New developments in hepatitis B, C and G virus

Sentjens, R.E.J.H.

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CHAPTER 2

INTRODUCTION
**Discovery of the hepatitis C virus (HCV)**

After the discovery of the hepatitis B and hepatitis A virus in the nineteen seventies (16,52), it appeared that neither hepatitis A nor hepatitis B virus caused the majority of blood transfusion-associated hepatitis cases (53) and subsequently the term post transfusion hepatitis non-A, non-B and hepatitis C was introduced (149). At the end of the eighties Houghton and co-workers cloned the genome of the etiologic agent of non-A, non-B hepatitis and named it hepatitis C virus (HCV) (18). Subsequently specific diagnostic tests were developed and it turned out that the majority of non-A/non-B hepatitis cases were associated with this newly detected virus (176).

**Fig 1.**

1. ALAT=alanine aminotransferase, PCR=polymerase chain reaction
2. Serologic sequence in chronic hepatitis C virus (HCV). HCV-RNA appears approximately 2 to 4 weeks after exposure. Symptoms and jaundice may appear 6 to 24 weeks after exposure. Antibody to HCV (anti-HCV) appears 20 to 150 days (mean 50 days) after exposure and its presence is usually lifelong. Anti-HCV does not denote chronicity or active infection. HCV-RNA denotes active infection. Most patients have no clinical disease the acute phase of the illness but instead are diagnosed years later with chronic HCV (5;172). Adapted from Hoofnagle (72).
Natural history of HCV infection

HCV infection is characterized by its propensity to evolve into chronicity. About 85% of patients infected by HCV will develop chronic infection and resolution of acute hepatitis C is observed in only 15% (113). After exposure to HCV, clinical symptoms such as jaundice occur in less than 20% of acute hepatitis C infections. When jaundice occurs it is often accompanied by general malaise, nausea and vomiting and right upper quadrant pain. Clinical symptoms of acute HCV infection may occur within 6 – 24 weeks after exposure to HCV. HCV RNA becomes detectable in the blood 2 – 4 weeks after exposure, and specific antibodies appear within 20-150 days (mean of 50 days). Elevation of alanine aminotransferase (ALAT) generally may occur 4 weeks after exposure (7:8:49). (see fig1).

The natural history of chronic hepatitis C virus infection in 280 patients with chronic HCV infection for 8 - 16 years were studied in 3 prospective studies (43:85:130:171). After this period of time cirrhosis had developed in 7 - 16% of patients and hepatocellular carcinoma (HCC) in 0.7 - 1.5%. In several retrospective studies with a HCV infection period between 10 – 28 years, 17 - 55% of patients had developed cirrhosis and HCC in 1 - 23% (82;169). It is estimated that overall in 20-30% of patients, chronically infected with HCV will ultimately develop cirrhosis (145). In patients with established cirrhosis the risk of development of HCC maybe is 1-4% per year (173). Factors that accelerate clinical progression to cirrhosis include excessive alcohol intake (> 50 gr/day), co-infection with hepatitis B or HIV, male sex and older age at the time of infection (145).
Epidemiology of HCV

Worldwide approximately 170 million persons are infected with HCV. Therefore HCV infection is a viral pandemic, which is five times as widespread as infection with the human immunodeficiency virus type 1 (93). The prevalence of HCV infection varies greatly in different countries. The various prevalence rates of chronic HCV infection throughout the world are described in Table 1.

Table 1: Prevalence of chronic HCV infection in various countries throughout the world

<table>
<thead>
<tr>
<th>Prevalence rate of Chronic HCV infection</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1%</td>
<td>Western Europe, Australia, Canada, Argentina.</td>
</tr>
<tr>
<td>1 - 2.5%</td>
<td>USA, Russia, Eastern Europe, India, Saudi Arabia, Indonesia.</td>
</tr>
<tr>
<td>2.5 - 5%</td>
<td>China, Brazil.</td>
</tr>
<tr>
<td>5 - 10%</td>
<td>Libya, Central Africa, Vietnam, Taiwan</td>
</tr>
<tr>
<td>&gt; 10%</td>
<td>Egypt, Mongolia, Bolivia</td>
</tr>
</tbody>
</table>

Adapted from Poynard et al (143).

Transmission routes of HCV

Risk factors for HCV infection include intravenous (I.V.) drug abuse, renal dialysis, transfusion of blood products and transplantation of organs, tattooing, accidental parenteral exposure in the health care setting, exchange of straws used for cocaine snorting, perinatally and infection with HIV (33;159). Of all population subsets I.V. drug abusers have the highest rate of HCV infection, with a prevalence between 31-98% (120). Oral drug abusers had a much lower prevalence of HCV, 4% as compared to I.V. drug abusers (120). Before 1990 the incidence of post transfusion hepatitis non-A, non-B ranged from 5-13%. Since the introduction of anti-HCV screening of all blood donors in 1990 the incidence of post transfusion HCV infection declined to <1%. Introduction of new HCV screening tests, based on nucleic acid technology (NAT) can in fact eliminate the risk of post transfusion hepatitis C (13). Hemophiliacs had also a very high prevalence of HCV infection i.e., 46-76% (110). Since several virucidal procedures for preparation of plasma Factor VIII concentrate were implemented, no new HCV infections in haemophilia patients were observed (115). Other risk factors for HCV transmission are listed in Table 2.
Table 2 Risk factors for HCV transmission

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Prevalence of HCV infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infective route</strong></td>
<td></td>
</tr>
<tr>
<td>• Long term cancer survivors</td>
<td>20</td>
</tr>
<tr>
<td>• Bone marrow transplants</td>
<td>29</td>
</tr>
<tr>
<td>• Renal dialysis</td>
<td>15 - 20</td>
</tr>
<tr>
<td><strong>Healthcare professionals</strong></td>
<td></td>
</tr>
<tr>
<td>• Needle stick accidents</td>
<td>0 - 5.6</td>
</tr>
<tr>
<td>• Intrafamilial transmission</td>
<td>3.6</td>
</tr>
<tr>
<td>• Sexual transmission</td>
<td>Infrequent or absent</td>
</tr>
<tr>
<td>• Perinatal</td>
<td>0 - 15</td>
</tr>
<tr>
<td>• Unknown</td>
<td>20 - 50</td>
</tr>
</tbody>
</table>

Adapted from Memon et al (120)

As described in table 2 20 -50% of chronic HCV carriers have no identifiable route of transmission. Several studies showed the presence of HCV in body fluids. HCV was detected in seminal fluid, (56) semen (87), tear fluid (121) saliva, ascites (105), duodenal bile (88), gastric juice (84) and amniotic fluid (42). An HCV infection can be established due to I.V. inoculation of saliva from HCV infected chimpanzees (1;2). Thus body fluids from patients infected with HCV may account for the unknown rate of transmission of HCV.

Molecular structure of Hepatitis C virus

HCV is a member of the family of Flaviviridae. All viruses of this family have a positive-sense single stranded RNA genomes with a similar organization (81:133) Other members of the Flaviviridae are Pestivirus (bovine viral diarrhoea, swine fever), Flavivirus (yellow fever, dengue viruses) and GB viruses.

