Hidden pathogens- identificantion of etiological factors of urianry tract infections in dialysis patients and renal transplant recipients using 16S rRNA sequencing

Hidden pathogens - detection of etiological factors of urinary tract infection in patients who are on dialysis and renal transplant recipients, using molecular biology techniques. Urinary tract infection (UTI) is the most common infectious disease. It occurs in the presence of microorganisms in the urinary tract Dialysis patients are a group at high risk of urinary tract infections. Infection in the urinary system are most often caused by Gram positive type of staphylococci (Staphylococcus aureus and Staphylococcus epidermidis) and Gram negative bacteria Pseudomonas aeruginosa. Fungal infections are much rarer observed as a complications factor of UTI. In renal transplant, urinary tract infections are the most common infectious complications, with an incidence in the first year after transplantation up to 60%. The most common etiologic agents of UTI in patients after renal transplantation are similar as in the case of complicated UTI in the general population. The most frequently identified pathogen in urine culture is Escherichia coli. It is also noted the presence of *Pseudomonas* spp. and *Klebsiella* spp. Infectious complications occurring in patients treated with dialysis methods are a significant problem in the process of renal replacement therapy. Dialysis infection of the exit of the catheter, and a tunnel catheter pose serious complications, which increase the rate of hospitalization for these patients and may even be the direct cause of threat their lives. Urinary tract infections are a major complication, not only because of the widespread occurrence, but potentially negative effects on the graft excretory function, survival of the patient and transplanted kidney. Infections, dominated by UTIs are the most common cause of acute injury of the transplanted kidney. Therefore, as a studied group for this project, dialysis patients and kidney transplant recipients were selected, in whom, in urine samples microbiological examination of uropathogens responsible for the occurrence of infections will be performed. Among postransplantation complications, bacteraemia is an independent risk factor for graft loss as a result of death or transplanted kidney failure, and total renal mortality. Therefore, the aim of this project is an early, full identification of uropathogens responsible for the occurrence of the infection, i.e. detection of both culturable and fastidious etiological factors of UTI. The basic diagnosic test of occuring urinary tract infection in these groups of patients is a microbiological examination of urine because the characteristic for these people, often oligosymptomatic clinical course, resulting in decreased inflammatory response, due to immunosuppression. In routine diagnostic laboratories, in testing of urine samples, only typhic pathogens were detected, however the isolation and identification of many fastidious microorganisms is often impossible. The limitation of their isolation is mainly due to their sensitivity to oxygen, which requires appropriate methods of collection, transportation and strictly anaerobic cultivation of specimens. Also, the slow growth of these organisms combined with time-consuming phenotypic identification methods have often resulted in inconclusive identification species. In addition, those microorganisms are often isolated from polymicrobial infections with known pathogens and therefore, their relevance has been largely overlooked. It is estimated that about 14% of the microorganisms is not detected using phenotypic assays, wherein taking into account the results of the latest research on human microbiome, this estimated value is probably an underestimate. Dialysis patients and kidney transplant recipients are predisposed to urinary tract infections caused by both opportunistic bacteria and uropathogens, which often causing mixed infections. In proposed project, we hypothesize that ”difficult to culture” microorganisms are common etiologic agents of UTI, but are currently not detectable by methods of classical microbiology. Project’ hypothesis will be verified using molecular biology methods, including sequencing of 16S rRNA (next-generation sequencing, NGS) using Illumina Miseq system. Selected NGS results will be verified using Sanger type sequencing. The study will provide the complete characteristics of urinary tract infection bacterial pathogens. 16S rRNA sequencing will allow to expand the knowledge of bladder bacteria and its diversity. 16S rRNA sequencing is a method able to detect bacteria that classical microbiology could not isolate. Differences in the composition of microbial communities in dialysis patients, renal transplant recipients and in individuals without renal failure will be characterized. Molecular identification of unidentifiable bacterial isolates will offer an opportunity for the description of novel or less known uropathogen species encountered in clinical microbiology laboratories. Inclusion of dialysis patients and renal transplant recipients as the studied groups makes this project the first such study in the world. Applied molecular biology methods will allow to assess the presence of fastidious and difficult-to-cultured uropathogens and will shorten the time of identification of pathogens through the isolation of the genetic bacterial material directly from a clinical sample of urine
Detailed understanding and knowledge of pathogens causing UTI in dialysis patients and kidney transplant recipients will also lead antibiotic therapy according to the rules and provide new prospects for reducing the risk of transplant rejection.