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HEPATITIS C VIRUS KINETICS DURING ANTIVIRAL TREATMENT

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To my relief...

ABSTRACT

It is estimated that 170 million individuals have chronic hepatitis C virus (HCV) infection, whereof 20-30% will develop end stage liver disease within 20-30 years. Antiviral therapy can stop this deterioration and clear the infection in 40-80% of the patients depending on treatment and HCV genotype. Suboptimal therapy, however, is likely to promote selection of resistance and emergence of escape mutants and an unfavourable treatment outcome. We studied the early HCV RNA kinetics during different antiviral treatment schemes and correlated the early viral decay to the eventual virological outcome with regard to the different genotypes.

In **paper I** the addition of amantadine to IFN and ribavirin during 24 weeks treatment of 10 former responder/relapsers and 13 non-responders to standard IFN and ribavirin combination therapy was found to have only limited beneficial effect. At end of treatment, only one previous non-responder and 5 previous response/relapsers were HCV RNA negative but only one former response/relapser had sustained response.

In **paper II** a more pronounced viral decline was seen with induction therapy, when standard IFN was given as mono-therapy daily initially during treatment, as compared to when it was given as standard dosing three times a week (t.i.w.). This difference persisted during the initial 12 weeks for patients infected with genotype 1, but was not maintained from day 14 and onwards for patients infected with genotype non-1. Eighty percent of patients in the induction group versus 16% in the standard t.i.w. group achieved undetectable HCV RNA levels (<600 IU/mL) at week 12 ($p < 0.05$).

In **paper IV** IFN induction mono-therapy resulted in a more pronounced HCV RNA decline at weeks 4, 8, and 12 compared to standard treatment. Most sustained responders were found to have a $>3 \log_{10}$ drop in serum HCV RNA levels week 4. Addition of ribavirin after 12 weeks IFN mono-therapy in two non-responders reverted them to sustained responders.

In **paper III** IFN induction therapy, in combination with ribavirin during treatment of naive patients, also resulted in a small but significantly more pronounced HCV RNA decline compared to standard dosing, in genotype non-1 infected patients but only day 2 and 7 during treatment, in contrast to what was found when IFN mono-therapy was given when this difference was more pronounced. The mean HCV RNA decline from baseline already day 1 was significantly greater in patients who became sustained virological responders as compared to non-responders. Hence, all sustained responders had a viral load decline of minimum 0.7 log (79%) after the first dose of standard IFN, whereas a lack of such response predicted a non-response.

In **paper V** pegylated-IFN alpha-2a (peg-IFN) was used in combination with ribavirin in naive patients. No difference in the decline of HCV RNA levels was noted between responders and non-responders after the first treatment day. At week 1 and week 4, however, sustained responders had a significantly more pronounced drop in HCV RNA levels as compared to response/relapsers and non-responders. Hence, the HCV RNA decline day 1 could not be used for prediction of response to peg-IFN treatment. At week 1, however, a positive predictive value of 92% was noted for a final virological sustained response in the subgroup of patients who had achieved a 2 \log_{10} drop in HCV RNA levels. The best time point for prediction of a non-response was week 12 when a negative predictive value of 92% was noted in patients who did not meet this criterion.

Keywords: Hepatitis C virus RNA, genotype, kinetics, interferon-alpha, ribavirin.
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LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I **Carlsson T**, Lindahl K, Schvarcz R, Wejstal R, Uhnöo I, Shev S, Reichard O. HCV RNA levels during therapy with amantadine in addition to interferon and ribavirin in chronic hepatitis C patients with previous nonresponse or response/relapse to interferon and ribavirin. *Journal of Viral Hepatitis*. 2000; 7: 409-413.
- II **Carlsson T**, Reichard O, Weiland O. Hepatitis C virus kinetics during induction and standard 3 times a week interferon-alpha therapy. *Scand J Infect Dis*. 2001; 33: 838-842.
- III **Carlsson T**, Weiland O, Reichard O. Viral kinetics and treatment response in patients with hepatitis C during induction and standard interferon therapy in combination with ribavirin. *Scand J Gastroenterol*. 2002; 37: 1228-1234.
- IV Balciuniene L, **Carlsson T**, Ambrozaitis A, Reichard O, Weiland O. Hepatitis C virus RNA levels during initial induction or standard interferon therapy: influence of continued treatment on sustained response. *Scand J Infect Dis*. 2003; 35: 180-185.
- V **Carlsson T**, Reichard O, Bläckberg J, Norkrans G, Sangfelt P, Wallmark E, Weiland O. Hepatitis C virus RNA kinetics during the initial 12 weeks treatment with pegylated interferon-alpha 2a and ribavirin according to virological response. (In manuscript).

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LIST OF ABBREVIATIONS

aa	amino acids
ALT	alanine aminotransferase
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
IFN	interferon-alpha
IU/mL	international units/millilitre
kD	kilo Dalton
MEIA	microparticle enzyme immunoassay
MU	million units
ORF	open reading frame
PMBC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
peg-IFN	pegylated interferon-alpha
PKR	RNA dependent protein kinase
RIBA	recombinant immunoblot assay
RNA	ribonucleic acid
RVR	rapid viral response
s.c.	subcutaneously
t.i.w.	thrice weekly
2', 5'OAS	2', 5'-oligoadenylate synthetase
UTR	untranslated regions

1 INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health problem with an estimated worldwide prevalence of 3% corresponding to at least 170 million infected persons (1). In Sweden the prevalence is estimated to be 0.5% corresponding to 40.000 infected individuals.

1.1 NATURAL COURSE OF HEPATITIS C VIRUS (HCV) INFECTION

After HCV has been transmitted to an individual, the virus will replicate preferentially in hepatocytes but also to some extent at extra-hepatic sites such as in peripheral blood mononuclear cells (PBMC), including lymphocytes, monocytes, dendritic cells, and granulocytes (2-4). HCV generally causes a slowly progressive liver disease, and it is estimated that 20-30% of infected individuals will develop liver cirrhosis within 20-30 years, many of whom eventually will develop liver failure and hepatocellular cancer (HCC) (5-7). In the US, it is estimated that approximately 10.000 patients will die from HCV related disease yearly and that this number will triple during the next 2-3 decades (8). Liver disease due to HCV infection is now the main indication for liver transplantation in the US (9). After development of a chronic HCV infection there is no sporadic clearance of the virus, hence treatment is the only option to achieve viral eradication. If treatment is successful and HCV is eradicated, the individual will not acquire immunity which protects against reinfection of HCV, not even with the original infecting viral strain. Patients who clear the virus spontaneously, however, seem to develop some immunity, as indicated by a lower incidence of new HCV infections in intravenous drug users (IVDUs) who have experienced an earlier infection as compared to IVDUs who are naive (10). Today there is no vaccine developed, nor any immune-globuline available for prophylactic use.