The HCV genome consists of approximately 9600 nucleotides. It has a single open reading frame (ORF), of about 9000 nucleotides in length. The ORF is flanked at each terminus by untranslated regions (UTR). Translation of the HCV ORF produces a polyprotein of about 3000 amino acids consistent of at least 10 structural or non-structural (NS) proteins (29;81). These structural and non structural proteins are described in table 3 (23).
Table 3. HCV structural and non-structural proteins after cleavage of the polyprotein by host signalises and viral proteases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Nucleocapsid protein</td>
</tr>
<tr>
<td>E1</td>
<td>Virion envelope protein</td>
</tr>
<tr>
<td>E2</td>
<td>Virion envelope protein</td>
</tr>
<tr>
<td>P7</td>
<td>Unknown</td>
</tr>
<tr>
<td>NS2</td>
<td>NS2-3 protease component</td>
</tr>
<tr>
<td>NS3</td>
<td>NS2-3 protease component, serine protease, NTPase and helicase</td>
</tr>
<tr>
<td>NS4A</td>
<td>Cofactor for NS3 serine-protease</td>
</tr>
<tr>
<td>NS4B</td>
<td>Unknown</td>
</tr>
<tr>
<td>NS5A</td>
<td>Unknown, possibly involved in interferon resistance</td>
</tr>
<tr>
<td>NS5B</td>
<td>RNA-dependent RNA polymerase</td>
</tr>
</tbody>
</table>

The 5'UTR of HCV is a highly conserved region of approximately 340 nucleotides. The 5'UTR is of great importance in diagnostic assays for the detection of HCV. The 5'UTR contains an internal ribosome entry site that controls translation, furthermore sequences within the 5'UTR may be used as replication signals, by direct interaction with the 3'end of the genome or by direct RNA protein interactions (23).

At the 3'end of the genome replication is initiated in analogy with other Flaviviridae. The 3'terminal sequences are, in analogue with the 5'UTR, highly conserved. HCV is an envelope virus which contains the 2 glycoproteins E1 and E2. The E1 and E2 of different HCV isolates exhibit a high degree of genetic heterogeneity (23). The N terminus of the E2 protein is highly variable and is designated HVR-1. In genotype 1b patients another hyper variable region was designated downstream of HVR 1, this region was designated HVR 2 (71). HVR1 is believed to play an important role in the persistence of HCV in the majority of infected people.
HCV genotypes and quasispecies

A distinctive characteristic of HCV is its genetic heterogeneity. The genomic heterogeneity occurs on two different levels. The widest variation is observed among HCV isolates of different genotypes. Isolates of the same genotype differ 5-10% in sequence and isolates of a different genotype differ up to 35% (132). The narrowest variation is observed among isolates of a single strain "quasispecies" in a single host, usually representing one dominant species and many minor ones. The quasispecies composition of HCV in infected individuals is the result of mutations and selection by immune-pressure during the course of the infection. The viral genomes of quasispecies differ from each other 1-2% (131). The variability of the quasispecies population may influence the outcome of acute infections, the severity of liver disease and the response to interferon therapy.

Six major genotypes along with more than 100 subtypes have been identified. The world wide distribution of HCV genotypes shows distinct geographic variation (119). HCV genotypes play an important role in the response to interferon treatment.

In table 4 The predominant HCV genotypes distributed in the world are described.

Table 4. Predominant genotype distribution throughout the world

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Predominant Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>North and South America, Europe, Russia, China, Japan, Australia, New Zealand</td>
</tr>
<tr>
<td>1b</td>
<td>Southern and Eastern Europe, China, Japan</td>
</tr>
<tr>
<td>2</td>
<td>North and South America, Europe, Russia, China, Japan, Australia, New Zealand</td>
</tr>
<tr>
<td>3</td>
<td>North and South America, Europe, Russia, China, Japan, Australia, New Zealand</td>
</tr>
<tr>
<td>4</td>
<td>Egypt, Central Africa</td>
</tr>
<tr>
<td>5</td>
<td>South Africa</td>
</tr>
<tr>
<td>6</td>
<td>South East Asia</td>
</tr>
</tbody>
</table>
Diagnostic tests for HCV Infection

Several diagnostic test have been developed for the detection of HCV infection (48).

Sero logical tests
At present third generation ELISA’s have a high sensitivity for detection of HCV (62). During an acute HCV infection anti-HCV antibodies can be detected 7-8 weeks earlier when these third generation tests are used (62).
Furthermore a HCV core antigen (HCV Ag) test has been developed with high sensitivity to detect HCV infection before development of antibodies(62;74;80;83;162). Evaluation of this test is needed to prove its efficiency for routine use (this thesis).

Qualitative HCV RNA tests
Polymerase chain reaction (PCR) is a sensitive method for the detection of HCV RNA and at present the detection limit is 5 - 50 IU/mL. Viral RNA must first be isolated and converted into cDNA prior to amplification by PCR. Another recently developed qualitative test for the detection of HCV RNA is Transcription-Mediated Amplification (TMA) with a detection limit of 5 IU/mL (122).
Qualitative HCV RNA tests discriminate between presence or absence of HCV infection. These tests are widely used to monitor the efficacy of anti viral therapy for chronic HCV patients.
Nucleic acid technology based tests (NAT) have also been introduced for blood donor screening to diminish the infection window period from approximately 60 days to 10 – 30 days (13;86).

Quantitative HCV RNA tests
Measurement of the amount of HCV RNA in the plasma or serum of patient is referred to as determination of HCV viral load.
The branched chain DNA (bDNA) assay is a molecular test for determination of HCV RNA viral load. The sensitivity of this assay is based upon amplification of the detection signal, in comparison to PCR in which the target RNA/DNA molecule is amplified. The detection limit of this assay is 615 IU/mL (122). The quantitative competitive PCR for measuring viral nucleic acid relies on the addition of a known amount of competitor RNA or DNA template to
test sample to permit accurate quantification. The detection limit of this test is 500 IU/mL HCV RNA.

Real time PCR based on the Taqman technology system has a sensitivity of $10^2$ copies/mL HCV RNA (122).

**Indications for therapy in chronic HCV infection**

The National Institutes of Health (NIH) consensus Conference and the Development statement and the European Association for Study of the liver (EASL) formulated inclusion criteria for treatment of patients with chronic hepatitis C infection who never received any treatment for HCV.

The combined criteria formulated for treatment are listed in table 5 (47;126).

<table>
<thead>
<tr>
<th>Inclusion criteria for treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated ALAT levels for more than 6 months</td>
</tr>
<tr>
<td>Detectable serum HCV RNA as measured by PCR</td>
</tr>
<tr>
<td>Compensated liver disease</td>
</tr>
<tr>
<td>A compliant patient</td>
</tr>
<tr>
<td>Abstinence of drugs and alcohol</td>
</tr>
<tr>
<td>Liver biopsy with portal fibrosis</td>
</tr>
<tr>
<td>No contraindications for interferon alpha or ribavirin</td>
</tr>
</tbody>
</table>

**Aim of antiviral treatment**

The optimal goal is to eliminate the virus completely and permanently and therewith reducing the change of cirrhosis and hepatocellular carcinoma.

Definitions of response:

1. Biochemical response: Normalization of ALAT
2. Virological response: HCV RNA negative as measured by PCR (50 IU/mL)
A. End of treatment response (ETR)  
A1. Biochemical  
Normalization of ALAT at the end of treatment.

A2. Virological  
HCV RNA negative as measured by PCR at the end of treatment

After cessation of therapy the biochemical and virological ETR were not always maintained. Therefore the recent clinical trials have been evaluated six months after cessation of therapy (end of follow up).

B. End of follow up response  
B1. Biochemical  
(Sustained biochemical response)

B2. Virological  
(Sustained virological response [SVR])

Patients with an SVR are regarded to be cured from chronic HCV infection.

At the moment there are three drugs approved in the treatment of HCV, interferon alpha (IFN), pegylated IFN (PEG IFN) and ribavirin only in combination with Interferon alpha or pegylated IFN.

**Interferon alpha (IFN)**

IFN's are a group of glycoproteins which are produced in response to a viral infection and which inhibit intracellular viral replication in a non infected cell. IFN binds to specific cell receptors and produces immunomodulatory and antiviral effects. Furthermore IFN is believed to have some anti-fibrotic activity. All effects of IFN are summarised in table 6.
Table 6. Different effects of IFN. IFN has antiviral effect, immunomodulatory effect and an anti-fibrotic effect.

