

# Hepatitis B Virus RNA in serum and liver tissue – quantification using digital PCR

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## ABSTRACT

Hepatitis B virus (HBV) infection is a global health issue that is responsible for approximately 900,000 deaths each year, by inducing liver cirrhosis and hepatocellular carcinoma (HCC). A few markers are used to classify HBV infection and monitor treatment efficacy, including HBV DNA, surface antigen (HBsAg) and e antigen (HBeAg) in serum as well as HBV DNA and RNA in liver tissue. The recent discovery of the receptor NTCP facilitates *in vitro* studies of HBV.

The aims of this thesis were (I) to characterize a new marker of HBV infection, HBV RNA in serum (II) to investigate *in vitro* the neutralizing effect of HBV encoded subviral (HBsAg) particles (III) to develop and apply a new method to discriminate viral and integrated DNA in liver tissue (IV) to analyze focal differences within the liver of HBV and hepatitis D virus (HDV) and (V) to explore HBV RNA profile in liver biopsies by digital PCR.

High levels of serum HBV RNA was found in the majority of 95 patient samples utilized in this study. This RNA was of full genome length, appeared in fractionation together with HBV DNA. Sequencing data supported that HBV RNA in serum represents virus-like particles with failing reverse transcription of the pregenomic RNA (pgRNA).

The role of subviral particles (SVP) during HBV infection was explored in HepG2-NTCP cell line. The results support that SVP functions as a decoy to neutralize antibodies synthesized by the host.

A novel droplet digital PCR (ddPCR) method was developed and applied on 70 liver biopsies to quantify circular and linear HBV DNA, in order to estimate the amount of integrated HBV DNA in the human genome. A complimentary study on the same material was performed to obtain an RNA profile using ddPCR to amplify six target regions. Together, these results indicate that integrated DNA represents the majority of intrahepatic HBV DNA in late stages of infection and is responsible for maintaining high HBsAg levels in serum. The results also suggest that reduced transcription of pgRNA via a novel mechanism may contribute to low HBV replication in HBeAg-negative phase.

ddPCR analysis of a range of HBV markers was used to study focal differences in infection in 15-30 pieces of liver explant tissue from six patients with HBV or HDV induced cirrhosis. Large differences in focality was observed especially in patients with low degree of viral replication or with HDV coinfection and the results also support expression of S RNA from integrated HBV DNA. HDV infection was less focal with presence of high HDV RNA levels in the absence of HBV.

In summary, this thesis compilation contributes to better understanding of HBV serum and tissue markers and their relationship to replication and integration.

**Keywords :** hepatitis B virus, NTCP, HBV DNA, HBV RNA, subviral particles, integrations, droplet digital PCR

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Avdelningen för Gastroenterologi och Reumatologi, Sektionen för Hepatologi,  
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## Avhandlingen baseras på följande delarbeten

- I **Kasthuri Prakash**, Gustaf E. Rydell, Simon B. Larsson, Maria Andersson, Gunnar Norkrans, Heléne Norder, Magnus Lindh. High serum levels of pregenomic RNA reflect frequently failing reverse transcription in hepatitis B virus particles. *Virology Journal* 2018; 15 : 86.
- II Gustaf E. Rydell, **Kasthuri Prakash**, Heléne Norder, Magnus Lindh. Hepatitis B surface antigen on subviral particles reduces the neutralizing effect of anti-HBs antibodies on hepatitis B viral particles *in vitro*. *Virology* 2017; 509: 67-70.
- III Gustaf E. Rydell, Simon B. Larsson, **Kasthuri Prakash**, Maria Andersson, Heléne Norder, Kristoffer Hellstrand, Gunnar Norkrans, Magnus Lindh. Abundance of non-circular HBV DNA suggests integrations to be the predominant form of viral DNA in chronic hepatitis B. Manuscript.
- IV **Kasthuri Prakash**, Simon B. Larsson, Catarina Skoglund, Gustaf E. Rydell, Johan Ringlander, Maria Andersson, Maria Castedal, Heléne Norder, Magnus Lindh. Intrahepatic focality of hepatitis B infection analysed by quantification of viral RNA in multiple tissue pieces from explanted livers. Manuscript.
- V **Kasthuri Prakash**, Simon B. Larsson, Gustaf E. Rydell, Maria Andersson, Johan Ringlander, Gunnar Norkrans, Heléne Norder, Magnus Lindh. Analysis of HBV RNA in liver biopsies by digital PCR reveals differences in transcript levels, length and origin between e antigen positive and negative individuals. Manuscript.

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