

Real-time PCR studies of genotypes, mutations and replication of hepatitis B virus

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Avhandlingen baseras på följande arbeten:

- I. **Malmström S, Hannoun C, Lindh M.**
Mutation analysis of lamivudine resistant hepatitis B virus strains by TaqMan PCR.
Journal of Virological Methods 2007; 143: 147-152.
- II. **Malmström S, Berglin-Enquist I, Lindh M.**
Novel method for genotyping hepatitis B virus on the basis of TaqMan real-time PCR.
Journal of Clinical Microbiology 2010; 48: 1105-1111.
- III. **Malmström S, Eilard A, Larsson SB, Hannoun C, Norkrans G, Lindh M.**
Genotype impact on long-term virological outcome of chronic hepatitis B. *Submitted*.
- IV. **Malmström S, Larsson SB, Hannoun C, Lindh M.**
Hepatitis B virus RNA levels in human liver biopsies and in transfected and non-transfected hepatoma cell lines. *Submitted*.

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Abstract:

Infection with hepatitis B virus (HBV) is an important cause of liver disease and affects 350 million people worldwide, causing 600,000 deaths/year. Treatment includes interferon and nucleoside analogues (NAs) such as lamivudine, entecavir, and tenofovir. During treatment with NAs, substitutions may arise in the viral genome that confer resistance to treatment, impairing or abolishing the effect. Clinical prognosis and outcome of treatment are affected by viral genotype, and to date there are eight established (A-H) and two putative (I-J) genotypes, as well as several subgenotype strains described.

Levels of viral DNA and surface antigen (HBsAg) in serum are used to monitor the course of infection and the response to treatment. It is however not clear to what extent mechanisms that inhibit transcription of the pregenomic RNA (pgRNA), contribute to suppression of viremia, which mainly occurs in parallel with loss of HBsAg from blood. Likewise, it is unclear how the excessive production of HBsAg is regulated.

The aims of this thesis were to develop methods for genotyping and resistance mutation analysis, to investigate the impact of genotypes on clinical outcome, and to investigate the role of the regulation of viral transcripts for replication and HBsAg production.

Two real-time PCR based assays were designed and evaluated. The first focused on amino acid positions 180 and 204 in the viral polymerase enzyme, which are important for resistance against treatment with the NA lamivudine. The second aimed to include all established genotypes in a multiplex genotyping assay for accurate and rapid analysis. It was not possible to find one single genomic segment that could be used for amplification and identification of all genotypes. Instead, we chose to target a number of segments in different parts of the genome, and for genotypes A-C two segments each were targeted, to obtain reliable accuracy. Both methods showed high accuracy and concordance with earlier methods, adding the possibility to identify mixed infections and assign relative proportions to the strains in the mixture.

Genotype impact on virological outcome was investigated after 9.2 years in 124 chronically infected adults. HBV DNA levels declined in patients carrying genotype A, B, and D, among whom HBsAg loss was observed in 92%. Genotype A and D showed 36% and 11% loss of HBsAg. In contrast, viral activity and aminotransferase elevation persisted in genotype C infections.

In the final study, real-time PCR was used to analyse the levels of cccDNA and viral RNA in biopsies and cell lines with focus on differences between HBsAg positive and negative stage. Patients negative for HBsAg had 2.15 log lower levels of cccDNA in liver tissue, 4.84 log lower serum levels of HBV DNA and 1.45 log lower serum levels of HBsAg, than HBsAg-positive patients. The pgRNA in liver tissue correlated strongly with cccDNA ($R^2=0.87$) and HBV DNA levels in serum ($R^2=0.81$). The S-RNA/pgRNA ratio was higher in HBsAg-negative patients, which may reflect specific down-regulation of pgRNA, or enhanced S-RNA production. Transcription efficiency was lower in vitro than in biopsies, and was not influenced by HBV core promoter mutations in transfected Huh7.5 cells.

Keywords: hepatitis B virus, real-time PCR, lamivudine resistance, genotypes, replication

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