<table>
<thead>
<tr>
<th>Antiviral effect</th>
<th>Immunomodulatory effect</th>
<th>Anti-fibrotic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increase macrophage activity</td>
<td>• Increases Th1 effect (interleukin 12)</td>
<td>• Reduces stellate cell activation</td>
</tr>
<tr>
<td>• Increases NK cell activity</td>
<td>• Increases HLA I expression</td>
<td>• Activates collagenases</td>
</tr>
<tr>
<td>• Increases cytotoxic cell activity</td>
<td>• Increases B cell proliferation</td>
<td></td>
</tr>
<tr>
<td>• Activates 2-5-oligoanenylate synthetase, which reduces viral RNA</td>
<td>• Modulates Th2 effect</td>
<td></td>
</tr>
<tr>
<td>• Activates protein kinase P1, which reduces viral protein synthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adapted from (144)

In 1991 the Food and Drug Administration approved IFN in a dose of 3 million units (MU) subcutaneously thrice weekly for 6 months, as a treatment for patients with chronic non-A non-B hepatitis, later named HCV hepatitis.

In a meta analysis by Carithers et al 20 IFN mono therapy randomised placebo controlled trials were analysed. In total 552 chronic HCV patients were randomised (26). The biochemical ETR in the IFN treated patients was 47% compared with 4% in the control group. The biochemical sustained response was in the treated patients 23% and the untreated patients 2% (26).

The virological response could only be assessed in five studies because of the lack of adequate laboratory detection methods at that time. The virological ETR was 29% in the treated patients and 5% in the control group. The SVR was 8% in the treated patients and 1% in the untreated patient groups(26).

Since the low rate of sustained biochemical and virological responders in IFN mono therapy new strategies were needed.

**Pegylated IFN**

In a standard treatment course IFN is administered in thrice weekly (t.i.w) 3-6 MU. IFN has a half life of approximately 8 hours. therefore during such a treatment with IFN there are periods with no suppression of HCV. Thus an IFN mono therapy treatment schedule may be associated with viral breakthrough (90;187) With the attachment of polyethylene glycol (PEG) to the IFN molecule a delay in protein clearance and prolongation of plasma half life was induced (68). Since the plasma half life of PEG IFN is approximately 10-fold longer than
that of IFN a constant antiviral pressure on HCV replication is maintained. Two Pegylated IFN's are currently under investigation in clinical trials.

1. PEG (12 kDA) IFN 2b. Attachment of a 12 kilo Dalton linear PEG to the IFN alpha 2b molecule.

2. PEG (40kDA) IFN 2a. Attachment of a 40 kilo Dalton branched PEG to the IFN alpha 2a molecule.

In table 7 the difference in response between IFN monotherapy and PEG INF monotherapy are listed.

Table 7 Sustained virological response (SVR) in chronic HCV patients treated for one year with IFN (3 x 3MU a week) in comparison with mono therapy Pegylated IFN 12 kDA (1.5 µkg wk) and Pegylated IFN 40 kDA (180 µg wk) (103;185). These studies included comparable patient groups.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Therapy</th>
<th>n</th>
<th>ETR %</th>
<th>SVR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindsay K.</td>
<td>2001</td>
<td>IFN 3 x 3MU</td>
<td>303</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>(103)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lindsay K.</td>
<td>2001</td>
<td>PEG IFN 12kDA (1.5 µkg wk)</td>
<td>304</td>
<td>49</td>
<td>23</td>
</tr>
<tr>
<td>(103)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zeuzem S.</td>
<td>2001</td>
<td>PEG IFN 40kDA (180 µg wk)</td>
<td>531</td>
<td>69</td>
<td>39</td>
</tr>
<tr>
<td>(185)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ribavirin**

Reichard et al (150) found a significantly higher SVR rate in patients treated with a combination of IFN and ribavirin as compared to patients treated with IFN mono therapy. They observed the same virological ETR in the two treatment groups but a significantly higher SVR. Thus they assumed that the better results in the combination therapy group were due to reduced relapse rate after virological ETR. Ribavirin is a guanosine analogue, the mechanism of action is poorly understood. Possible mechanism include depletion of the intracellular triphosphate pools through direct inhibition of inosine monophosphate dehydrogenase, inhibition of the 5'cap structure of viral mRNA and inhibition of the viral dependent RNA polymerases. Furthermore it has been suggested that ribavirin does not have a direct antiviral effect but rather acts as an immune modulator, preserving Th1 and reducing Th2 cytokine production (73). Two large trials with IFN ribavirin versus the IFN mono therapy were conducted (118;146). These two large studies are summarised in table 8.
Table 8 Sustained virological response rates (SVR) of 2 trials compared (IFN ribavirin combination) with IFN mono therapy for chronic hepatitis C patients. All genotypes included

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Treatment Groups</th>
<th>n</th>
<th>ETR %</th>
<th>SVR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>McHutchinson</td>
<td>1998</td>
<td>Group A IFN monotherapy 3 x 3 MU wk 48 wks</td>
<td>225</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B IFN 3x 3 MU wk in combination with ribavirin 1000-1200mg day 24 wks</td>
<td>228</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group C IFN 3x 3 MU wk in combination with ribavirin 1000-1200mg day 48 wks</td>
<td>228</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>Poynard</td>
<td>1998</td>
<td>Group A IFN monotherapy 3 x 3 MU wk 48 wks</td>
<td>278</td>
<td>33</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B IFN 3x 3 MU wk in combination with ribavirin 1000-1200mg day 24 wks</td>
<td>277</td>
<td>57</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group C IFN 3x 3 MU wk in combination with ribavirin 1000-1200mg day 48 wks</td>
<td>227</td>
<td>52</td>
<td>43</td>
</tr>
</tbody>
</table>

These results showed that there was a significantly higher rate of SVR in patients treated with the combination of IFN and ribavirin as compared to patients treated with IFN monotherapy. In 1999 in an international consensus meeting (EASL) it was decided that a combination of IFN and ribavirin was the first choice for treatment of naïve chronic hepatitis C patients. Recently the first results of combination treatment with PEG IFN (12 kDA) and ribavirin in treatment naïve HCV patients was published (112). The SVR results of this study are summarised in table 9.
Table 9. Comparison of the virological response at ETR and SVR of HCV patients treated with PEG IFN and ribavirin or IFN and ribavirin. All genotypes included.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Treatment groups</th>
<th>n</th>
<th>ETR</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manns (112)</td>
<td>2001</td>
<td>Group A IFN 3x3MU wk in combination with ribavirin 1000-1200mg day 48 wks</td>
<td>501</td>
<td>54</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B PEG IFN 1.5 μg kg wk in combination with ribavirin 800mg day 48 wks</td>
<td>511</td>
<td>65</td>
<td>54</td>
</tr>
</tbody>
</table>

With the combination therapy of IFN and ribavirin or PEG IFN and ribavirin 47-54% of the patients became HCV RNA negative as measured by PCR at the end of follow up.

Several pre-treatment factors were associated with a better response to combination therapy. These favourable factors were described by Poynard et al and listed in table 10 (146).

Table 10. Pre-treatment favourable factors for sustained virological response in patients treated with IFN in combination with ribavirin.

<table>
<thead>
<tr>
<th>Favourable factors for sustained virological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 2 or 3</td>
</tr>
<tr>
<td>Low viral load before start of treatment</td>
</tr>
<tr>
<td>Female gender</td>
</tr>
<tr>
<td>Age - 40 years at time of infection</td>
</tr>
<tr>
<td>No liver fibrosis</td>
</tr>
</tbody>
</table>

Viral dynamics and Induction treatment

The kinetics of the viral decline in patients responding to IFN show a biphasic decline (Fig 3) (90:128:140:188).

During IFN therapy for hepatitis C, the viral decline can be divided in three phases (see fig 3)

A. After administration of IFN a delay in HCV RNA decline of 7-10 hours occurs. This is probably due to the pharmacokinetics of IFN. The peak plasma level of IFN is reached 7-10 hours after injection. Therefore viral decline starts 7-10 hours after the first injection (90:128)
B. In this phase almost every patient treated with IFN shows a rapid dose dependent HCV RNA decline for during 24-48 hours. This phase is a known as the first rapid phase (90:128).

C. After the first rapid phase a slower second phase of HCV RNA decline is observed. This phase is not IFN dose dependent (128).

