Abstract:

Circulating cell-free DNA (cfDNA) and its tumour-derived circulating tumour DNA (ctDNA) fraction are considered an innovative prognostic and predictive biomarker in oncological diagnostics. Many studies have demonstrated higher levels of cfDNA concentration and integrity, as an indicator of the amount of ctDNA in cfDNA, in body fluids from patients with cancer diseases in comparison with healthy individuals, which suggest its potential as an effective biomarker for monitoring of the tumour dynamics. This study focused on optimisation and validation of measurement methods later used for analysis of cfDNA concentration and integrity in blood samples from patients with four different solid cancers. Two different commercial isolation kits have been tested in plasma and serum samples. Quantitative real-time polymerase reaction (qPCR) and PicoGreen dsDNA assay were optimized to effectively quantify low concentrations of cfDNA, subsequently compared to each other and to droplet digital PCR assay tested on selected samples. The concentration and integrity of cfDNA from plasma samples of breast, ovarian, colorectal and pancreatic cancer patients were evaluated. Higher amounts of cfDNA were obtained by the QIAamp Circulating Nucleic Acid isolation kit (Qiagen) in comparison to Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit (Norgen). The cfDNA levels in plasma samples from patients with mentioned diseases were higher compared to plasma samples obtained from healthy individuals. On the other hand, cfDNA integrity behaved differently. While cfDNA integrity in plasma samples of breast and pancreatic carcinoma patients did not differ from controls, the same parameter was lower in patients with colorectal and ovarian carcinoma in comparison to healthy individuals. These results support the hypothesis, that cfDNA which originates in tumour may be more fragmented compared to cfDNA from healthy cells and prove that cfDNA is a good candidate for detailed study of its dynamics in patients with solid tumours.

Key words: circulating biomarkers, cell-free DNA, DNA integrity, DNA quantification, optimisation