Objectives: Peritoneal dialysis (PD) is a life-saving renal replacement therapy for patients suffering for kidney failure. In this form of treatment, peritoneum that lines the abdominal wall and protects viscera, acts as a dialyzing membrane. The method relies on introducing the peritoneal dialysis fluids into peritoneal cavity enabling elimination of uremic waste products and excess of water. Unfortunately, long-term exposure of the peritoneal membrane to peritoneal dialysis fluids may provoke structural alterations that eventually lead to technique failure. Peritoneal mesothelial cells (HPMC) consists the biggest fraction of peritoneal cells and play an important role in solute transport, inflammation, and wound healing. These functions of mesothelial cells are important also in the context of PD. Numerous studies have shown that both long-term exposure to PD fluids and PD-associated inflammation impact adversely on viability and function of peritoneal mesothelial cells. One of the well documented processes is epithelial-to-mesenchymal transition (EMT) of HPMC. It contributes to fibrotic thickening of the peritoneum, that develops in patients on peritoneal dialysis. The process is thought to be largely mediated by transforming growth factor-beta (TGF-β). Interestingly, TGF-β has also been implicated in senescence of HPMC. In this respect, TGF-β has been shown to mediate such aspects of senescence as growth arrest, cell hypertrophy and expression of senescence-associated β-galactosidase (SA-β-Gal).
Results of earlier experimental and clinical studies suggest that in response to PD solutions mesothelial cells may undergo either EMT or senescence and these phenotypes are associated with pro-angiogenic and pro-fibrotic activities that may impact on the peritoneal structure and function. Although transforming growth factor-β (TGF-β) has been identified as a key mediator in both EMT and senescence, it is not clear what determines the choice of cellular response to TGF-β in the milieu of PD. To gain a detailed insight into how mesothelial cells react to TGF-β in the context of PD-fluids, we propose to asses in detail the gene expression profile of ‘young’ and ‘senescent’ HPMC.

Methodology: HPMC will be isolated from the pieces of omentum from patients undergoing planned abdominal surgery. Freshly isolated cells will be exposured to TGF-β and appropriate samples for further analysis will be collected. Parallel the HPMC will be culture till senescence, as it will be asses by staining against SA-β-Gal. Senescent cells will be next exposed to TGF-β, in the same manner as young cells. At first, gene expression profile of studied cells will be obtained by applying microarray analysis. Identified in this assay differences in expression profile of genes will be verify by qPCR, whereas in protein production by immunobloting and immunofluorescence staining.

Significance: Although some kind of a relationship between cellular senescence and EMT seems to exist, its nature is not clear. Early data suggested that senescent cells promoted EMT and, thereby, cancer development These effects were partly attributed to the senescence-associated secretory phenotype (SASP), in particular to increased release of proinflammatory cytokines. While this concept may well explain the interaction between cellular senescence and EMT at the tissue level, the link between these processes at a single cell level is probably much more complex. Comparison of gene and protein expression by young and senescent cells in response to TGF-β and further identification of signalling pathways linking EMT and senescence may shed new light on peritoneal mesothelial cells biology and help to understand its changes in the course of clinical PD.