Among individuals with an acute HCV infection, only 10-15% experience clinical symptoms such as jaundice, stomach pain, or nausea (11). The majority will not develop any symptoms, and hence their infection will pass unnoticed. Fifteen to 50% of patients with acute HCV infection clear HCV RNA from serum within 6 months and recover spontaneously (12-14). Earlier, only 15-20% of post transfusion non-A, non-B/HCV cases were believed to resolve their infection spontaneously without

treatment (15, 16). More recent data, however, have shown that patients with sporadic acute HCV infection will clear their infection more often. Hence, women infected by anti-D prophylaxis in Ireland and Germany, IVDUs with sporadic HCV infection, and children with post-transfusion HCV infection seem to recover spontaneously in up to 40-50 % (13, 14, 17, 18). The remaining patients develop a chronic HCV infection. The chronic infection is usually asymptomatic and is often recognised only by intermittently raised liver enzymes (15). A majority of patients will not have any symptoms until they have developed more advanced liver disease such as liver cirrhosis or HCC. However, symptoms such as fatigue, arthralgias, myalgias, paresthesias, and pruritus have been reported also in patients with less advanced liver disease, however, less frequently (19). Furthermore, extrahepatic manifestations such as essential mixed cryoglobulinemia, porphyria cutanea tarda, membranous glomerulonephritis, sicca syndrome, and thyroiditis have also been associated with chronic HCV infection (20-23).

1.2 HEPATITIS C VIROLOGY

In the 1970s a presumed virus, different from hepatitis A and B virus, was known to cause the majority of transfusion-associated hepatitis cases (24, 25). This type of hepatitis was known as non-A, non-B hepatitis. The post-transfusion non-A, non-B hepatitis was most well recognised. However, sporadic cases were also reported both internationally and in Sweden (26-28). It was not until 1989 that the virus causing this type of hepatitis was discovered by Choo et al and given the name HCV (29). It has later been classified as a member of the Flaviviridae family, and as a separate genus named Hepacivirus. HCV is approximately 50 nm in diameter (30). It is bound to low-density lipoproteins (31), which is thought to facilitate its entry into the hepatocytes by endocytosis. The HCV genome is a single stranded linear RNA of positive polarity of approximately 9500 nucleotides length. It has a long open reading frame (ORF) flanked by highly conserved untranslated regions (UTR) at the 5' and 3' termini. The ORF encodes a precursor poly-protein of about 3000 aminoacids (aa), which is processed into at least 10 different polypeptides (Figure1).

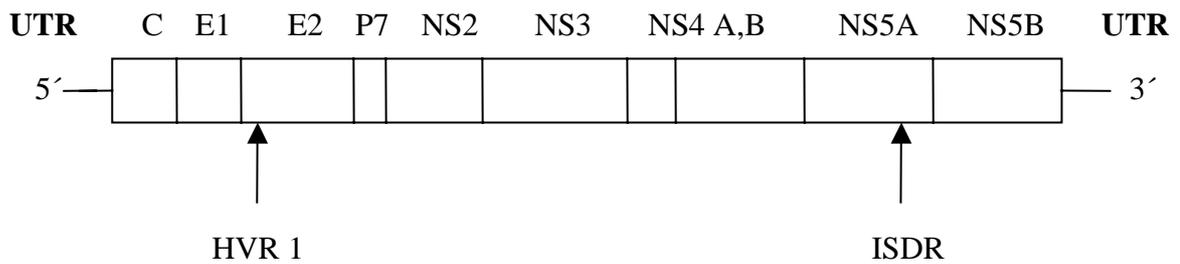


Figure 1. Genomic organization of hepatitis C virus. UTR, untranslated region; HVR 1, hypervariable region 1; ISDR, interferon sensitivity-determining region.

The processing of the poly-protein is mediated by cellular enzyme host signal peptidase, and two viral proteases, the NS2-3 encoded protease and the NS3-4A encoded serine protease. The process results in the production of a core or capsid protein (C), two envelope glycoproteins (E1 and E2), a small hydrophobic protein (p7), and six non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (32, 33). The E2 and NS5A proteins are believed to have a certain influence on the response to IFN (34-38). The E2 protein interacts with the E1 protein to form the HCV envelope protein complex. The aa sequence 1—27 of the N-terminus of the E2 protein is extremely variable and is referred to as hypervariable region 1 (HVR 1). The heterogeneity in this region is the result of the selective pressure induced by the host immune system. A second hypervariable region 2 (HVR 2) has been found in genotype 1 isolates, but the function of this region is not yet fully understood. The NS5A protein is assumed to be important for RNA replication and encodes for a RNA dependent RNA-polymerase. Mutations in this region may be related to increased IFN responsiveness (39, 40). This region has been denominated the IFN sensitive determining region (ISDR). The NS5A protein may promote resistance to IFN by repressing IFN-induced cellular protein kinase (PKR) (41). A similar interaction with PKR has been reported for a region within the E2 protein (42). Hence, interaction between the E2 and/or NS5A proteins can represent mechanisms that HCV utilizes to avoid the antiviral activity caused by IFN. Another mechanism of HCV resistance against IFN is the NS3/4A serine protease, which recently was shown to block the phosphorylation and effector action of IFN regulatory factor-3 (IRF-3), which is a key component in the cellular antiviral signalling system (43). Nucleotide sequence data have shown a high degree of diversity among different viral

isolates. Several systems to classify isolates have been proposed, but the system by Simmonds et al. is now generally accepted (44). In this system, six genotypes are defined and represented by Arabic numerals and subtypes by lower case letters (e.g. a, b, c). Genotypes 1a and 1b are predominant in America and Europe followed by genotype 2b and 3a (45). Genotype 1 also predominates in Japan and some other parts of Asia, whereas genotype 4 is predominant in Egypt and Central Africa (44, 46), genotype 5 in South Africa (47). Genotype 6 has only been isolated in south East Asia (48). Within a genotype there are genetic variants called quasispecies, which are thought to cause further opportunities for HCV to evade both the humoral and cellular host immune responses (49).

