	20%
Lithuania	1 449.22	276.22	19%
Luxembourg	917.54	260.71	28%
Hungary	1 455.45	348.14	24%
Malta	939.38	233.53	25%
Netherlands	957.96	282.2	29%
Austria	957.15	249.28	26%
Poland	1 241.04	292.31	24%
Portugal	991.4	242.14	24%
Romania	1 531.08	273.21	18%
Slovenia	1 018.63	299.91	29%
Slovakia	1 353.43	324.05	24%
Finland	994.74	218.57	22%
Sweden	922.1	234.75	25%
United Kingdom	971.17	278.43	29%

by massive parallel sequencing (MPS) in ctDNA[7]. In a breast cancer patient, mutation profile of ctDNA, primary tumor and metastasis was compared by targeted MPS. In metastatic lesion, there were identified mutations that could not be detected in the primary tumor. However, sequencing of ctDNA could capture all mutations that were present in both the primary tumor and metastases. This study provided evidence that ctDNA contains representative tumor genetic material and can solve the problem of intra-tumor heterogeneity[9]. Whole genome sequencing approaches have also been published to identify tumor-specific chromosomal abnormalities in cfDNA of breast and colorectal cancer patients. It has been shown that using this approach, ctDNA with concentration of ≥ 0.75% can be detected in the blood of oncological patients with a 90% sensitivity and > 99% specificity[10]. Both the targeted and whole genome analysis of ctDNA has been proven, but because of the high cost of whole genome analysis, the research focuses on targeted MPS analyzes that are more cost-effective for routine diagnostic practice.

Cell-free RNA

Cell-free RNAs (cfRNAs) are instable molecules that are subjected to degradation, resulting in relatively low levels

Figure 1. Liquid biopsy scheme. Blood of oncological patients contains analytes such as circulating tumor cells (CTC); extracellular vesicles (EV); cell-free DNA (cfDNA); cell-free RNA (cfRNA) and proteins that can be used as biomarkers for cancer.



in the blood. Because of these limitations, cfRNA is poorly explored and do not receive much attention. However, a recent study has shown that circulating mRNA and microRNA can be used as effective diagnostic markers for prostate cancer. Four circulating candidate molecules (miR-200c, miR-200 b, OR51E2 and SIM2) were used to predict cancer risk, which were able to diagnose prostate cancer with 67% sensitivity and 75% specificity[11].

MicroRNAs (miRNAs) are small non-coding RNA molecules that play a significant role in regulating important physiological processes in the human body. Cell-free miRNA can be enclosed in EVs, especially exosomes, but also occurs in complexes with ribonucleoproteins Argonaute 2 or Nucleomorphin 1. These ribonucleoprotein complexes protect miRNAs from degradation so they represent stable circulating biomarkers[12,13,14]. Many studies have shown that impaired expression of some miRNAs is associated with the development of cancer, therefore quantification of these molecules could be an effective non-invasive tool for the detection and monitoring of tumor diseases[7,15,16]. In addition, experiments on animal models have shown that if the impaired levels of certain miRNA molecules recover to normal values, it is possible to alleviate the symptoms of the disease or even eliminate the disease. Thus, miRNA molecules have potential in cancer therapy[17].

Extracellular vesicles

EVs are membrane structures that mediate intercellular communication[18]. Based on their size and origin, they are divided into several categories including exosomes, microvesicles and apoptotic bodies[19]. These vesicles can be released from both healthy and tumor cells, but tumor cells have been shown to release more EVs compared to normal cells. EVs that are released from tumor cells are called oncosomes because they contain various oncogenic components in the form of oncoproteins and nucleic acids[20,21]. When oncosome is introduced into recipient cells, oncogenic factors trigger signaling pathways that can transfer the aggressive phenotype of tumor cells to idle cells[19]. Since EVs serve as carriers of macromolecules, they contain proteins, lipids and nucleic acids that are protected from degradation[22]. So, this is the reason why high-quality RNA molecules can be extracted

by exosomes. Another benefit of these vesicles is, that they originate from living cells, so they represent the active processes running in the cell[7].

Conclusion

Statistical data of mortality showed that the current situation in the field of cancer is very poor in Slovakia and also within the European Union. In 2014, up to 24% of all deaths were caused by cancer. Outdated procedures used in clinical practice are also responsible for the worse situation in Slovakia. Therefore, it is necessary to introduce methods for detection and monitoring of tumor diseases that would help to early identification of oncological patients and avoiding the side effects of therapy. The introduction of liquid biopsy method and next generation sequencing technology for routine clinical practice should contribute to the overall improvement and personalization of anticancer therapy.

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