Introduction: Cyclosporine A (CsA), a calcineurin inhibitor, is widely used in renal transplantation. TDM of CsA in whole blood is recommended for dose adjustment. CsA dose and its blood level do not correlate well with the degree of immunosuppression generally. Monitoring of CsA level at the site of its action (lymphpocytes) should be more advantageous. Delayed graft function (DF) is a form of acute renal failure resulting in post-transplant oligouria.

Aim: Development and application of LC-MS/MS method for therapeutic drug monitoring of cyclosporine A and its first line metabolites in whole blood and isolated peripheral lymphocytes. TDM of CsA and metabolites in early and chronic phase after renal transplantation (Tx).

Methods: 14 adult renal transplant recipients (8 males) were prospectively included in the study and were followed during first three months after Tx. On the basis of clinical status confirmed by serum creatinine level and creatinine clearance all subjects were divided into 2 groups. IF group involved subjects with immediate graft function after transplantation, DF group involved patients with delayed graft function.

54 renal transplant patients were followed up for 4 years during chronic phase (>1year) after renal Tx. 31 of them had ambulatory blood pressure monitoring (ABPM) performed. Patients were divided into the group of "non-dipper" (N; nocturnal blood pressure decrease <10%; 25 measurements) and the group of "dipper" (D; nocturnal blood pressure decrease >10%; 26 measurements)

Concentration of CsA and its primary metabolites (AM1, AM9 and AM4N) before and 2h after administration was measured in whole blood and isolated lymphocytes using liquid chromatography-tandem mass spectrometry method (LC-MS/MS). Differences between both groups were compared by Mann-Whitney test. Correlations were investigated using Spearman's correlation coefficient.

Results: During the first three months after transplantation no acute rejection was observed. Creatinine levels were significantly lower while creatinine clearance was significantly higher in the IF group. C_0 of CsA and metabolites in blood was higher in the DF group. In the DF group C_2 of CsA was lower, C_2 of AM1 and AM9 were higher during first week, C_2 of AM4N was higher during follow up. The highest differences between groups were found in C_0 of AM4N metabolite. Similar results were found in lymphocytes. Patients from the DF group had higher C_0 of CsA and metabolites. The greatest differences were in AM4N in both sampling times.

Ratios of AM1/CsA, AM4N/CsA and AM9/CsA were calculated, but significant results were obtained only in AM4N/CsA ratio before administration. Higher AUC_{0-4} were in the DF group in metabolites AM1 and AM4N, AUC_{0-4} of CsA and AM9 was higher in the IF group. The greatest differences were AUC in lipophilic metabolite AM4N.

The concentration of CsA and its metabolites in both samples gradually decreased, as well as the total daily dose. Higher C_0 concentrations of AM1 and AM9 than CsA were found, AM4N levels were low. In contrast to the findings in the blood concentrations in lymphocytes remained stable. CsA concentrations in blood correlated with concentrations in lymphocytes significant, while metabolites results were not clear.

When comparing measured concentrations we found no statistically significant difference between groups "dipper" and "non-dipper" in the case of CsA or primary metabolites and AM9 AM1, the only statistically significant difference ($p = 0.0339$) was found in AM4N metabolite, whose concentration in the lymphocytes was in the group "non-dipper" 4-fold higher.

Conclusion: New LC-MS/MS method for determination of CsA and its first line metabolites not only in blood but also in lymphocytes was developed. Significantly higher concentrations of AM4N in blood and lymphocytes during early phase after renal transplantation may have relation to delayed onset of normal renal function after renal transplantation. During long-term CsA therapy 50% of patients switched to “non-dipper” status. Significantly higher concentration of metabolite AM4N in lymphocytes were observed in this group of patients.