1.3 DIAGNOSIS OF HEPATITIS C

Diagnosis of both acute and chronic HCV infection is based on detection of antibodies to HCV and detection of HCV RNA by a polymerase chain reaction (PCR) test. The anti-HCV test used as a screening test, is a microparticle enzyme immunoassay (MEIA) based test for detection of antibodies against different recombinant viral epitopes. In low HCV prevalence populations this test is usually confirmed with a supplementary test. The most often used confirmatory test is the recombinant immunoblot assay (RIBA, Chiron Corporation, Emeryville, CA). This test uses recombinant antigens of HCV coated in bands on a nitrocellulose strip. Two out of four bands must be positive for confirmation of a positive test. An anti-HCV test cannot discriminate between a resolved and a chronic infection. For this a qualitative HCV RNA PCR test is used. With the latest commercially available test the detection limit is approximately 50 international units/millilitre (IU/mL) (50). Lately an even more sensitive test (TaqMan PCR-test) has been developed with a lower detection limit of 5-10 IU/mL. It is expected that this test will be approved for routine use in the near future. Quantification of HCV RNA in serum is performed at baseline before treatment, and usually week 12 during treatment if the virus is not already cleared from serum. This is done in order to evaluate to what extent the viral load has declined, especially in patients infected with genotype 1. In clinical practice two quantitative methods have been used; either a bDNA test or a PCR based test, represented by the Versant HCV RNA 3.0 Assay (Bayer Diagnostics) and the Cobas AmpliCor HCV Monitor assay v. 2.0 (Roche Diagnostics), respectively. The HCV RNA titres in serum are reported as IU/mL in accordance to international agreement,

which facilitates comparison of results performed with different test methods in different studies (51). Genotyping of HCV is performed by direct sequencing of the NS5B or E1 regions (52). The most often used commercial test for genotyping of HCV is the Innolipa® (Innogenetics, Belgium) (53).

1.4 HEPATITIS C EPIDEMIOLOGY AND TRANSMISSION ROUTES

As earlier mentioned at least 170 million people worldwide are estimated to be infected with HCV (1) corresponding to 3% of the world population. A wide geographical variation in prevalence is seen. In northern Europe, the prevalence is estimated to be 0.3% and in southern Europe and North America 1-1.5% (54), and in northern and central Africa over 10% (55-57).

Blood transfusions and intravenous drug use have been the predominant modes of transmission for HCV infections in the Western world (58). After 1990, when the first generation of anti-HCV test was developed, mandatory screening of blood donors and blood units was introduced. This virtually eliminated transfusion as a source for HCV transmission (59, 60). At present, intravenous drug use is the major route for transmission of HCV in the Western world. Nosocomial transmission has also been reported (61, 62). The risk for perinatal transmission of HCV from an infected mother to the newborn has been estimated to be less than 5%, and the risk for sexual transmission even lower (63). Acupuncture and tattooing have a potential to cause transmission but have not been considered to be routes of major importance (58).

1.5 TREATMENT OF ACUTE HEPATITIS C

Since most patients do not develop any symptoms after having been infected with HCV, the majority of patients with acute hepatitis C will not be treated, due to the fact that they will not seek medical attention. In individuals with symptoms, the risk for developing a chronic HCV infection seems to be smaller than in asymptomatic cases (64, 65). When treatment is given, almost 100% will clear the infection when IFN 5 million units (MU) is given daily during four weeks, followed by 5 MU three times per week (t.i.w.) for another 20 weeks (66).

1.6 TREATMENT OF CHRONIC HEPATITIS C

The rationale for antiviral treatment in HCV is to halt the progression towards end stage liver disease, and to diminish the risk for HCC. Previously IFN administered

subcutaneously (s.c.) at a dose of 3 MU t.i.w. in combination with ribavirin orally at a dose of 1000–1200 mg/day depending on bodyweight was standard therapy (67-69). Treatment duration was 24 weeks for patients infected with genotype non-1 (i.e. genotype 2 or 3) and for genotype 1 infected patients with low baseline HCV RNA levels ($<1.2 \times 10^6$ IU/mL) whereas 48 weeks was used for patients infected with genotype 1 with high baseline HCV RNA levels (70, 71). Recently, pegylated (peg) IFNs (peg-IFN), where a polyethylene glycol molecule has been attached to the IFN molecule, have replaced standard IFNs. Peg-IFNs are dosed once weekly because of their slower elimination and prolonged serum half-life. Currently two different peg-IFNs are approved, peg-IFN-alpha 2a (Pegasys®) which has a 40 kilo Dalton (kD) branched peg-molecule attached to IFN, and peg-IFN-alpha 2b (PegIntron®) which has a 12 kD linear peg-molecule attached to IFN. After a single s.c. dose of standard IFN maximum serum concentration is reached after 6-8 hours, and serum IFN levels remain detectable for 20-24 hours. Thus when IFN is given with a standard t.i.w. dosing scheme, IFN serum concentrations will vary considerably from day to day with no measurable levels detected on the alternating days when no injections are given (72). The new peg-IFNs reach maximum serum concentrations after 72-96 hours (peg-IFN-alfa 2a) and 15-44 hours (peg-IFN-alfa 2b) and plasma half-lives are 75 and 31 hours, respectively (73). Ribavirin reaches maximum plasma concentrations after 1.7 hours and 3 hours after a single dose and a twice daily dose of 600 mg, respectively. The steady-state plasma concentration after oral administration of ribavirin 600 mg twice daily is reached after 4 weeks (74). Ribavirin seems to have little or no impact on the initial phase of HCV RNA elimination (75) but reduces the risk for relapse after treatment cessation.

Roughly 80% of genotype non-1 and 50% of genotype 1 patients will achieve a virological sustained response and clear their HCV RNA when peg-IFN in combination with ribavirin is used (76-78). For patients with a virological non-response (HCV RNA continuously detectable during treatment) or response/relapse (HCV RNA is cleared during treatment but reappears after treatment cessation) to the old standard combination treatment, the new combination therapy including peg-IFN and ribavirin offers some hope. Amantadine has been proposed as a complement to standard combination therapy for treatment of naive patients, and for patients with a prior non-response or response/relapse (79-84).

Induction treatment, where IFN initially is given on a daily basis and/or at a higher dose has been proposed as an alternative to the old standard t.i.w. scheme in order to

improve treatment outcome in difficult to treat patient categories. The theoretical basis for this is to avoid peaks and troughs in IFN serum levels during t.i.w. dosing which theoretically can carry a risk for emergence of viral escape mutants. With peg-IFN such peaks and troughs in IFN serum concentrations can be avoided, but if this equals induction treatment with standard IFN has not been confirmed.