Fig 3 The periods of HCV RNA decline during successful standard treatment with IFN in patients treated with chronic HCV. Phase A: Delay in HCV RNA decline; Phase B: dose dependent rapid phase (first phase); Phase C: slower dose independent phase (second phase).

The biphasic HCV RNA decline can be explained by a conceptual model of HCV replication (140). This model involves free virions (V), which can infect susceptible cells (T), at a rate (K). These infected cells (I), will again produce new free virions at a rate (P), and die at a rate (δ). Virions are cleared from the serum at rate (C). This is described in fig 4.
Fig 4 Schematic overview of HCV kinetics and the effects of IFN possible according to Layden et al and Perelson et al (94;140). I=infected cell, T=target cell, k=infec tion rate of the target cell by the virion, 
p=production rate of new virions, δ=death rate of the infected cell, C=clearance rate of the virion from the serum.

The first rapid phase (B) is best explained by an effect of IFN in blocking viral production in infected cells. The second phase (C) is best explained by clearance of infected hepatocytes by IFN (95). Recently Bekkering et al(11)observed a raise in HCV RNA 32-120 hours after initiation of therapy. This so called third phase starts after the end of the first phase and before the beginning of the second phase. One possible explanation for this third phase is that the efficacy of IFN may change after the first phase due to down regulation of IFN receptors. Since the immune mediated second phase does not start before 2-3 days after the first doses of IFN, a lag-period of diminished viral suppression may be present (11).

Neumann et al showed that the rate of viral decline in the second phase is a good predictor of HCV RNA becoming undetectable after 3 months of anti viral-therapy (128). Furthermore a study by Zeuzem et al showed that a 3 log decline of HCV RNA in the first 4 weeks of IFN monotherapy was a strong predictor of SVR. The predictive value of HCV RNA decline in the first 4 weeks even exceeds the significance of HCV genotype and pre-treatment viral loads as predictors for successful IFN treatment (186). More early predictors of SVR during IFN were published (see table 11).
Table 11. Published predictors of SVR during treatment for chronic HCV

<table>
<thead>
<tr>
<th>Positive predictors of SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 3 log decline in HCV RNA in the first 4 weeks (186)</td>
</tr>
<tr>
<td>• High rate of HCV RNA decline in the second phase (128)</td>
</tr>
<tr>
<td>• Viral load decline in the first 24 hours after administration of IFN beta (58)</td>
</tr>
<tr>
<td>• HCV RNA negative at week 4 (19)</td>
</tr>
<tr>
<td>• HCV RNA negative by PCR three months after start IFN therapy (168)</td>
</tr>
<tr>
<td>• HCV RNA negative as measured by PCR two weeks after start of treatment (12)</td>
</tr>
</tbody>
</table>

A negative predictor for SVR was presence of HCV RNA at 12 weeks after start of IFN therapy. Patients with chronic hepatitis C treated for 24 or 48 weeks with IFN monotherapy, 93% and 98-100% respectively did not achieve a SVR when HCV RNA was present at week 12 of therapy (60;116;117;168).

Recently McHutchinson et al studied the predictive value of HCV RNA at different time points during combination therapy with IFN and ribavirin. During combination therapy, testing for HCV RNA as predictor of non virological response is most accurate at week 24 of therapy. A positive HCV RNA test at week 24 identified 99% of non virological responders (60). Taking these positive and negative predictive factors for SVR into consideration daily administration of IFN by one or more dosage (induction therapy) may provide potential benefits in terms of achieving a higher rate of SVR.

Several studies have been published investigating the SVR rate after treatment schedules with an IFN induction period (table 12)
<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith</td>
<td>Example 1</td>
<td>Value 1</td>
</tr>
<tr>
<td>John</td>
<td>Example 2</td>
<td>Value 2</td>
</tr>
<tr>
<td>Jane</td>
<td>Example 3</td>
<td>Value 3</td>
</tr>
</tbody>
</table>

Table 1: Results of controlled trials with H1 in induction schedules for treatment of chronic hepatitis C patients.
In conclusion some studies show benefit of IFN induction therapy and therewith increasing the SVR rates, however other studies fail to show the benefit of induction therapy. Thus the value of IFN induction therapy remains unclear.

Future therapies for chronic HCV infection

Inhibitors of viral replication

With the introduction of PEG IFN in combination with ribavirin the chance of successful therapy increased for patients with chronic HCV. However there are still patients who never achieved a SVR, when treated with the current combination therapy. In HIV infected patients treatment with protease inhibitors successfully suppressed HIV. Therefore specially developed protease inhibitors for HCV suppression may be an optional target for HCV clearance.

Potential targets are depicted in figure 5 include 1). The HCV viral protease which is a three dimensional compound similar in appearance to chymotrypsin (153). NS3 encodes a serine protease domain in its N-terminal one third and an NTPase/helicase domain in the C-terminal portion. The NS3 serine protease is responsible for cleavage of the polyprotein. NS4a acts as integral cofactor for the serine protease and is found as a stable heterodimer in vivo, and appears to function in part by stabilizing the protease molecule (44).

2). The HCV helicase is a nonsubstrate specific enzyme needed to unwind plus and minus strands of the RNA genome following polymerase replication. HCV helicase is also encoded at the NS3 region (153).

3). The RNA dependent RNA polymerase used to produce the negative strand template for replication is encoded in region NS5b (153). The three dimensional structure of NS5b and its function has been studied and it appeared that this target offers great potential for the development of new antiviral agents (44).

4) Currently synthetic nuclease resistant ribozymes are designed to cleave the HCV internal ribozyme entry site (IRES). The IRES is located in the 5’ nontranslated region and mediates cap-independent initiation of translation of the viral polyprotein mechanism that is unprecedented in eukaryotes (69). Cell culture studies using an HCV poliovirus chimera that contains the HCV IRES have identified ribozymes that target sequences within the IRES and inhibit viral replication (175).
Patients with chronic HCV who have no response to anti-viral therapy and who have a high risk for developing severe liver disease may benefit from therapies that halt fibrosis progression. In a small study 24 patients with chronic hepatitis C, who had not previously responded to IFN-based therapy, were enrolled in a randomised double-blinded 2-dose trial in which they received either 4 or 8 microgram/kg IL-10 subcutaneously daily for 90 days. In 14/24 patients fibrosis decreased (127). In another pilot study patients with chronic HCV and bridging fibrosis or cirrhosis were treated with IL-10 for 12 months and it appeared that only 4/27 treated patients had a decrease in hepatic fibrosis. When IL-10 treatment was prolonged for more then 3-4 months HCV RNA levels rise by as much as a 3 fold (44).

In a recent retrospective analysis Poynard et al (147) compared 4 different treatment regimens for chronic hepatitis C (103:112;118;146), for fibrosis progression. In total 3010 patients had paired biopsies. In table 13 the main results of this study are summarised.

Table 13. Results of 4 large randomised trials on liver fibrosis progression and inflammation activity progression between baseline and post treatment biopsies

