The influence of hepatitis B virus (HBV) polymorphism on the response to antiviral therapy in chronically infected patients

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Hepatitis B virus infection remains a serious health problem affecting about 2 billion people globally. There are estimated to be 350 million chronic carriers of hepatitis B infection worldwide. These people are at risk of liver cirrhosis with associated mortality because of hepatocellular carcinoma and other complications. Long-term therapy, that is required because of the existence of extremely stable cccDNA within the hepatocyte nuclei, can lead to the emergence and selection of drug resistance mutations and treatment failure. Moreover, it is possible to have drug resistance mutations present already in the HBV population of treatment naive patients. Currently available methods detecting drug resistance mutations are able to detect mutants that constitute 5% or more (≥20% in direct Sanger sequencing) of viral population. What is more, drug resistant mutants can be detected in patients with a viral load of more than 10^4 copies/mL.

The aim of this PhD thesis was to optimize the detection method of HBV genetic material in clinical samples with low viral load and to develop a sensitive assay for determination of HBV quasispecies. Subsequently, the use of proposed methodology to estimate how heterogeneity of HBV genetic variants influences on the course of antiviral treatment and to examine the association between antiviral therapy failure and the number as well as diversity of HBV variants before treatment.

This study enrolled chronically infected patients that were qualified and received analogues treatment. The detection method of HBV genetic material in clinical samples with low viral load was developed in this study. Moreover, two extremely sensitive methods (MSSCP and MALDI-TOF mass spectrometry) for identification of HBV quasispecies were proposed and compared. The use of both methods in this study allowed for a precise monitoring of changes occurring in viral quasispecies during therapy. As a result, it was demonstrated that the increase in the percentage of drug resistant variants during treatment is the first sign of emerging HBV resistance and can become a useful diagnostic tool. Additionally, it was demonstrated that the sensitivity of the conventionally used methods for determination of HBV drug resistance is not sufficient.