Eva Vejražková: Human Cytomegalovirus Infection in Patients after Allogeneic Hematopoietic Stem Cell Transplantation - Summary

Introduction: Specific anti-cytomegalovirus (CMV) treatment reduces CMV-related morbidity and mortality in patients after allogeneic hematopoietic stem cell transplantation (HSCT) but carries a risk of viral resistance. The viral resistance after HSCT treatment is still a relatively unexplored phenomenon.

The aim of the study was to determine the incidence of CMV infection in patients within one year after HSCT in our centre. The goal was also to analyse CMV infection data, incidence of graft-versus-host disease and other clinical endpoints by comparing two patients' cohorts that were administered two different Thymoglobuline Genzyme (TG) doses as part of the HSCT conditioning regimen. Incidence of treatment failure cases and viral CMV drug resistance were also to be determined. Two methods of sequence analysis (Sanger and Next-Generation Sequencing, NGS) were compared in cases of proven viral resistance.

Patients cohort and methods: The study included 101 adult patients after allogeneic HSCT for haematological diseases treated between July 2012 and December 2014 at the Hradec Kralove University Hospital, Czech Republic. CMV DNAemia was determined by quantitative real-time PCR, the diagnosis of CMV disease was confirmed by immunohistochemistry. UL97 and UL54 gene analysis were performed retrospectively in cases of treatment failure diagnoses (defined as the viral load increase > 1 $\log_{10}$ after at least 2 weeks of antiviral therapy). The results were compared with reference CMV strain and compared to previously published polymorphism and mutation data. Both Sanger and NGS sequence analyses were performed in cases of proven viral resistance.

Results: Incidence of CMV primoinfection/reactivation was 69%. Mild Thymoglobuline dose reduction from 7,5 mg/kg to 6 mg/kg as a part of the fludarabine/busulfan/TG conditioning regimen did not significantly influence CMV infection data. No impact of TG dose reduction on incidence of graft-versus-host-disease, relapse of underlying disease or mortality within first year after transplantation were observed. Treatment failure was observed in 7% of patients (i.e. 12% of patients treated by antiviral therapy), viral resistance was proven in 3% (5% of patients with anti-CMV therapy). Three cases of treatment failure diagnoses were caused by CMV UL97 mutation coding resistance (L595F, M460I, A594V). For patients with proven resistance, the mutation was confirmed by NGS up to one week prior to the standard sequencing using the Sanger method.

Conclusions: The mild ATG dose reduction in the fludarabine/busulfan/TG conditioning regimen had no impact on incidence of graft-versus-host-disease, relapse of underlying disease, or mortality within first year after transplantation. Therefore, such reduction can allow for lower toxicity of conditioning regimen, while keeping the performance.

Patients with prolonged antiviral therapy and high viral load should be monitored closely, testing of viral susceptibility should be performed when the response to antiviral therapy is not satisfactory. A portion of such treatment failure cases can be linked to viral resistant mutations.

Thanks to increased sensitivity, the NGS analysis can prove mutated gene in smaller ratios than Sanger and can also provide quantitative measures. However, Sanger is more viable in clinical praxis due to higher workload requirements for NGS.