Methodology challenges in isolation of circulating cell-free DNA from liquid biopsy samples in patients with colorectal adenoma

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Background: Circulating cell-free DNA (ccfDNA) has an underexplored diagnostic potential in colorectal cancer (CRC) and its sufficient quantity and quality is of key importance in obtaining accurate results. The aim was to select the most suitable method for the isolation of ccfDNA from liquid biopsy samples in patients with colorectal adenoma (preCRC), which will be used in further assessment of CRC patient samples.

Methods: ccfDNA was isolated from liquid biopsy samples obtained by collecting peripheral blood from 11 preCRC patients in CellSave tubes (Menarini Silicon Biosystems) using two spin-column-based isolation kits (NucleoSpin cfDNA XS Kit, Machery Nagel and QIAamp ccfDNA/RNA Kit, Qiagen) and one vacuum-based (QIAamp Circulating Nucleic Acid Kit, Qiagen). To verify the presence of fragment corresponding to ccfDNA (160-170 bp) and the absence of genomic DNA (gDNA) contamination, all isolates were analysed using High Sensitivity DNA Kit on Bioanalyzer 2100 (Agilent Technologies). Concentrations of ccfDNA were determined using Qubit dsDNA HS Assay Kit (Invitrogen) on DS-11 FX (DeNovix), from which the quantities of isolated ccfDNA *per* mL of plasma were calculated and presented as median and range. Statistical analysis of paired amounts of ccfDNA isolates was performed using Friedman test (MedCalc software, v22.020).

Results: The fragment corresponding to ccfDNA was found in all eleven vacuum-based method isolates, nine QIAamp ccfDNA/RNA Kit isolates (gDNA contamination suspected in two isolates), and two NucleoSpin Kit isolates. The quantities of isolated ccfDNA per mL of plasma were significantly different ($P<0.001$) showing the highest amounts using vacuum-based method, than QIAamp ccfDNA/RNA Kit and the lowest quantities when using NucleoSpin Kit: 12.24 (7.47-78.70), 1.80 (0.49-15.56) and 0.6 (0.0-7.17) ng of ccfDNA *per* mL of plasma, respectively.

Conclusion: The vacuum-based method proved to be the most suitable for ccfDNA isolation and therefore was selected for further analysis of liquid biopsy samples from CRC patients.

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