HBV load in treated and untreated individuals

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Chapter 8

General discussion
Impact of HBV and HCV infection in an endemic area

The sheer size of the nationwide anti-schistosomiasis campaigns in Egypt, combined with the methods applied, has provided an effective mechanism for establishment of HCV and HBV in the Egyptian population. This is the world's largest occurrence of iatrogenic transmission of blood-borne pathogens known to date. It most likely led to a massive increase in the reservoir for HCV and HBV in the general population. Because of the high rate (85%) of chronicity in HCV infections, this reservoir is probably responsible for the high incidence of HCV transmission in Egypt today.

The rate and geographical pattern of prevalence of antibodies to HCV in Egypt found in our study (chapter 2) confirm the high prevalence and its distribution reported in rates of HCV seropositivity. HCV and HCV/HBV double infection but not HBV or HEV infection alone were correlated with hepatocellular carcinoma (HCC). HCC was strongly related to HCV infection irrespective of HBV infection. This is in agreement with other reports, which showed a stronger association of HCV and HCV/HBV double infection with HCC than with HBV infection alone.

We did also find that HCV/HBV double infection leads to an increased prevalence of HCC. We found no significant difference in mean HCV RNA load in HBV+ and HBV- samples indicating that in this setting, HBV infection does not have an impact on HCV replication.

HBV and HCV are endemic in for instance South-East Asia and parts of Africa. HCC is indeed becoming one of the most important causes of death in these parts of the world. In these regions, infection with HBV and/or HCV is the major cause of HCC. Contrary to HCV, HBV can be treated fairly well and good vaccines exist. HCV infection alone has a lesser impact on HCC compared to HBV or HCV/HBV double infection in endemic regions. Prevention of HCC by hepatitis B vaccination should therefore have the highest benefit in decreasing the incidence of HCC in these areas.

We found that HCV infection rather than level of replication was associated with HCC. This suggests that, in the case of HCV, it is not the load that is a determinant of disease progression, but the immune response raised against it. Khakoo et al. reported that CD4 positive lymphocytes may play an important role in the hepatic injury found in patients with chronic hepatitis C, by inducing cytotoxic T-cell responses against infected liver cells. This continuing process of hepatic damage may then eventually lead to the development of HCC. The same may hold true for HBV infection.
**Quantification of HBV DNA**

The pattern found during screening by nucleic acid amplification testing on transmission of hepatitis B virus DNA is complex. A serological response to a virus infection generally develops after a few weeks, while the DNA or RNA of the virus may already be detectable in a matter of days. Screening by nucleic acid amplification is therefore useful to narrow the infectious window in the early stage of acute HBV infection. Another even more important application is during treatment of persistently infected individuals, in which extremely low concentrations of HBV antigen and antibody are often observed.

We developed a detection and quantification method for HBV DNA based on NASBA, even though NASBA is in principle more suited for RNA amplification (chapter 3). The method developed is easy to use, accurate, specific and sensitive, making the assay well suited for routine serological screening for HBV.

**Impact of HIV coinfection on acute HBV infection**

HBV itself is normally not cytotoxic, and it has been well accepted that the liver damage during acute HBV infection is mainly immune mediated. CD8 cell function is disturbed in HIV-1 infection because of the effect of HIV-1 on CD4 cells which are subsequently unable to properly regulate CD8 proliferation and action. HIV-1 infection may alter the duration of HBV infection by alteration of the balance between humoral (TH1) and cytotoxic (TH2) immune responses through its immunomodulatory action. It is well known that the clearance of HBV is greatly determined by this balance.

A very common marker for liver damage is the elevation of ALT and AST, two enzymes mainly found in liver cells. Following liver damage, these enzymes may leak into the blood and elevated levels may subsequently be detected. We have used this marker to estimate the occurrence of liver damage.

