Title of the project: Genetic plymorphisms, MicroRNAs and bioindicators of activity: interrelations in the diagnostics and therapy of severe familial hypercholesterolemia

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Summary of 2018 results

Title of the presentation: Amount of RNA contamination in rinsing solution from the apheresis column

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Background: LDL- apheresis therapy is a type of 'extracorporeal' procedure to remove low-density lipoprotein (LDL) cholesterol from the blood. In our research, we target molecular markers - small noncoding RNAs in patients under long-term apheresis treatment. The aim of the current experiment was to detected possible RNA contamination of apheresis column.

Materials and methods: We performed real-time PCR for measurement of 19 samples from the rinsing solution of apheresis column before procedures. As negative controls, we used 3 samples of PCR clean water included in three different RNA isolations. As positive controls, we used 3 plasma samples. We compared Ct (threshold cycle) values of three RNA Spike-ins used for control of RNA isolation (UniSp2, UniSp4, UniSp5) and 3 reference miRNAs stably expressed miRNAs in plasma (hsa-miR-191-5p; hsa-miR-103-3p; hsa-let7a). Ct value is inverse to the amount of template.

Results: RNA controls and reference miRNAs were in negative controls and in 4 rinsing solution samples undetectable. Ct values of RNA Spike-in controls in other 15 samples were significantly higher than in plasma samples (for UniSp2: 26.9 vs 19.5, for UniSp4: 33.0 vs 25.7, for UniSp5: 36.0 vs 32.1), which indicates very poor or any amount of RNA. Reference miRNAs were in all rinsing solution samples undetectable.

Conclusions: Samples from the washing solution of apheresis columns showed an only limited amount of templates for control of RNA isolation. We detected no RNA contamination of the apheresis column.

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