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>3010</td>
</tr>
<tr>
<td>Sustained responders</td>
<td>1094</td>
</tr>
<tr>
<td>Relapsers</td>
<td>464</td>
</tr>
<tr>
<td>Non responders</td>
<td>1452</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrosis progression</th>
<th>Improved</th>
<th>Stabilized</th>
<th>Worsened</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>All patients</td>
<td>590 (20)</td>
<td>1955 (65)</td>
<td>465 (15)</td>
</tr>
<tr>
<td>Sustained responders</td>
<td>277 (25)</td>
<td>740 (68)</td>
<td>77 (7)</td>
</tr>
<tr>
<td>Relapsers</td>
<td>74 (16)</td>
<td>311 (67)</td>
<td>79 (17)</td>
</tr>
<tr>
<td>Non responders</td>
<td>239 (17)</td>
<td>904 (62)</td>
<td>309 (21)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inflammation activity progression</th>
<th>Improved</th>
<th>Stabilized</th>
<th>Worsened</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>All patients</td>
<td>1660 (55)</td>
<td>924 (31)</td>
<td>426 (14)</td>
</tr>
<tr>
<td>Sustained responders</td>
<td>944 (86)</td>
<td>131 (12)</td>
<td>19 (2)</td>
</tr>
<tr>
<td>Relapsers</td>
<td>200 (43)</td>
<td>168 (36)</td>
<td>96 (21)</td>
</tr>
<tr>
<td>Non responders</td>
<td>516 (36)</td>
<td>625 (43)</td>
<td>311 (21)</td>
</tr>
</tbody>
</table>
In total 153 patients had cirrhosis and in 75 (49%) a reversal of cirrhosis (change in fibrosis score based on the biopsy sample) was observed. Among these 75 patients in 23, 26, 23 and 3 patients, the second biopsy was graded F3, F2, F1 and no fibrosis respectively. The authors of this study concluded that combination of PEG IFN and ribavirin had the potential to reduce the morbidity and mortality of chronic hepatitis C patients, by reducing fibrosis progression and development of cirrhosis. Furthermore patients without extensive fibrosis at baseline, patients younger than 40 years of age and with a body mass index lower than 27 had a much lower progression to liver fibrosis which was independent of presence or absence of a SVR (147). So there may be a possible role for IFN alpha as anti fibrotic medicine. Several studies have been started to investigate the efficacy of long term Pegylated IFN (>3 years), on fibrosis progression and cirrhosis.
Discovery of Hepatitis B virus (HBV)

In 1965 Blumberg et al discovered an antigen in an Australian aborigine which was later associated with Hepatitis B virus (HBV) (34:148). It is estimated that there are worldwide now 350 million chronic HBV carriers, defined as individuals positive for Hepatitis B surface antigen (HBsAg) for more than 6 months. Carriers of HBV are at increased risk of developing liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (9).

Natural History

The risk of developing chronic HBV carrier state after exposure to HBV ranges from 90% in new born infants of HBeAg positive mothers to <10% in adults(75:163:179) (10).

The life cycle of an HBV infection can be divided in four stages(97).

First stage: Immune tolerant stage: In healthy adults this is the incubation period and last for 2-4 weeks. HBsAg, HBeAg and HBV DNA are present. There is no elevation of alanine aminotransferase (ALAT) and no symptoms.

Second phase: Immune response stage: In acute hepatitis B this stage is marked by the presence of jaundice, malaise and elevated ALAT and last for 2-4 weeks. HBsAg, HBeAg are present and HBV DNA levels are declining. When the host is able to provoke a response that eliminates infected cells, active viral replication ends and the third phase begins.

Third phase: Clearance stage: The number of infected hepatocytes are declining. HBeAg is no longer detectable and antibodies against HBeAg (anti-HBe) are developed. The levels of HBV DNA are declining and the ALAT levels become normal.

Fourth phase: Immune stage: In this stage most patients become HBsAg negative and anti-HBs becomes detectable. HBV DNA is no longer present.

In patients who become chronic carriers of HBV, the following possibilities are known:

- Group A. HBV (HBsAg) positive without signs of chronic hepatitis (healthy carriers).
• Group B. HBV (HBsAg) positive with signs of chronic hepatitis, divided in 2 subgroups:

1. HBeAg positive with chronic hepatitis: HBsAg, HBeAg and HBV DNA positive with or without elevated ALAT values.

2. HBeAg negative with chronic hepatitis: HBsAg, anti HBe and HBV DNA positive. HBeAg is negative and with or without elevated ALAT values.

The rate of progression to cirrhosis ranges between 10-40% over a period of 10 years or more. In patients with cirrhosis, due to chronic hepatitis B, the probability that they will develop liver decompensation (ascites, jaundice, encephalopathy, bleeding due to portal hypertension) ranges from 4-10% (36:51:129). In the western world the annual rate of development of hepatocellular carcinoma (HCC) among patients with chronic hepatitis B and cirrhosis ranges from 0.2-9% (36:51:129).
Epidemiology of HBV

High (> 9%) prevalence rate areas (of HBV carriers) are Africa and Asia. medium prevalence rate areas (1.1 - <9%) are southern and eastern Europe and low prevalence rate areas (0 - <1.1%) are North America and Western Europe (109).

Transmission

In countries with a high rate of HBV carrier state (south east Asia, China and Africa) more than half of the population has been infected with HBV at some time in their lives, as a result of either perinatal (vertical) via HBsAg and HBeAg positive mothers or from one person to person (horizontal) transmission. In countries with a low rate of HBV carriers, horizontal transmission is the main route of HBV transmission. In developed countries most horizontal HBV infections result from sexual contact, injection of drugs and occupational exposure. Other less frequent causes of infection include household contacts, renal dialysis and receipt of organs or blood products. No clear risk factors are found in approximately 20-30 percent of individuals positive for HBsAg.

HBV genotypes

Seven HBV genotypes have been classified. Epidemiological studies demonstrated that the prevalence of these genotypes varies in different parts of the world (table 14).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NW Europe, N America, Central Africa</td>
</tr>
<tr>
<td>B</td>
<td>SE Asia, China, Japan</td>
</tr>
<tr>
<td>C</td>
<td>SE Asia, China, Japan</td>
</tr>
<tr>
<td>D</td>
<td>S Europe, Middle East, India</td>
</tr>
<tr>
<td>E</td>
<td>Africa</td>
</tr>
<tr>
<td>F</td>
<td>American natives, Polynesia, Central and South America</td>
</tr>
<tr>
<td>G</td>
<td>United States, France</td>
</tr>
</tbody>
</table>
There is evidence that HBV genotype (C and B) may influence the tendency to seroconversion from HBeAg to anti HBe and the severity of liver disease, however further studies are needed to confirm these observations (30).

**Structure of HBV**

Hepatitis B virus is a small enveloped DNA virus which replicates by reverse transcription. It belongs to the group of hepadna viridae, other members of this group are the woodchuck hepatitis virus (WHV), beechy ground squirrel hepatitis virus (GSHV) and the peking duck hepatitis B virus (DHBV) (167). All hepadna viruses have the same life cycle in their host. The viral genome of HBV is a partial double stranded circular DNA of approximately 3200 base pairs that encodes four overlapping reading frames (ORF). The four open reading frames are: S, core, pol and X. The first ORF is the S ORF which encodes for the surface antigen (HBsAg) and is composed of the pre-S₁, pre-S₂ and S region. Translation from the pre-S₁, pre-S₂ and S region yield a 38-kDa L-protein, 31-kDa M-protein, and a 24 kDa S-protein. The second ORF is the core ORF which encodes for the core antigen (HBeAg) and hepatitis B e antigen (HBeAg) and is composed of the pre-core and core region. The pre-core region encodes for a hydrophobic leader peptide that directs the translation product to the endoplasmatic reticulum to form HBeAg which is secreted in the blood and it’s presence is associated with active viral replication. HBcAg and HBeAg are important targets for immune response against HBV. The third ORF is the pol ORF which encodes the DNA polymerase. DNA polymerase has a function in reverse transcription. The fourth ORF is the X ORF which encodes for HBX, the function of this protein is not fully understood. HBX may play an important role in the development of hepatocellular carcinoma. The proteins from the S ORF (HBsAg), core ORF (HBcAg) and pol ORF (DNA polymerase) are essential structures for viral replication and assembly. How the proteins from the core ORF (HBeAg, HBcAg) and X ORF (HBX) are interacting with the host is not well understood (157).

**Viral replication (fig 6)**

The pre S domains of the surface protein is involved in the attachment of the mature HBV to the membrane of the hepatocytes (39). Entry of the virus is a process of fusion of the viral and
host membranes when the nucleocapsid is released in the cytoplasm. This endosomal uptake pathway is probably mediated through a fusion like peptide on the pre-S2 domain (108). Delivery of the partially double stranded viral DNA into the cell nucleus is still poorly understood. After translocation in the cell nucleus, nucleotides are added to the partly double stranded viral DNA so that a completely double stranded circular DNA is formed. The circular DNA forms a supercoiled covalently closed circular DNA (cccDNA). cccDNA is resistant to degradation and remains intact throughout the life cycle of the hepatocytes. The cccDNA in the nucleus serves as template for transcription.

