Introduction

Since its introduction, HSCT is performed annually in over 30,000 patients worldwide for a wide range of hematological disorders. Patients with viral infection B or C and underwent HSCT may change the natural course of the disease.

Patients undergoing HSCT and are already infected with HBV are at risk for developing VOD and/or recurrent HBV infection after transplantation ***(Locascilli et al., 1994)*** with recovery of cellular immunity after HSCT sever hepatitis and fulminant liver failure may occur ***(Ivantes et al., 2004).***

Hepatitis C is universally transmitted from HCV positive allogenic or syngenic donors to their recipients. Recipients become viremic within days of stem cell transfusion. However they do not immediately develop clinical or biochemical hepatitis because of lack of cellular immunity after myeloablation and transplantation. Transmission of HCV from an infected donor to a recipient may be preventable by treating the donor pre-transplant with interferon-α until serum HCV RNA is not detectable at time of stem cell harvesting ***(Ivantes et al., 2004).***

Aim of the Work

Evaluation of hepatitis B and C infection in patients treated with Hematopoetic Stem Cell Transplantation (HSCT) and its relation to the short term outcome on the transplanted patients.

Chapter I: Hepatitis B Virus

It is estimated that 40% of the world's population has had contact with or are carriers of the hepatitis B virus (HBV). This corresponds to an estimated 350 million HBV carriers. Thus, HBV infection is one of the most important infectious diseases worldwide ***(Goldstein et al., 2005).***

Around one million persons die of HBV-related causes annually. There is a wide range of HBV prevalence rates in different parts of the world. HBV prevalence varies from 0.1% up to 20%. Low prevalence areas (0.1-2%) are Western Europe (with wide variation within Europe), United States and Canada, Australia and New Zealand; intermediate prevalence (3-5%) are the Mediterranean countries, Japan, Central Asia, the Middle East, and Latin and South America; and high prevalence areas (10-20%) southeast Asia, China, and sub-Saharan Africa.

This diversity is probably related to differences in the age at infection, which correlates with the risk of chronicity. The progression rate from acute to chronic HBV infection decreases with age. It is approximately 90% for an infection acquired perinatally, and is as low as 5% (or even lower) for adults ***(Wasley et al., 2008).***

Although the incidence of acute HBV infection has decreased in most countries due to the implementation of vaccination programs, HBV-related complications such as cancers and deaths have been on the increase ***(Gomaa et al., 2008).***

Reasons might be the delay of vaccination effects, improved diagnosis, and better documentation of HBV cases. Although a drop in prevalence has been observed in many countries, estimates are difficult due to a continuously growing migration from high or medium prevalence areas to low prevalence areas ***(Belongia et al., 2008).***

Structure of the viral particles and organization of the viral genome

The family name Hepadnaviridae is based on the clinical picture of infection and the target organ (hepatitis; liver, classical Greek: επαρ, phonetic: to hepar) and its nucleic acid, the DNA. The family of Hepadnaviridae contains two genera, the *orthohepadnaviruses* that infect only mammals, and the *avihepadnaviruses* that infect birds.

The *Hepadnaviridae* are enveloped DNA viruses with a circular partially double stranded DNA that in concert with the core protein forms the nucleocapsid. The infectious virus, i.e., the Dane particle, is of a spherical shape with a diameter of 42-47 nm.

As with nearly all enveloped viruses there is also evidence that the viral particle contains proteins assumed to be of host origin for HBV ***(Albin & Robinson 1980).***

The average size of the viral genome is around 3.3 kbp, varying slightly from genotype to genotype and from isolate to isolate ***(Lüsebrink et al., 2010).***

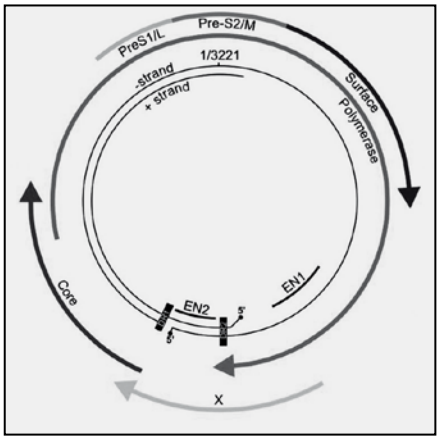

Figure (1): Genome organization and transcripts of the human hepatitis B virus ***(Lüsebrink et al., 2010).***