Our study (chapter 4) indicates that (con)current HIV-1 infection causes a more rapid clearance of HBV infection. HBV positive drug addicts were more often presenting with elevated aminotransferases than HBV positive homosexuals. Apart from this,
development of chronic HBV-infection in our study population does not seem to be correlated with sex, age, CD4 or CD8 count before HBe seroconversion. Our findings strengthen the evidence for a significant effect of concurrent HIV-1 infection on the natural history of HBV infection, which could have an important impact on HBV infection in regions, or patient groups, with high HIV-1 seroprevalence.

Effect of therapy on HIV/HBV coinfected individuals

New HBV-specific therapies are currently being evaluated. Some of these therapies, such as lamivudine treatment, have an excellent safety profile and potent antiviral efficacy. Central to developments in HBV therapy has been the improved knowledge of the HBV lifecycle and lessons learned from the HIV field, where multidrug regimens are standard. Nucleoside analogues, the major agents evaluated in clinical trials for the treatment of chronic HBV infection, have been shown to inhibit viral replication, and improve liver enzymes and histology in infected individuals. HIV-related immunosuppression may reduce necroinflammatory lesions in the liver and serum alanine transaminase (ALT) level in HBV-infected patients. Nevertheless, HBV infection has also been shown to be associated with more severe liver fibrosis in HIV-coinfected patients, which may increase mortality. Loss or absence of serum HBeAg can be associated with increased inflammation and liver injury. We show that the same may hold true for HBV DNA in HBV/HIV-1 coinfected patients during treatment with 3TC (chapter 5).

An improved immune response associated with reductions in HIV-1 viremia alone may have a significant anti-HBV effect. This raises the question whether hepatotoxicity observed in HIV-1/HBV coinfected patients during HAART including 3TC is caused by the drug therapy or the restoration of the immune system by elimination of HIV-1.

CD4 nor CD8 cell count in our case-control study was correlated with moderate hepatotoxicity, although increase in CD8 counts were associated with severe LEE with borderline significance. These findings are in favor of the hypothesis that not 3TC therapy by itself, but rather the active clearance of virus particles from the blood by the host's immune system through the cytotoxic pathway may be causing damage to the liver.

The hepatotoxicity observed in our patients did not seem to be related to the usage of antiviral drugs or other risk factors (chapter 5). This was also illustrated by the fact that
all patients clear HIV-1 after initiating HAART, but hepatotoxicity is only observed in individuals with a good HBV response.

We therefore propose that this hepatotoxicity is not immediately due to the drugs used during HAART, but that it is mainly associated with the recovery of the immune system and the associated anti-HBV response.

**Detection of HBV RNA in serum**

HBV is a DNA virus that replicates through an mRNA intermediate \(^{18,31}\). Hence, it has been long accepted that the only HBV genetic material to be found in viruses circulating in the blood should be DNA. Nevertheless, more and more studies have indicated that HBV RNA can also be detected in serum\(^{32-34}\). Whether this HBV RNA is contained within virus particles remains unclear. However, we convincingly show in a different study that HBV RNA in serum is actually contained in a particle. Furthermore, we also show in that paper that nude HBV RNA is rapidly degraded in serum (Penning et al., submitted to PNAS).

We developed a real-time assay for the quantification of HBV RNA, based on NASBA technology (chapter 6). The HBV Retina™ has been tested in more than 1000 patient serum samples and has proven to be easy to perform. It has a dynamic range from \(10^2\) to \(10^8\) HBV RNA copies/reaction. The assay performs best in the range from \(10^4 \text{ - } 10^7\) copies/reaction, and has in this range a very good reproducibility and precision. Studies in different groups of patients showed that presence of HBV RNA is correlated strongly to the presence of HBV DNA, HBsAg and HBeAg. The correlations found were strongest in the group of chronic HBV/HIV double infected patients. In the chronically infected patients, time points at which HBV RNA is positive, but HBV DNA is below the quantification limit of the assay (10000 copies/ml serum) seemed to occur more often than in patients with an acute infection.