Cellular RNA polymerase II produces a genomic and three subgenomic transcripts. The 3.5 kb genomic transcripts consist of two species with different 5' ends: the pregenomic and precore RNA's. The pregenomic RNA serves as a template for reverse transcription of the mRNA for the core and polymerase proteins. The polymerase protein reverse-transcribes the pregenomic RNA into a viral negative DNA strand. The negative strand can than serve as a template for the formation of the positive viral strand. After reverse transcription, the nucleocapsid interacts with the S protein to initiate assembly at the endoplasmatic reticulum. However the newly formed nucleocapsids may re-enter the cell nucleus and therewith increasing the pool of cccDNA. Integration of HBV DNA into the host chromosome is not an obligatory step of the HBV life cycle, but in long term HBV carriers it may occur (125). Subsequently the virions and the viral envelope particles are excreted from the hepatocytes.

Fig 6. Replication of HBV
HBeAg positive chronic hepatitis B

The group of HBeAg HBV carriers (group B1) will be shortly described, since this thesis will focus on HBeAg negative hepatitis B (group B2).

In most patients with HBeAg positive chronic hepatitis B the HBV DNA load is high (HBV DNA > $10^5$ copies mL) and the transaminase levels are elevated. The presence of HBeAg positivity is a negative predictive factor for the development of cirrhosis (100). In patients with cirrhosis the one risk factor for accelerated liver decompensation is the presence of HBeAg in serum (51). Patients with compensated cirrhosis with detectable levels of HBeAg have a 5 year survival rate of 76% (36). Seroconversion from HBeAg to anti-HBe resulted in a decrease of viral replication and subsequently influences the natural history of chronic HBV infection. It's estimated that HBeAg positive hepatitis B patients seroconvert spontaneously from HBeAg to anti-HBe in 5-15%. After seroconversion patients usually have normal ALAT values and the chronic liver disease is less progressive (35).
Diagnostic criteria for HBeAg negative chronic hepatitis B

The following criteria are applicable to HBeAg negative chronic hepatitis B (135):

1. Presence of HBsAg for at least 6 months
2. Absence of HBeAg for at least 6 or 12 months
3. Increased ALAT (≥ 1.5 x upper limit of normal determined twice in at least one month)
4. Hepatocellular damage (Histology activity index >4)
5. HBV DNA levels >10^5 copies/mL

The difference between a chronic HBeAg negative chronic hepatitis B patient and "healthy" HBV carrier is that the last group has:

1. Persistently normal ALAT levels
2. Levels of HBV DNA < 10^5 copies/mL

Thus the HBV DNA level and ALAT level discriminates between a HBsAg positive HBeAg negative "healthy carrier" and the HBeAg negative chronic hepatitis B patient. However the detection and quantitation of HBV DNA is dependable on the sensitivity and standardization of the method used (25; 139) It has o be noted that the proposed level of 10^5 copies/mL HBV DNA has as yet not been adequately validated in large to discriminate between these groups.

Natural History of HBeAg negative chronic hepatitis B

In most studies the age of patients with HBeAg negative chronic hepatitis B is between 40-50 years. Males predominate 5-17 fold over females (67).

Interfamilial acquisition of HBV infection is the most common mode of transmission in this group (67:99).

In patients with HBeAg negative chronic hepatitis B the mean HBV DNA levels are significantly lower as compared to HBeAg positive chronic hepatitis B patients (76).

Furthermore the ALAT levels of HBeAg negative chronic hepatitis B patients may have a fluctuating course, or are persistently elevated (99:184).

Two main patterns of disease activity are observed in HBeAg negative chronic hepatitis:

1. Persistent (3-4 fold) increased ALAT levels, without a tendency for spontaneous normalisation (30-40% of cases)
2. ALAT fluctuation with frequent flares of disease activity (45-65% of cases) (21.99) Sometimes these flares are severe resembling acute hepatitis B with ALAT levels over >1000 U/L and development of IgM anti-HBe levels (65).

In HBeAg negative chronic hepatitis patients, with histological proven chronic hepatitis, Liaw et al reported an annual incidence of cirrhosis of 1.3% (100). Other studies in Italy indicated that 33% of the patients develop cirrhosis within a period of 6 years (17). In the group of “healthy” HBV carriers the mortality over a period of 15 years was only 0.5% (177).

**Molecular basis of HBeAg negative chronic hepatitis B**

In HBeAg negative chronic hepatitis B the disease condition is caused by strains of HBV that are not producing HBeAg (114). HBeAg negative hepatitis B is a late phase of the natural course of chronic hepatitis B that develops after HBeAg/anti-HBe seroconversion. In 1989 the molecular basis of this form of chronic hepatitis B was discovered (27). HBeAg negative chronic hepatitis B is associated with the selection of replication competent pre-core HBV mutants. The most common pre-core mutation is a replacement of guanine (G) to adenine (A) at the level of nucleotide 1896 (G1896A). This mutation creates a novel translational stop codon (pre-core codon 28) leading to premature termination of the translation of the pre-core protein (27). However in HBeAg negative chronic hepatitis B with genotype A, pre-core mutants are a rare occurrence. In HBV genotype A patients cytosine (C) is present at position 1858 (C1858) preventing the selection of the G1896A mutation (99). The prevalence of genotype A is high in Northern Europe, North America and some parts of Africa, therefore the prevalence of pre-core mutants is rare in these parts of the world (59). In Mediterranean countries the pre-core mutation is present in 85% (59) of HBeAg negative chronic hepatitis patients. The geographical variation in prevalence of the pre-core variants is related to the fact that the occurrence of the G1896A mutation is restricted to HBV genotypes with thymidine at position 1858 (T1858) (99; 102). HBV genotype D harbours T1858 and therefore the prevalence of HBeAg negative chronic hepatitis B is high in areas with a high prevalence rate of genotype D. However in areas were non-D genotypes are present, HBeAg negative chronic hepatitis B also exist in Asia 50 - 77% of these patients have a mutation in the basic core promoter (BCP) (59). This double core promoter mutation is found on A1762T and G1764A.
There is evidence that the core promoter mutations are most likely to be selected in HBV genotypes with cytosine at position 1858 (28;99).

Pre-core and core mutants are already developed in the early phase of the Hepatitis B infection, however only during the seroconversion phase from HBeAg to anti-HBe they predominate when immune tolerance (third phase) is lost (20;99). This type of chronic hepatitis will develop in 25-33% of patients who have lost their HBeAg and convert to anti-HBe (99;134).

**Detection of HBV DNA**

As discussed before HBV DNA detection is important in the differentiation between HBsAg “healthy” carriers and HBeAg negative chronic hepatitis B.

HBV DNA tests are widely used, but they are limited by a lack of standardization and variability in sensitivity. Recently WHO and related standards are available to improve the standardisation of HBV DNA tests. In table 15 currently used HBV DNA assays are summarized.

Table 15. Assays for detection of HBV DNA

<table>
<thead>
<tr>
<th>Assay (manufacturer)</th>
<th>Sensitivity a</th>
<th>Dynamic range cop/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/ml.</td>
<td>cop/ml.</td>
</tr>
<tr>
<td>Branched DNA (Bayer)</td>
<td>7 x 10^7</td>
<td>7 x 10^7 - 5 x 10^7</td>
</tr>
<tr>
<td>Hybrid Capture (Digene)</td>
<td>5 x 10^7</td>
<td>5 x 10^7 - 3 x 10^7</td>
</tr>
<tr>
<td>Liquid hybridisation (Abbott)</td>
<td>4.5 x 10^2</td>
<td>4 x 10^2 - 1 x 10^2</td>
</tr>
<tr>
<td>PCR (Roche)</td>
<td>4 x 10^2</td>
<td>1 x 10^2</td>
</tr>
<tr>
<td>Molecular Beacons (Organon)</td>
<td>50</td>
<td>50 - 1 x 10^2</td>
</tr>
</tbody>
</table>

Adapted from Lok (107)

*a* 1 pg HBV DNA = 283,000 copies (3 x 10^5 cop ml/L) (107)
Treatment of chronic hepatitis B

The optimal goal is to eliminate the HBV virus completely and permanently. There are two different goals for the treatment of chronic hepatitis B. The first is to achieve sustained clearance of the HBV and seroconversion from HBsAg to anti-HBs. When HBsAg is not eradicated, the second goal is to reduce liver injury to prevent development of cirrhosis, to reduce infectivity and to improve quality of life (143). There are now two drugs registered for the treatment of chronic hepatitis B. In 1992 IFN was the first drug approved in the United States and Europe for HBeAg positive chronic hepatitis B. In 1999 lamivudine was registered for the treatment of chronic HBeAg positive and HBeAg negative hepatitis B.