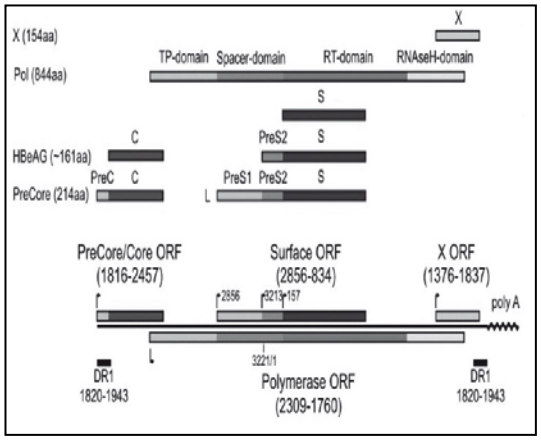

Figure (2): The open reading frame organization of the HBV genome ***(Lüsebrink et al., 2010).***

Pathogenesis of hepadnavirus infections

The transmission of HBV and other members of the Hepadnaviridae family occur both vertically and horizontally via body fluids. A maximum of 10^{10} to 10^{12} genome copies / ml serum or body fluid can be found. In chronic infections, the viremia is subject to natural fluctuations of one $\log^{10}$ The rate for chronicity, varies from >90% in neonates and approximately 10-15% in adults ***(Schildgen 2010).***

There is considerable variation in the predominance of transmission modes in different geographic areas. For example, in low prevalence areas such as Western Europe, the routes are mainly unprotected sexual intercourse and intravenous drug use. In high prevalence areas like Sub-Saharan Africa perinatal infection is the predominant mode of transmission. Horizontal transmission, particularly in early childhood, is regarded as the major route of transmission in intermediate prevalence areas ***(Wasmuth 2010).***

Natural history and clinical manifestations

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Acute hepatitis

After HBV transmission, the incubation period lasts from one to four months. A prodromal phase may appear before acute hepatitis develops. During this period a serum sickness-like syndrome may develop. This syndrome manifests with fever, skin rash, arthralgia and arthritis. It will usually cease with the onset of hepatitis. At least 70% of patients will then have subclinical or anicteric hepatitis, while less than 30% will develop icteric hepatitis.

The most prominent clinical symptoms of hepatitis are right upper quadrant discomfort, nausea, jaundice and other unspecific constitutional symptoms. In case of co-infection with other hepatitis viruses or other underlying liver disease the clinical course may be more severe. The symptoms including jaundice generally disappear after one to three months, but some patients have prolonged fatigue even after normalization of serum aminotransferase concentrations.

Concentrations of alanine and aspartate aminotransferase levels (ALT and AST) may rise to 1000-2000 IU/L in the acute phase. ALT is typically higher than AST ***(Wasmuth 2010).***

Bilirubin concentration may be normal in a substantial portion of patients. In patients who recover, normalization of serum aminotransferases usually occurs within one to four months. Persistent elevation of serum ALT for more than six months indicates progression to chronic hepatitis.

There are lots of evidence that even in patients positive for anti-HBs and anti-HBc, HBV DNA may persist for long periods of time and this latent infection maintains the T cell response that keeps the virus under control**.** Complete eradication rarely occurs, this is an important finding, as immunosuppression can lead to reactivation of the virus, e.g., after organ transplant or during chemotherapy. Fulminant hepatic failure is unusual, occurring in approximately 0.1-0.5% of patients ***(Cornberg 2007).***

Therefore, treatment of acute hepatitis B is mainly supportive in the majority of patients. Treatment can be considered in certain subsets of patients, e.g., patients with a severe or prolonged course of hepatitis B, patients co-infected with other hepatitis viruses or underlying liver diseases, patients with immunosuppression, or patients with fulminant liver failure undergoing liver-transplantation ***(Tillmann et al., 2006).***

Chronic hepatitis

The HBV chronicity rate is around 5% or less in adult-acquired infection, approximately 90%, in perinatally acquired

infection, and is estimated to be 20-50% for infections between the age of one and five years. However, most patients will not have a history of acute hepatitis ***(Ganem & Prince 2004).***

Most patients with chronic hepatitis B are clinically asymptomatic. Some may have nonspecific symptoms such as fatigue. In most instances, significant clinical symptoms will develop only if liver disease progresses to decompensated cirrhosis. In addition, extrahepatic manifestations may cause symptoms.

Accordingly, physical examination will be normal in most instances. In advanced liver disease there may be stigmata of chronic liver disease such as splenomegaly, spider angiomata, Caput medusae, palmar erythema, testicular atrophy, gynecomastia, etc. In patients with decompensated cirrhosis jaundice, ascites, peripheral edema, and encephalopathy may be present.