So far, treatment indications for chronic HCV infection has been based on the severity of the histological damage in the liver (70, 71). In general, moderate to severe inflammation and/or fibrosis has been required (70, 71). During recent years, however, treatment outcome for genotype 2 and 3 infected patients has improved so that 80% will achieve a virological sustained response and a liver biopsy before treatment is not always considered necessary (85). Recent data on the influence of steatosis on fibrosis progression also speaks in favour for early treatment of in particular genotype 3 infected patients. Genotype 3 is associated with steatosis (86) and treatment induced viral clearance is known to reduce steatosis (87). The main purpose with treatment of chronic HCV infection is to eradicate the infection, and by this reduce the inflammation in the liver, and halt the down hill course of the liver disease towards end stage liver disease and cirrhosis, and also reduce the risk of developing HCC (6, 7, 88). However, roughly 50% of patients receiving standard IFN and ribavirin combination treatment will not achieve viral eradication (67-69). For these non-responders long-term maintenance IFN therapy has been proposed to reduce further evolution of the fibrosis (89-91). Besides HCV genotype 1 and non-1 (usually referring to genotype 2 and 3) genotype 4 has become relatively common in Western countries due to immigration of patients from parts of the world where this genotype is prevalent. Genotype 4 seems to respond to the same extent as genotype 2 and 3 if the treatment strategy applied for genotype 1 (i.e. higher IFN and ribavirin doses and treatment length of 48 weeks) is used (92).

1.7 MECHANISMS BY WHICH DRUGS USED FOR THERAPY OF HCV INFECTION EXERT THEIR EFFECT

1.7.1 Interferon

The exact mechanisms by which IFN exerts its effect during treatment of chronic HCV infection are unknown. It is thought to have both a direct and an indirect antiviral effect (93). The direct antiviral effect is thought to be initiated by IFN through its receptor on the target cell, which leads to the production of antiviral

polypeptides. The indirect antiviral effect is exerted via the host immune response. After IFN has attached to its receptor on the cell surface, phosphorylation of Janus kinases (JAKs) in the cytoplasm occurs, which in turn leads to phosphorylation of a family of proteins functioning as signal transducers and transactivators in the JAK-STAT pathway. These induce transcription of IFN-sensitive genes (94). HCV proteins have been shown to inhibit the IFN induced signalling through the JAK-STAT pathway (95). Some of the IFN-sensitive genes produce proteins with antiviral effect such as 2', 5'-oligoadenylate synthetase (2', 5'OAS), which inhibit the replication of different viruses (96). Reduced baseline and post IFN stimulated activity of 2', 5'OAS has been noted in peripheral blood mononuclear cells (PBMC) originating from HCV infected patients with poor response to IFN in vitro (97). IFN also induces the PKR, which leads to phosphorylation and inactivation of the eukaryotic translation initiation factor-2 alpha, which is required for efficient viral synthesis (94). Furthermore, IFN has been shown to augment cell-mediated immune responses (98).

1.7.2 Ribavirin

The guanosine analogue ribavirin was discovered in 1972 and was first approved for use in severe respiratory syncytial virus infections. It has a broad spectrum of antiviral activities against various RNA and DNA viruses (99) but the exact mechanism by which ribavirin exerts its effect against HCV has not been fully elucidated. Mono-therapy with ribavirin does not induce any substantial direct antiviral effect, neither on the HCV RNA serum levels nor on the evolution on HCV quasispecies development (100, 101). One possible mechanism by which ribavirin exerts its action against the hepatitis C virus is through immune modulation, by preserving the Th1 and reducing the Th2 cytokine response (102). Others have suggested that ribavirins primary effect on RNA viruses is by inducing lethal mutagenesis of the RNA virus genome, resulting in an error catastrophe (103, 104).

1.7.3 Amantadine

Amantadine is a tricyclic symmetric amine, which inhibits different viruses such as influenza type A virus and flaviviruses (105, 106). Amantadine reduces viral replication by interfering with the uncoating of the virus or by interfering with the transcription of viral RNA (105). Amantadine alone or in combination with IFN, also

reduces the HCV content in peripheral blood mononuclear cells in a dose-dependent way (107). The optimal dose to suppress HCV RNA seems to be reached with 100 mg amantadine twice daily (107). In contrast to IFN, amantadine has no effect on the 2', 5'OAS activity (107). Antiviral therapy studies with inclusion of amantadine as therapy for HCV either as mono-therapy or in combination with IFN or with IFN and ribavirin in trippel therapy have yielded conflicting results (81, 83, 84, 108-111). Recently a meta-analysis failed to show any benefit with amantadine in the treatment arsenal for chronic HCV infection (84).

1.8 HCV RNA KINETICS

The serum HCV RNA levels in untreated patients are in a steady state balance between the amount of HCV produced from infected hepatocytes and released into serum, and the amount eliminated from serum by the immune system. Reinfection of uninfected hepatocytes occurs continuously, and infected hepatocytes are continuously cleared by the immune system (Figure 2).

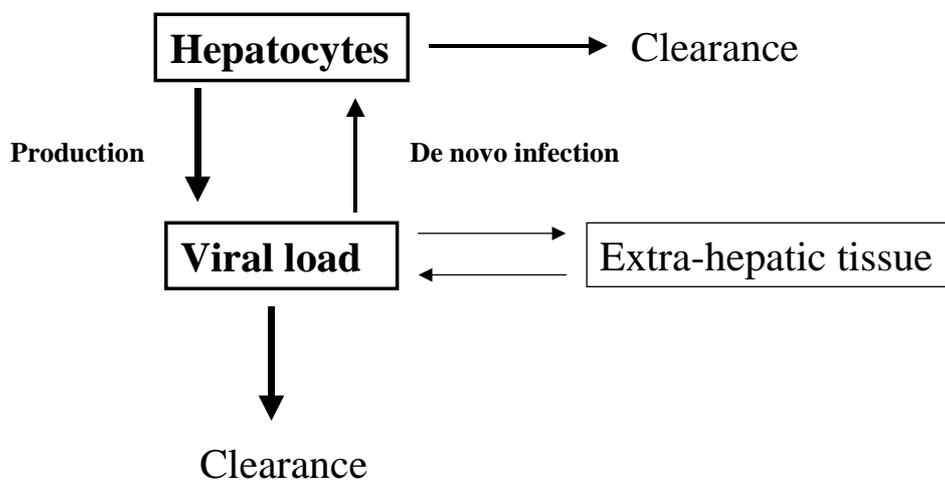


Figure 2. HCV production and elimination schematically.

The turnover rate of HCV in serum has been calculated to be 2.7 hours with a production rate of 10^{12} virions per day (112). The serum viral load in the untreated patient is relatively constant over time. It does, however, tend to fluctuate to some degree corresponding to $0.75 \log_{10}$ in the individual patient (113, 114). Furthermore,

the intra assay variation for most quantitative HCV RNA assays is approximately 0.5 \log_{10} (51).

When IFN treatment is initiated, this balance is disturbed and during treatment serum HCV RNA levels decline in a biphasic manner (112) (Figure 3).