Treatment of HBeAg positive chronic hepatitis B with IFN

The mode of action of IFN is described in table 7. The treatment of HBeAg positive hepatitis B will be shortly described, since this thesis will focus on HBeAg negative hepatitis B.

A meta-analysis published in 1993 reviewed 15 randomised controlled studies involving 837 patients who received IFN in doses of 5–10 million units (MU) given daily or thrice weekly (t.i.w.) for 4–6 months (178). The results are summarised in table 16.

Table 16. Results of the meta-analysis by Wong et al.

<table>
<thead>
<tr>
<th>Results</th>
<th>Treated patients (n=498) (%)</th>
<th>Control group (n=339) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of HBeAg</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>Loss of HBsAg</td>
<td>7.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Loss of HBV DNA*</td>
<td>37</td>
<td>17</td>
</tr>
<tr>
<td>Normalization of ALAT</td>
<td>57</td>
<td>27</td>
</tr>
</tbody>
</table>

* HBV DNA measured by hybridisation assay (cut off < 10^5 copies/mL)

Treatment of HBeAg negative chronic hepatitis B with IFN

IFN treatment for HBeAg negative chronic hepatitis B is associated with a virological (bDNA < 10^5 copies/mL) ETR of 38–72% and a biochemical (normalization of ALAT) ETR of 38–72% (22:50:64:78:91:111). However the majority of the patients with an ETR will relapse after
cessation of IFN therapy. At the end of follow up (6–12 months after cessation of therapy) the virological response rate as measured by hybridisation assays (lower limit of detection < 10^4 cop/mL) was only 10-15% and the biochemical response rate varied between 18-65% (22:50:64:78:91:111).

In HBeAg negative chronic hepatitis B patients, a sustained biochemical response is associated with improved long term outcome (reduction of liver decompensation and or hepatocellular carcinoma) (136).

**Lamivudine**

Lamivudine is the orally administered (-) enantiomer of the racemic mixture 2',3'-dideoxy-3'-thiacytidine (SddC, 3'TC) which have a potent activity against HIV infection by suppressing reverse transcriptase activity (61). It was found that lamivudine had also antiviral activity against HBV by suppressing the replication of this virus. However it has also some indirect immunomodulatory activities.

There are 4 possible modes of action of lamivudine on the HBV replication cycle.

1. Inhibition of reverse transcription of the pregenomic mRNA into nascent minus-strand DNA.
2. Inhibition of formation of plus-strand DNA from the nascent minus-strand DNA (polymerase dependent).
3. Inhibition of completion of double-stranded DNA (reducing formation of cccDNA)
4. Inhibition of the formation of cccDNA during initial entry of the virus into the hepatocytes nucleus (183)

The major drawback of the use of lamivudine as a single agent is the emergence of resistance due to the development of mutant HBV strains. The major site of mutation is the methionine residue in the tyrosine-methionine-aspartate-aspartate (YMDD) aminoacid motif. lamivudine has little or no inhibitory effect on the replication of the lamivudine-resistant HBV mutant (183)
Treatment of HBeAg positive chronic hepatitis B with lamivudine

The response to lamivudine of HBeAg positive hepatitis B is summarized in table 17.

Table 17 Antiviral response to lamivudine therapy in patients with HBeAg positive chronic hepatitis B

<table>
<thead>
<tr>
<th>Loss of HBV DNA*</th>
<th>Lamivudine 100mg/day (52 wks) (n=71)</th>
<th>Placebo (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of HBeAg</td>
<td>44%</td>
<td>16%</td>
</tr>
<tr>
<td>HBeAg seroconversion</td>
<td>32%</td>
<td>11%</td>
</tr>
<tr>
<td>Loss of HBsAg</td>
<td>17%</td>
<td>6%</td>
</tr>
<tr>
<td>Normalization of ALAT</td>
<td>2%</td>
<td>0</td>
</tr>
<tr>
<td>Loss of HBsAg</td>
<td>41%</td>
<td>7%</td>
</tr>
</tbody>
</table>

* measured by hybridisation assay (cut off 10^5 cop/mL)

Adapted from (45).

The sustained virological response (HBV DNA < 10^5 cop/mL) is maintained only in patients who lost HBeAg (81%) or in patients who had a HBeAg seroconversion (73%). In patients who do not lose HBeAg during lamivudine treatment, stopping therapy after 3 to 12 months is usually followed up by a return of HBV DNA to pretreatment values (45;89).

Treatment of HBeAg negative chronic hepatitis B with Lamivudine

In table 18 studies are summarized which applied lamivudine treatment for HBeAg negative chronic hepatitis B.

Table 18 Antiviral response and biochemical to lamivudine therapy in patients with HBeAg negative chronic hepatitis B
As shown in table 18 the virological ETR as determined by the HBV DNA assay used is between 41-90%. Little is known about the end of follow up response in these lamivudine treated patients. In HBeAg positive hepatitis B patients the main goal of anti-viral therapy is HBeAg anti HBe conversion, resulting in suppression of HBV DNA (<10⁵ cop/mL) in 70-80% of patients associated with long term improvement. However in HBeAg negative hepatitis B patients the end points for cessation of anti-viral therapy are not well defined. Since the virological ETR is between 41-90% continuing treatment with lamivudine (lifelong) has been proposed, however in the study by Hadziyannis et al it was shown that when treatment was continued up till 24 months, the virological response rate assessed by PCR, decreased over time 68% at 6 and 12 months, 52% at 18 months and 41% at 24 months.

(66).

**Future therapies for chronic hepatitis B**

**Famciclovir**

The oral prodrug of penciclovir, is a nucleoside analogue that has been licensed for the treatment of varicella zoster infections. Penciclovir triphosphatase inhibits viral replication through competitive inhibition of viral DNA polymerase. In a large controlled study HBeAg positive chronic hepatitis B patients were treated for 12 months with either famciclovir (3 x 500mg/day or 1500 mg daily) or placebo. In the famciclovir group, HBV DNA levels were significantly reduced within the first 8 weeks of therapy as compared to the placebo group. However at the end of treatment and at end of 6 months follow up no significant difference in virological and biochemical response between famciclovir and placebo groups was observed. Only 9% of the patients seroconverted from HBeAg to anti-HBe during famciclovir treatment.

(37).
Furthermore viral resistance due to mutations in YMDD motif and the B domain of the polymerase gene is a frequent problem with famciclovir treatment (158). From a small pilot study, in which Chinese HBeAg positive chronic hepatitis B patients were treated with lamivudine and famciclovir it was suggested that this combination therapy was superior in suppressing HBV DNA than either lamivudine or famciclovir as monotherapy. Famciclovir suppresses the synthesis of HBV cccDNA, which is normally resistant to lamivudine therapy (92).

**Adefovir**

Adefovir is an acyclic analogue of dAMP which has broad spectrum antiviral activity. It is a potent inhibitor of HBV DNA replication. Treatment with adefovir results in a rapid decrease in serum HBV DNA, within 1-2 weeks after start of the therapy. Furthermore a study by Perillo et al showed that adefovir is still in suppression of HBV DNA in patients who developed resistance to lamivudine. Resistance to adefovir has not been observed after more than 52 weeks of continuous treatment in immunosuppressed liver transplant recipients (141).