Taken together, the presence of HBV RNA in serum might be a marker for active replication. Studies to test whether the presence of HBV RNA in serum can be used to make predictions on disease progression or outcome of therapy are underway (see also chapter 7).
Determinants of a beneficial HBV response to lamivudine

We studied patients longitudinally during a period of lamivudine treatment, to establish which factors determine the outcome of infection and which laboratory markers respond most sensitively to therapy (chapter 7). We used the newly developed quantitative NASBA-based assays (see chapters 3 and 6) to monitor HBV DNA and RNA in serum.

In chapter 7, we show that the best way to treat HBsAg positive patients with HIV-1 coinfection is with a triple therapy regimen including lamivudine. Lamivudine suppresses both HIV-1 and HBV replication, making lamivudine-containing regimens the therapy of choice for HIV-1/HBV coinfected individuals. We found faster clearance of HBV DNA and RNA in patients receiving triple therapy as compared with mono therapy. This is probably due to the immune system being more competent to eradicate HBV because of a more efficient suppression of the HIV-1 infection.

Furthermore, immune reconstitution with HAART has been reported to shift the spectrum of HBV disease toward an enhanced inflammatory response to hepatitis B, followed by decreased viremia and seroconversion. Finally, in patients with low CD4 counts at the start of lamivudine therapy, the immune system may have been too weak to resolve the HBV infection. Due to the antiretroviral therapy their immune system may improve, resulting in a higher chance of clearing HBeAg. This relation with CD4 cell count is not seen with HBV DNA and RNA clearance. Clearance of HBeAg is only immune mediated, while clearance of HBV DNA and RNA is a result of the immune reaction, combined with the direct influence of lamivudine on the replication of HBV.

The effect of lamivudine therapy could be detected earliest by HBV RNA clearance, followed by the clearance of HBV DNA. HBeAg was the last marker to disappear from the serum, and this feature is in line with commonly observed clinical patterns. As noted previously, HBV DNA containing particles can be detected in serum longer than virions containing HBV RNA. This may be explained by the hypothesis that during HBV replication DNA containing particles develop from particles with RNA. When the replication of HBV is hampered by the effect of lamivudine, the RNA containing particles which arise in the beginning of the replication cycle will be the first to be affected.

In conclusion, we show that HBV RNA is cleared more often from the serum of patients...
than DNA, and that HBV RNA is cleared faster to levels below detection level than HBV DNA. The effect of therapy on HBV may be measured best by monitoring HBV RNA, instead of HBV DNA.

Concluding remarks

The liver is a tremendously resilient organ unless it is subjected to a continuous assault. Chronic infection with the hepatitis B virus is such an assault, and can result in cirrhosis, cancer, and death. HBV infects more than 500 million people worldwide. Hepatitis B is most prevalent in underdeveloped regions like parts of Southeast Asia, China, and Africa.

The usual diagnostic hepatitis B laboratory profile consists of assays for (I) hepatitis B surface antigen, (II) antibody to hepatitis B surface antigen, and (III) hepatitis B core antibody. All three tests are commonly performed, to preclude missing the diagnosis during a window period. A window period occurs when the surface antigen has been cleared and the surface antibody has not yet risen during the convalescence period. The assays presented in this thesis (chapter 2 and 6) provide additional means to monitor HBV infection especially in these stages. The usefulness of the HBV DNA and RNA Retina is further demonstrated in chapters 4-7. In these chapters, multiple clinical findings are presented which could not have been made as easily with different assays: the confirmation of the hypothesis that HIV coinfection alters the natural history of HBV infection; triple therapy being more effective for treating HBV in HIV-1 coinfected individuals than mono or duo therapy; HBV RNA which may be a more sensitive marker for effectiveness of therapy.

It may be clear that still a lot can be learnt about the mechanisms behind HBV/HIV coinfection. To achieve this, new techniques and new ideas should be continued to be explored. We have made a first step into exploring some of these new directions.
References