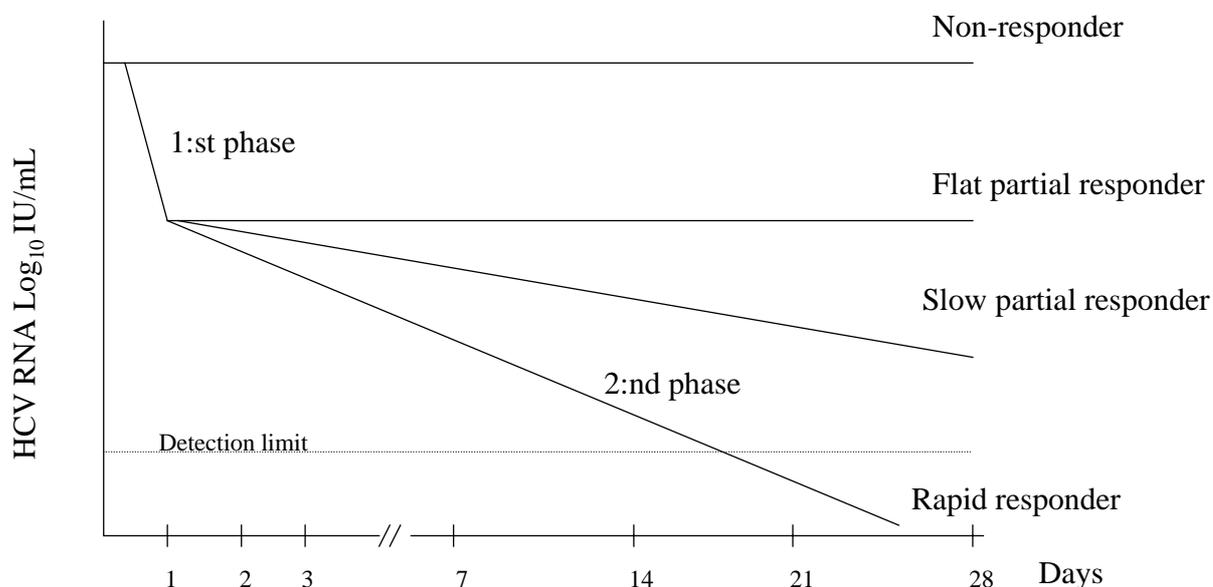


Figure 3. HCV kinetics during antiviral treatment with the rapid first (24-48 hours) and the slow second phase (days-months) HCV RNA declines, and examples of different patterns in responders are depicted.

The first rapid phase is dose-dependent and lasts 24-48 hours. This phase is thought to correlate with the elimination of free virus particles from serum due to blocking of the viral production by IFN. The second slower phase lasts from days to months and is probably correlated to the elimination of infected hepatocytes, which have a calculated half-life of 1.7- >70 days (112). During the second phase, HCV RNA levels continue to decline in responders, but minimal or no further decline can be detected in non-responders (Figure 3). Some investigators have noticed a third phase which appears after 7 to 28 days treatment, which seemed to be correlated with a sustained virological response. The HCV RNA decay during this third phase was faster in patients treated with the combination of peg-IFN-alpha 2a and ribavirin, as compared to peg-IFN alone, and was thought to represent treatment enhanced degradation of infected cells (115). Furthermore, the decline in HCV RNA levels is

more rapid in patients who display a NS3-specific T-cell response during treatment, which seems to be related to the genotype (116). HCV genotype non-1 has recently been shown to have a more rapid first and second phase decline of HCV RNA levels as compared to genotype 1 (117, 118).

Furthermore, a more rapid decline in HCV RNA levels has been noted with 6 MU as compared to 3 MU IFN t.i.w. indicating that higher IFN doses will increase the viral clearance rate (75). A $> 3 \log_{10}$ decline in viral load within the first 4 weeks of treatment has also been shown to predict sustained virologic response (119).

Since a large proportion of genotype 1 infected patients do not respond with clearance of HCV RNA from serum during treatment or have a relapse after treatment cessation, a time point where a prediction of a later non-response can be made is highly desirable. It is also desirable that this prediction can be done as early as possible after treatment initiation to reduce the economical costs and adverse events. In one study, a $< 70\%$ decline of the HCV RNA levels day 1 after the first IFN dose predicted a non-response to standard IFN and ribavirin (120).

1.9 PRACTICAL APPLICATIONS OF HCV RNA KINETICS

Induction treatment with IFN given on a daily basis during the initial phase of therapy has been utilized as an alternative treatment scheme to standard t.i.w. dosing. The rationale for using induction treatment is to avoid low IFN serum levels on the alternating days when no IFN dose is given. Low IFN serum concentrations are thus thought to promote emergence of resistant HCV escape mutants. Furthermore, induction treatment is presumed to result in a faster decline of HCV RNA levels, leading to earlier viral clearance. When HCV RNA has been cleared from serum, continued suppressive treatment has to be given in order to clear also the infected hepatocytes which produces new virus particles. So far, studies of IFN induction treatment in combination with ribavirin have yielded conflicting results (121-124). In a recent study, however, high induction dosing followed by high every other day IFN dosing was shown to increase the virological sustained response rate in difficult to treat patients with genotype 1 infection who had a high viral load (125).

New peg-IFNs have now replaced standard IFNs. Peg-IFNs offer continuous serum IFN levels throughout the week in practical terms corresponding to daily induction treatment with standard IFNs.

If a rapid viral response, meaning a more than 2 log₁₀ drop in viral levels from baseline by week 12 during therapy, has not been reached in the individual patient during treatment with the new peg-IFNs in combination with ribavirin, the negative predictive value for not achieving a sustained virological response is 98-100% (77, 126). A lack of a rapid viral response is generally used as a stop rule in most treatment algorithms. The aim, however, is to improve this rule so it can be used at an earlier time point during treatment.

2 AIMS OF THE STUDY

The overall aim of this thesis was to study the initial HCV RNA kinetics during different antiviral treatment regimens and to determine what impact the genotype has on the kinetics.

The specific aims were:

1. To study the HCV RNA kinetics during triple treatment with IFN, ribavirin and amantadine and to evaluate the virological outcome with this treatment in patients with a previous non-response or response/relapse to standard combination treatment (Paper I).
2. To evaluate and compare the HCV RNA kinetics during IFN induction and standard treatment (Paper II, IV).
3. To evaluate and compare the HCV RNA kinetics during IFN induction and standard treatment when it is combined with ribavirin, and to correlate the early virological response to the end of treatment, and sustained virological outcome 24 weeks after treatment cessation (Paper III).
4. To evaluate the early HCV RNA kinetics during treatment with peg-IFN-alpha 2a in combination with ribavirin, and to correlate it to the sustained virological response, and to evaluate if the response can be predicted earlier than week 12 during treatment (Paper V).