**Entacavir**

Entacavir is a carbocyclic deoxyguanosine analogue with potent activity against the group of herpes and hepadna viruses. In patients with HBeAg positive chronic hepatitis B treated for 28 days with entecavir a mean log decline of 2.45 HBV DNA was observed in these patients. No significant side effects were observed in this study (38).

**Combination therapy of IFN and Lamivudine**

In a pilot study IFN in combination with lamivudine was given to HBeAg negative chronic hepatitis B patients. At the end of treatment (52 wks) 93% of the patients had a biochemical and a virological response as measured by a hybridisation assay (cut off 9 x 10^5 cop/mL). However only 14% of the patients achieved a sustained virological response one year after cessation of therapy. It was remarkable that during this combination treatment no YMDD mutants developed. (165).

Many new immunomodulatory therapies and other antiviral agents are currently under investigation. **Emtricitabine**, **DAPD** and **clevudine** appear to be at least as potent as lamivudine in suppressing HBV DNA replication. In vitro studies showed that YMDD
mutations confer cross-resistance between lamivudine and emtricitabine. However, adefovir, dipivoxil, lobucavir, DAPD and possibly clevudine suppress HBV DNA in patients with YMDD mutants as well as wild types of HBV. Immunomodulatory approaches for treatment of chronic hepatitis B are conceptually attractive, but some agents used to date (thymosin-alpha, interleukin-12, therapeutic vaccines) have not demonstrated sufficient efficacy as yet. Combinations of an immunomodulatory agent and nucleoside analogue may improve the therapeutic efficacy and may reduce the emergence of drug resistance, like HAART therapy in HIV infected patients.
Discovery of HGV/GBV-C

GB agent was originally described by Deinhardt and co workers, who in occluded tamarins (Sanguinus sp.) with the serum of a 34 year old patient with hepatitis (a surgeon with the initials GB) (41). Animals that were inoculated with GB serum developed hepatitis (hepatitis defined as elevated liver enzymes and histological signs of inflammation in the liver). Although GB had the characteristics of a virus. It was questioned of GB agents was a human virus (137;138). Simons et al characterized two new flavivirus in a tamarin infected with the GB agent, which were termed GBV-A and GBV-B (161). Subsequently it was found that both viruses were not present in humans and that GBV-B caused hepatitis only in tamarins and GBV-A did not cause hepatitis (155). Next a third member of the GB virus family was isolated in serum from patients with cryptogenic non A-E hepatitis (160). Phylogenetic analysis demonstrated that this third member was an additional member of the Flaviviridae and therefore was named GBV-C (96). In addition Linnen et al independently identified a similar RNA virus from plasma of a patient with chronic hepatitis (104). This virus was designated Hepatitis G virus (HGV). Subsequently sequence comparison revealed that GBV-C and HGV virus were isolates of the same virus genus (123).

In the further part of this thesis this virus will be referred to as HGV/GBV-C.

Natural history

HGV/GBV-C was first identified in individuals with non-A, non- B and non-C hepatitis. Several studies demonstrated a relation between HGV/GBV-C infection and liver damage (31:32:55). Yoshiaba et al suggested that HGV/GBV-C was an important aetiological agent of fulminant hepatitis (182). However in more recent studies it was found that HGV/GBV-C did not cause acute or chronic hepatitis (4:6). Infection with HGV/GBV-C can persist for decades with no apparent morbidity or mortality (3). It was found that HGV GBV-C infection could be transient with clearance of the virus and development of antibodies. However the virus could persist for months or years (124). It was now established that HGV/GBV-C is not a hepatotropic but a lymphotropic virus (57:142:174).
Molecular structure and diagnostic tests

HGV GBV-C is an enveloped RNA virus with a single positive sense RNA genome. The viral genome consists of 9100-9400 nucleotides (96). HGV GBV-C belongs to the family of *Flaviviridae*. The genomic organization of HGV GBV-C is for 29% homologous to HCV (96). The E2 region of the virus encodes for a glycoprotein which was located on the outer surface of the virion(156). This HGV GBV-C glycoprotein was expressed in eucaryotic cells and has been used as antigen in EIAAs to detected specific antibodies (46). These E2 antibodies are referred further as E2Ab. HGV GBV-C can be detected by PCR using primers from the NS3 region. Several groups developed primers on the 5'UTR, which are more conserved, which results in offered a higher sensitivity of the PCR technique used (77;79). A recent phylogenetic analysis of 33 complete HGV GBV-C genome sequences demonstrated 4 different genotypes of HGV/GBV-C (89).

Epidemiology

HGV/GBV-C is spread worldwide and infections with this virus were found among healthy individuals as well as in various patients groups. In table 19 the prevalence of HGV/GBV-C in serum as measured by PCR and E2Ab is noted.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>USA PCR %</th>
<th>USA E2Ab %</th>
<th>Europe PCR %</th>
<th>Europe E2Ab %</th>
<th>Africa PCR %</th>
<th>Africa E2Ab %</th>
<th>Asia PCR %</th>
<th>Asia E2Ab %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td>1.2</td>
<td>3.8</td>
<td>1.4</td>
<td>3.15</td>
<td>12.33</td>
<td>20.0</td>
<td>1.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Children</td>
<td>6.0</td>
<td>1.9</td>
<td>3.0</td>
<td>1.0</td>
<td>8.15</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous drug abusers</td>
<td>6.0</td>
<td>1.9</td>
<td>3.0</td>
<td>1.0</td>
<td>8.15</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual man</td>
<td>10.31</td>
<td>46.0</td>
<td>1.0</td>
<td>24.49</td>
<td>46.0</td>
<td>43.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>41.0</td>
<td>21.0</td>
<td></td>
<td></td>
<td></td>
<td>43.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polytransfused</td>
<td>18.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-A-non-C</td>
<td>12.39</td>
<td>16.22</td>
<td>22.0</td>
<td>14.50</td>
<td></td>
<td>43.75</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Fulminant hepatic failure</td>
<td>22.43</td>
<td>16.22</td>
<td>22.0</td>
<td>14.50</td>
<td></td>
<td>43.75</td>
<td>13.0</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from (151)*
Transmission

Several transmission routes have been postulated for HGV GBV-C infection. Alter et al showed that HGV GBV-C was transmitted via blood products (4). However in these studies the rate of infectivity of HGV GBV-C RNA positive blood products in recipients could not be shown. Also it was not well documented if E2Ab protects against an HGV GBV-C infection. However in the study by Alter et al the rate in which HGV GBV-C and the influence of E2Ab on HGV GBV-C transmission was not studied. Furthermore HGV GBV-C may be transmitted vertically perinatally from mother to child (101). It has also been suggested that HGV GBV-C can be transmitted by sexual contacts(154).

Co-infection with HIV

Recently 4 studies a significant longer survival of HIV infected patients who were infected with HGV GBV-C (70:98:170:181). It was postulated that there was a favourable interaction between HGV GBV-C and HIV infection. Xiang et al described 362 HIV infected patients of which 144 were HGV GBV-C positive. During a follow up of 4.1 years 41/144 (28.5%) of the HGV GBV-C positive and 123/218 (56.4%) of HGV GBV-C negative HIV patients died. Also HIV patients on HAART who were co-infected with HGV GBV-C had a significantly better survival (71%) than patients without this co-infection (180). A study by Tillmann et al showed that there was a slower progression to AIDS in patient which were co-infected with HGV GBV-C. This study also showed that the survival period, from the date of their first positive anti-HIV test, was significantly longer for patients who were E2Ab positive and HGV GBV-C RNA negative, as compared to patients who never were exposed to HGV GBV-C.

In conclusion HGV GBV-C infection is not known to cause a clinical disease, but co-infection with HIV is associated with a decrease in the mortality rate in this patient group(166).

Iatrogenic infection HGV GBV-C in HIV infected patients may offer an alternative therapy to improve survival and reduce morbidity, in this patient group.
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