Some alterations in the RBCs induced by the D-gal model are also characteristic for natural aging. This model causes a significant decrease in RBC deformability, which affects their rheological properties and

their ability to squeeze through microvessels. When compared with the control, deterioration of RBC morphology in the D-gal model follows the trend observed in natural aging of C57BL/6J mice, namely, a decrease in MCV and an increase in MCHC. It is worth highlighting that comparison of the dry smears of

fresh RBCs isolated from the studied groups of animals did not provide any statistically significant changes in their RBC shape (majority with a bio-concave shape). This stays in agreement with our previous results, where we have evaluated the morphological alterations and ultrastructural features among the control and natural aging groups in C57BL/6J RBCs using not only the classical dry smear analysis, but also nano-scale AFM to finely analyze the RBC ultrastructural features [3]. Those results clearly proved that only detailed AFM analysis provided insight into an age-dependent decrease in RBC height and no signs of ultrastructural deteriorations in RBC morphology were found. The biochemical composition of RBC membranes observed in the D-gal model and natural aging includes a decrease in the phospholipid amount, lipid unsaturation level, and an increase in acyl chain shortening. Even though some alterations are not statistically significant in the D-gal model, their direction remains the same as in natural aging. Here we may list the changes to the secondary structure of the proteins of intact RBCs, revealed by a decrease in the ratio of turns to  $\alpha$ -helices, a decrease in MCH and LDL, and lack of changes in RDW and blood plasma parameters, such as HDL, cholesterol, triglycerides, and LDH. Altogether, these suggest that the D-gal model mimics both the mechanical and functional properties of the RBCs of naturally aging mice. It was previously reported that alterations in the secondary structures of proteins in intact RBCs may be regarded as biomarkers of aging or specific diseases. Progressive