3 ETHICS

Written informed consent was obtained from all patients prior to treatment, and the ethics committees at Karolinska Institutet, Stockholm, Sweden, and Vilnius University, Vilnius, Lithuania (Paper IV), approved the studies. All studies were performed in accordance with the principles put forward in the Helsinki Declaration

4 SUBJECTS AND STUDY PROTOCOLS

4.1 PAPER I

In an open-label IFN/ribavirin/amantadine triple therapy pilot study 13 patients with previous virological non-response, and 10 patients with previous response/relapse to at least one course of IFN (3 MU t.i.w.) in combination with ribavirin (1000-1200 mg/day) were included. The definition of a non-response to a previous therapy was HCV RNA positivity by a qualitative PCR test after at least 12 weeks treatment with IFN/ribavirin, and the definition of a response/relapse was a negative HCV RNA at the end of at least 24 weeks combination treatment followed by a relapse during the post-treatment follow-up period. Previous IFN/ribavirin therapy was discontinued at least six months prior to study entrance. All patients were treated with IFN-alpha 2a (Roferon®, Roche) at a dose of 3 MU t.i.w., ribavirin (Rebetol®, Schering-Plough) at a dose of 1000 mg/day and amantadine (Virofral®, Ferrosan) at a dose of 200 mg/day for a total of 24 weeks. A qualitative HCV RNA test was performed at week 0, 12, 24 and at follow-up week 48 for evaluation of virological response. In order to measure the HCV RNA levels during treatment, serum samples were obtained and immediately frozen at -70°C at week 0, 2, 4, 8, 12, 16, 20 and 24, and at the end of follow-up (week 48) and were later analysed by a quantitative PCR test.

4.2 PAPER II + III

Thirty-one and 32 treatment naive patients, respectively, were included and randomised to treatment (1:1:1) with natural human leukocyte IFN (Interferon Alfa-nativo®; Bionative AB) 3 MU daily for 14 days (induction phase) followed by t.i.w. administration (Group A), natural human leukocyte IFN 3 MU given t.i.w. from the start (Group B) or recombinant IFN (Intron A®; Schering Plough) 3 MU given t.i.w. from the start (Group C). In Paper II, only patients with low baseline viral load ($<1.2 \times 10^6$ IU/mL) were included. In Paper III, ribavirin (Rebetol®, Schering Plough) was given orally (1000 mg or 1200 mg daily, depending on bodyweight; <75 kg or >75 kg, in two divided doses) in combination with the IFN. Sera were drawn at baseline day 0 and day 1, 2, 3, 7, 14, 28, 56 and 84 and immediately frozen at -70°C for later analysis of HCV RNA levels. In Paper III, patients infected with genotype 1 with a high baseline viral load ($>1.2 \times 10^6$ IU/mL) were treated for 48 weeks, and

those infected with genotype non-1 or genotype 1 with low viral load for 24 weeks. A virological response was defined as the absence of HCV RNA, as tested by a qualitative PCR method, at the end of treatment and at follow-up 24 weeks after treatment stop (end-of-treatment response and sustained response, respectively).

4.3 PAPER IV

Thirty-four patients received induction treatment the initial 10-14 days and thereafter standard t.i.w. dosing, and 31 patients standard t.i.w. dosing from the beginning. The induction group were given 10 days with IFN-alpha-2b 6 MU daily (n=24, Realdiron®; Biotechna, Lithuania) followed by either 6 MU t.i.w. (n=11; Realdiron®) or 3 MU t.i.w. (n=13, Realdiron®) or 14 days with natural human leukocyte IFN (Interferon Alfa-native®; Bionative AB) 3 MU daily followed by 3 MU t.i.w. (n=9, Interferon Alfa-native®) for a total of 24 and 36 weeks, respectively. The standard treatment group was treated with IFN 3 MU t.i.w. for 36 weeks (n=18) or 48 weeks (n=13). Realdiron®, Alfa-native®, and Intron A® was given to 12, 10, and 9 patients respectively. All patients treated for 36 weeks also had ribavirin (Rebetol®, Shering Plough) orally (1000 mg or 1200 mg daily, depending on bodyweight; <75 kg or >75 kg, in two divided doses) added during the last 24 weeks of treatment. Sera were drawn at baseline and at week 4, 8, and 12 and immediately frozen at -70°C for a later analysis of HCV RNA levels. The follow-up period after the end of treatment was 24 weeks.

4.4 PAPER V

Treatment naive patients were included from Swedish centres in two large international multicenter studies, either a randomized controlled (78) or an open non-randomized phase IV study. Sera were drawn within two hours from sampling at baseline and weeks 1, 4, and 12 during treatment and frozen at -70°. In addition, serum was drawn day 1 in the phase III study. Eighteen patients were selected from the phase III randomized study and 42 from the phase IV non-randomized study. All patients were treated with peg-IFN-alfa 2a (Pegasys®, Roche) 180 µg s.c. once weekly in combination with ribavirin (Copegus®) which was given daily at a dose of 800 or 1000-1200 mg in patients recruited from the randomized controlled study during 24 or 48 weeks as reported earlier (78) or at a dose of 800-1200 mg daily, depending on body weight, in patients recruited from the open uncontrolled study

during 24 weeks for infections caused by genotype non-1, and 48 weeks for genotype 1. A virological sustained response was defined as the absence of HCV RNA at the end of treatment and at follow-up 24 weeks after treatment cessation as tested by PCR; a response/relapse as the absence of HCV RNA at the end of treatment but reappearance of HCV RNA during follow-up; a non-response as the presence of HCV RNA during treatment and at the end of treatment.

5 METHODS

5.1 VIROLOGY TESTS

Anti-HCV was tested by a MEIA test (AxSym, version 3.0; Abbott Laboratories) and qualitative HCV RNA by a PCR test (Amplicor HCV; Roche Diagnostics) with a sensitivity of approximately 50 IU/mL (50) (Paper I-V). For quantification of HCV RNA, Cobas Amplicor HCV MonitorTM (Roche Diagnostics) was used (Paper I-V). This assay has a sensitivity of approximately 600 IU/mL (51).

HCV genotyping was performed by a line probe assay (Innolipa® HCV II, Innogenetics, Brussels, Belgium) (53) or an in-house method (127) (Paper I-V). All tests were performed according to the manufacturer's instructions.

5.2 HISTOLOGY

A scoring system was used that ranked liver inflammation (grade) and fibrosis (stage) on a scale 0-4 (128, 129) (Paper I-V). In brief grade 0 represented no or minimal inflammation and grade 4 severe inflammation; stage 0 was classified as no fibrosis and stage 4 was equivalent to cirrhosis. In the Lithuanian part of Paper IV the scoring system by Ishak was used (130).