Laboratory testing shows mild to moderate elevation in serum AST and ALT in most patients, whereas normal transaminases occur rarely. During exacerbation, serum ALT concentration may be as high as 50 times the upper limit of normal. Alfa-fetoprotein (AFP) concentrations correlate with disease activity. In exacerbations of hepatitis B concentrations as high as 1000 ng/mL may be seen.

The natural course of chronic HBV infection is determined by the interplay between viral replication and the host immune response. Other factors that may play a role in the progression of HBV-related liver disease include gender,

alcohol consumption, and concomitant infection with other hepatitis virus(es). The outcome of chronic HBV infection depends upon the severity of liver disease at the time HBV replication is arrested. Liver fibrosis is potentially reversible once HBV replication is controlled.

There are two different states that are distinguished in chronic HBV infection: firstly, a high-replicative state with active liver disease and elevated serum ALT. HBV DNA and HBeAg are present. Secondly, a low or non-replicative phase, where serum ALT may normalize, HBeAg disappears, and anti-HBe antibodies appear. In some patients, virus replication stops completely, as demonstrated by sensitive HBV DNA assays, although they remain HBsAg-positive. These patients have undetectable HBV DNA in serum and normal ALT concentrations. No sign of ongoing liver damage or inflammation is found on liver biopsy. This state is called inactive carrier state.

The first high-replicative phase may switch into the nonreplicative phase spontaneously or upon antiviral treatment. Conversely, the non-replicative phase may reactivate to the high-replicative phase either spontaneously or with immunosuppression (e.g., in HIV infection or with chemotherapy) ***(Wasmuth 2010).***

Table (1): Hepatitis B definitions ***(Wasmuth 2010).***

Chronic hepatitis B	• $HBsAg^+$ > 6 months • Serum HBV DNA > 2000 IU/mL (10^4 copies/mL) • Persistent or intermittent elevation in ALT/AST levels • Liver biopsy showing chronic hepatitis
Inactive HbsAg carrier state	• $HBsAg^+$ > 6 months • $HBeAg^-$, anti-HBe+ • Serum HBV DNA < 2000 IU/mL • Persistently normal ALT/AST levels • Liver biopsy confirms absence of significant hepatitis
Resolved hepatitis B	• Previous known history of acute or chronic hepatitis B or the presence of anti-HBc ± anti-HBs • $HBsAg^-$ • Undetectable serum HBV DNA
Reactivation of hepatitis B	• Reappearance of active liver disease in a person known to have the inactive HBsAg carrier state or resolved hepatitis B

Extrahepatic manifestations

Extrahepatic manifestations can occur in both acute and chronic infection. The two major extrahepatic complications of chronic HBV are polyarteritis nodosa and glomerular disease. They occur in 10-20% of patients with chronic hepatitis B and are thought to be mediated by circulating immune complexes ***(Han et al., 2000).***

- ***Polyarteritis nodosa:*** The clinical manifestations are similar to those in patients with polyarteritis who are HBV-negative. There may be some clinical benefit to antiviral therapy.

- ***Nephropathy / Glomerulonephritis:*** HBV can induce both membranous nephropathy and, less often, membrane oproliferative glomerulonephritis. Most cases occur in children. The clinical hallmark is proteinuria. In contrast to polyarteritis nodosa, there is no significant benefit of antiviral treatment ***(Wasmuth 2010).***

Prognosis

As clinical course varies among patients, there is a wide variation in clinical outcome and prognosis of chronic HBV infection. The lifetime risk of a liver-related death has been estimated to be 40-50% for men and 15% for women. The risk of progression appears to be higher, if immune activation occurs. The estimated five-year rates of progression ***(Fattovich 2008).***

- Chronic hepatitis to cirrhosis – 10-20%
- Compensated cirrhosis to hepatic decompensation-20-30%
- Compensated cirrhosis to HCC-5-15%

Accordingly, the survival rates are:

- Compensated cirrhosis-85% at five years
- Decompensated cirrhosis-55-70% at one year and 15-35% at five years.

There are several factors known to influence survival: viral replication, alcohol consumption, Hepatitis C co-infection and Hepatitis D co-infection ***(Wasmuth 2010).***

Treatment

Viral replication: In patients with signs of viral replication (i.e., HBe Ag-positive) there is consistently worse survival than in patients who are HBe Ag-negative. However, in recent decades, infections with HBe Ag-negative precore mutants prevail by far in newly-acquired infections, resulting in a different pattern of HBe Ag-negative and HBV DNA positive hepatitis with fibrosis progression and HCC in a substantial proportion of patients.

