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CELL-FREE DNA AND POLYPHOSPHATES AS ADDITIONAL MARKERS OF COAGULATION STATUS

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Aim. We aimed to develop a method for determining the cfDNA and PP in plasma and to conduct pilot studies assessing the level of these indicators in patients with cardiovascular disease.

Material and methods. The citrated plasma of healthy donors and patients with different forms of acute myocardial infarction were used.

A set of reagents with sorbent was used for DNA extraction.

To determine cfDNA and PP, the fluorescent dye 4,6-diamidino-2-phenylindole (DAPI) was used. 20 mM DAPI was diluted in a ratio of 1:500 to prepare the working solution. All manipulations were carried out in 96-well black glass-bottomed plates. Fluorescence was measured using a microplate fluorescence reader Bio-Tek FL600. Initially, the background level of the sample fluorescence was measured. Next, 5 μ l DAPI working solution was added to the samples and after 15 min of incubation in the dark, the fluorescence intensity was measured. DNA excitation and emission detection was performed at 360 nm and 460 nm. PP were detected at 400 nm/550 nm, respectively.

Results. PP with DAPI shifted the peak emission of DAPI to 525 nm when using excitation at 360 nm. However, in addition to PP-DAPI, complexes DNA-DAPI emit too. To prevent this and to increase the sensitivity of PP detection, excitation at 400 nm was used. In this case, the fluorescence of PP-DAPI is detected at 550 nm. The emission of cfDAPI and DNA-DAPI is minimal at this wavelength, which makes the PP-DAPI signal highly specific and does not essentially depend on the presence of DNA. According to our data the level of cfDNA in the blood plasma of patients with AMI is 93 ± 1 ng·ml⁻¹, which is increased twice comparatively to the control group. Significant changes of the PP level in these patients have not been identified, but there was a tendency of this index to increase in the coronary bloodstream.

Conclusion. The concentration of circulating DNA in the blood can be used as a measure to reflect the stage of the disease and to monitor the effectiveness of the therapy. Importantly, based on the fact that glycosaminoglycans (GAGs) also emit at 400 nm/550 nm and heparin is used as an anticoagulant in the treatment of patients with AMI, further study is needed to split GAGs and PP spectra.