5.3 STATISTICS

The non-parametric Wilcoxon signed-rank test (Paper I), Kruskal-Wallis test (Paper II+IV) and the Mann-Whitney U-test (Paper I-V) were used to test quantitative variables. The Fisher's exact two-tail test was used to test categorical variables (Paper I-V). A p-value <0.05 was considered statistically significant.

6 RESULTS AND DISCUSSION

6.1 PAPER I

Many patients do not respond to standard IFN and ribavirin therapy, or respond during treatment but relapse after treatment cessation. At present, no accepted treatment strategy exists for these patients. When the study in Paper I was planned, two small pilot studies had shown promising results with amantadine, given either as mono-therapy or in combination with IFN and ribavirin, in patients with previous non-response to IFN (108, 109). Eight of 13 (62%) and 6/10 (60%) of the patients in the non-responder and response/relapse groups, respectively, were infected with HCV genotype 1; and 4/13 (31%) and 2/10 (20%), respectively, had compensated cirrhosis. Of the 23 included patients, 21 completed the study. One from each group discontinued therapy prematurely due to sleeping disturbances, and alcohol abuse, respectively. At end-of-treatment, one out of 13 previous non-responders and five out of ten previous response/relapsers were HCV RNA negative by qualitative PCR. Geometric mean HCV RNA levels in the non-response and response/relapse groups were 6.3×10^5 and 8.6×10^5 IU/ml, respectively, at baseline; 1.4×10^5 and 1.6×10^4 IU/mL, respectively, at week 4; 5.4×10^4 and 1.0×10^4 IU/mL, respectively, at week 12; 8.5×10^4 and 1.2×10^4 IU/mL, respectively, at week 24 (week 0 vs. 24; $p < 0.05$ for response/relapsers, $p = ns$ for non-responders). Four previous non-responders became HCV RNA negative during triple therapy: two at week 8, one at week 12, and one at week 16 (Figure 4a). Three of these patients had a virological breakthrough at week 24, and one relapsed soon after end-of-treatment. Three previous response/relapsers did not respond with a significant decline in HCV RNA titres during triple therapy (Figure 4b). If this is due to development of resistance to IFN or ribavirin can only be assumed.

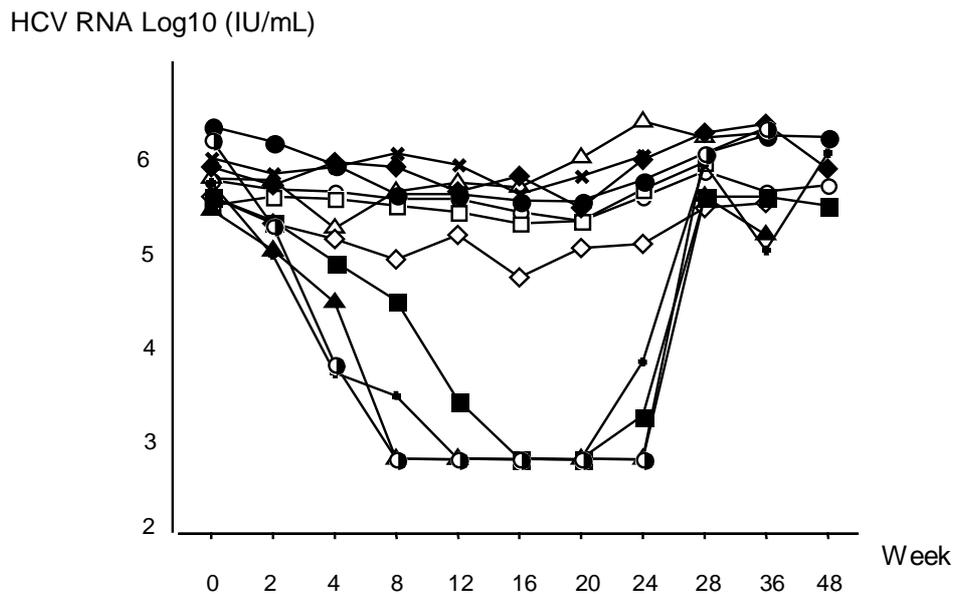


Figure 4a. Individual HCV RNA levels (IU/mL) during therapy with amantadine in addition to interferon and ribavirin in non-responders to previous interferon and ribavirin combination therapy.

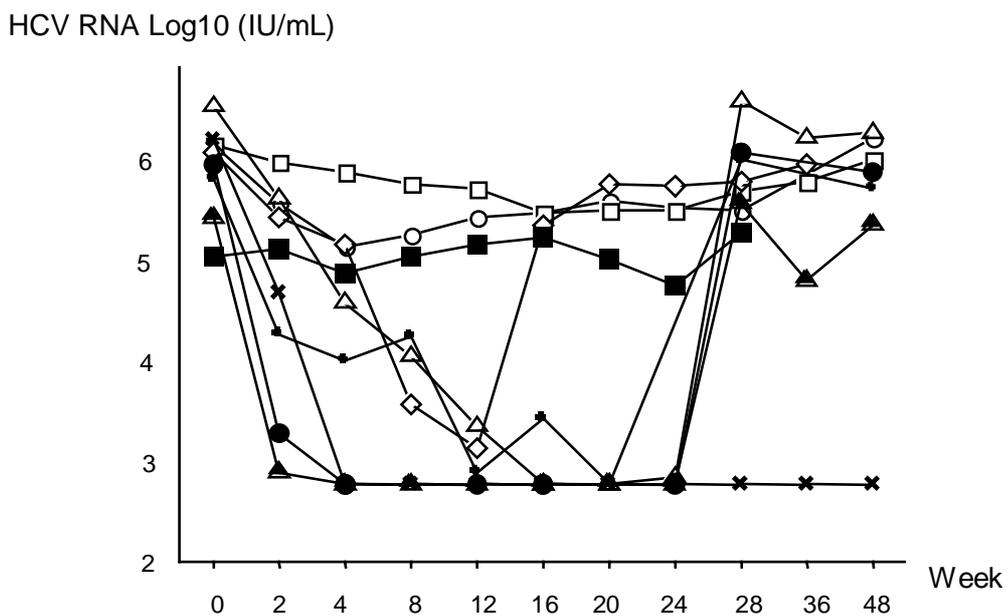


Figure 4b. Individual HCV RNA levels (IU/mL) during therapy with amantadine in addition to interferon and ribavirin in response/relapsers to previous interferon and ribavirin combination therapy.

At baseline patient characteristics for end-of-treatment responders and non-responders to triple therapy did not differ significantly except for mean ALT levels, which were lower for triple therapy responders (1.4 μ kat/L vs. 2.7 μ kat/L, $p < 0.05$).