In recent years, the amount of HBV DNA has also been linked to disease progression and has replaced HBeAg postivity as a marker for disease activity. This is true both for progression to cirrhosis as well as for the risk of HCC. Therefore, most treatment guidelines today are based on the level of HBV viremia. A reasonable cut-off to distinguish patients with a low risk of progression from patients with a high risk of progression and indication for antiviral treatment is 10^4 copies/ml (corresponding to approximately 2 x 10^3 IU/ml), although other cut-offs may be used. The duration of viral replication is obviously linked with the risk of development of cirrhosis and HCC. As necro-inflammation may persist longer in patients with a prolonged replicative phase, the risk of disease progression is elevated ***(Chen 2006).***

Conversely, even in patients with decompensated cirrhosis, suppression of HBV replication and early HBs Ag clearance can result in improvement in liver disease ***(Fung et al., 2006).***

Vaccination against hepatitis B

A response to HBV vaccination is determined by the development of anti-HBs antibodies which is detectable in 90-95% of individuals one month after a complete vaccination schedule. Responses are lower in elderly people and much weaker in immunocompromised persons such as organ transplant recipients, patients receiving haemodialysis and HIV-infected individuals ***(Wedemeyer 2007).***

In case of vaccine non-response, another three courses of vaccine should be administered and the dose of the vaccine should be increased. Other possibilities to increase the immunogenicity of HBV vaccines include intradermal application and co-administration of adjuvants and cytokines ***(Cornberg 2007).***

Aims of diagnostic tests in the management of HBV-infected patients

The first diagnosis of an HBV infection has to figure out whether the infection is acute or chronic. Therefore, as standard procedure, the patient with HBV infection diagnosed by clinical symptoms or elevated alanine aminotransferase (ALT) levels needs to test positive for anti-hepatitis B core antigen (HBc Ag) antibodies. HBc Ag is massively expressed in both acute and chronic infections and is a clear sign of HBV infection. After a positive result for anti-HBc Ag antibodies, antibodies reactant to the surface antigen (HBsAg) are looked for. If found, this indicates that the patient is cured from the infection or has been successfully vaccinated.

Based on these initial serologic diagnostics, further efforts to define the status of the infection are made. An anti-HBc Ag positive but anti-HBs Ag negative patient may be dealing with a chronic infection. In these cases a number of parameters should be investigated, namely early antigen (HBe Ag), anti-HBe Ag, HBs Ag, HBc Ag, and finally, the viral load measured as genome equivalents per ml in serum. HBe Ag is normally expressed only in case of an acute and/or ongoing infection with active replication. Unfortunately, so-called precore mutants exist that display active replication without expressing HBe Ag, still bearing a high risk for progression to HCC. It is worth noting that HBe Ab sero-conversion occurs in up to 98% of people and that this is not a marker for a cure of the infection, although it does act as a marker for healing.

These methods are generally used for monitoring treatment efficacy and treatment compliance, to identify resistant strains, and to identify precore mutant strains of HBV ***(Schildgen 2010).***

Molecular assays in the diagnosis and management of HBV Utility of quantitative HBV DNA assays

All of the published guidelines/consensus papers recommend an initial quantification of viral load and continuous measurements during follow-up monitoring chronically-infected HBV patients. Follow-up is considered important for deciding on initiation of treatment or changes to the patient's drug regimen.

One agreed-upon criterion for chronic HBV infection is a detectable viral load-measured as viral DNA in serum or plasma-for a minimum of 6 months ***(de Franchis 2003; Lok et al., 2001; Lok & McMahon 2004).*** In this case, replication is considered to be active if >2,000 IU/ml or >10,000 copies/ml can be detected. Also, in HBeAg-negative chronic hepatitis B virus infections, HBV DNA is the only marker for viral replication that consequently needs to be monitored. A cutoff limit of 2000 IU/ml differentiates active from inactive replication ***(Manesis & Hadziyannis 2001; Zacharakis et al., 2005).***

Qualitative and quantitative measurement of viral DNA is important to monitor occult hepatitis (characterized as HBV infection with measurable DNA levels in the absence of detectable HBsAg). measurement of viral load after starting therapy is a useful standard tool to help identify therapy non-responders ***(Sirma et al., 2007; Volz et al., 2007).*** Non-response to therapy can be induced by host factors, viral resistance, or non-compliance.

HBV Genotypes

Eight genotypes of HBV have been identified labelled A through H. The prevalence of HBV genotypes varies depending on the geographical location. Genotyping of the HBV genome can be useful. First, the viral genotype influences the success of therapy, e.g., patients with an HBV genotype B infection have a better chance for a more favorable outcome than those patients infected with genotype C ***(Sirma et al., 2007; Volz et al., 2007)***.