Only one patient had a sustained virological response. This was a man with HCV genotype 2 infection and a previous response/relapse, with a mild fibrosis, and a low baseline viral load. In general our results are in contrast to the two studies reported before ours (108, 109). In these studies, treatment with amantadine alone or in combination with IFN and ribavirin resulted in sustained HCV RNA clearance in a significant number of patients with previous non-response to IFN therapy. However, our study population consisted of previous non-responders or response/relapsers to IFN/ribavirin combination treatment. Furthermore, perhaps sustained virological response could have been achieved in a few more patients in our study if a higher dose and/or longer treatment period had been used. Thus several patients had substantial drops in viral titres indicating that if a longer treatment course had been used some may have reached a durable virological response. In conclusion, no obvious beneficial effect was noted by adding amantadine to IFN and ribavirin during 24 weeks treatment in this small pilot study. Hence, a more general use of amantadine in combination with IFN and ribavirin for non-responders and response/relapsers to standard IFN/ribavirin combination treatment does not seem to be warranted. A recent large German study has indicated a less than 10% improved sustained virological response rate if amantadine is added to IFN + ribavirin in patients with earlier non-response (131). Recently, however, a meta-analysis has failed to show any benefit of adding amantadine to standard combination therapy (84).

6.2 PAPER II + III

Thirty-one patients with a mean age of 43 years (range 24-63 years) and 32 patients with a mean age of 43 years (range 22-65) were included in study II and III, respectively. In study II, 20 patients were infected with genotype 1, five out of ten (50%) in the induction group and 15/21 (71%) in the standard treatment groups (including one with mixed genotype 1/3a). Eleven patients were infected with genotype non-1 (including one with genotype 4). In study III, 13 patients were infected with genotype 1, three out of ten (30%) in the induction group and 10/22 (45%) in the standard treatment groups, and 19 were infected with genotype 2 or 3. Twenty-nine patients completed the study in Paper II in which two patients were withdrawn after 2 weeks (one from the recombinant IFN standard group due to psychiatric illness and one from the natural human leukocyte IFN standard group due to non-compliance). In Paper III, 31 patients completed the study. One patient in the

standard natural human leukocyte IFN treatment group discontinued treatment after the first dose (due to severe headache, nausea and anxiety) and was not included in further analyses. At baseline, the median HCV RNA levels and other demographic data did not differ between the study groups. In both study II and III, HCV RNA levels did not differ significantly between the two standard treatment groups during treatment. Thus, these groups were combined in both Paper II and III for further comparisons with the induction group.

6.2.1 Paper II

At baseline, the median HCV RNA levels in the induction and standard treatment groups were 0.50×10^6 IU/mL and 0.78×10^6 IU/mL respectively ($p=ns$). Already at day 2, the median HCV RNA level in the induction group had decreased to a significantly lower level as compared to the standard treatment group (0.01×10^6 IU/mL vs. 0.32×10^6 IU/mL; $p<0.0001$). This difference in median HCV RNA levels was stable during the entire study period for all genotypes analysed together (day 3-84; $p<0.005$) (Figure 5).

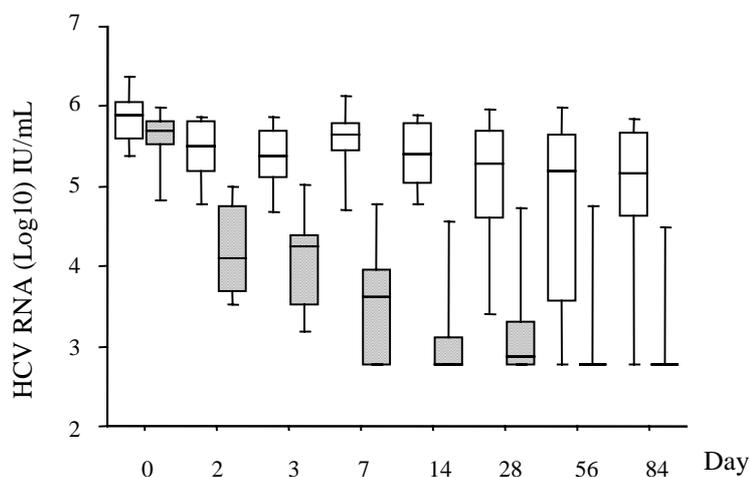


Figure 5. The median HCV RNA level (bar within the box) during treatment with IFN. Patients received either standard t.i.w. treatment from the start (open boxes; $n = 21$) or daily induction therapy for two weeks followed by t.i.w. treatment (filled boxes; $n = 10$). The boxes show the 25th to 75th percentiles and the lower and upper bars show the 10th and 90th percentiles, respectively.

For patients with genotype 1, the median HCV RNA level was lower in the induction group at every time point during the study period (day 2-84; $p<0.005$) (Figure 6a).

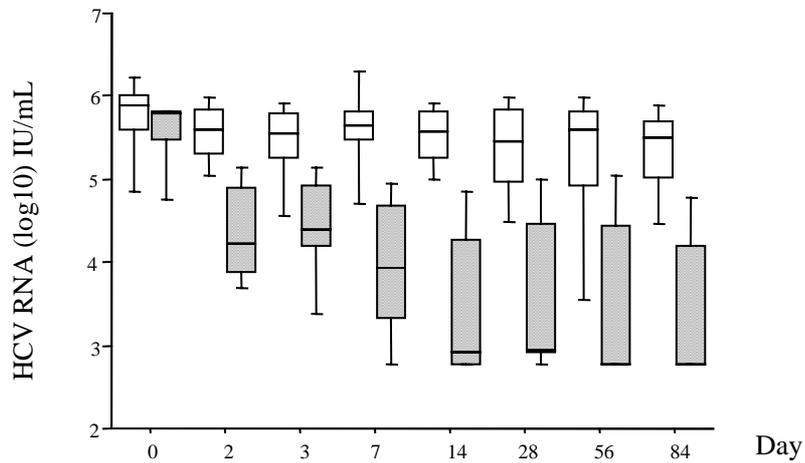


Figure 6a. The median HCV RNA level (bar within the box) during treatment with IFN in patients with genotype 1. Patients received either standard t.i.w. treatment from the start (open boxes; n = 15) or daily induction therapy for two weeks followed by t.i.w. treatment (filled boxes; n = 5). The boxes show the 25th to 75th percentiles and the lower and upper bars show the 10th and 90th percentiles, respectively.

For patients with genotype non-1, the median HCV RNA level was significantly lower only during the initial 14 days of treatment (day 2-14; $p < 0.05$, day 28-84; $p = ns$) (Figure 6b). However, a difference day 28-84 might have been missed due to a type II error, due to the low number of patients in the induction group.

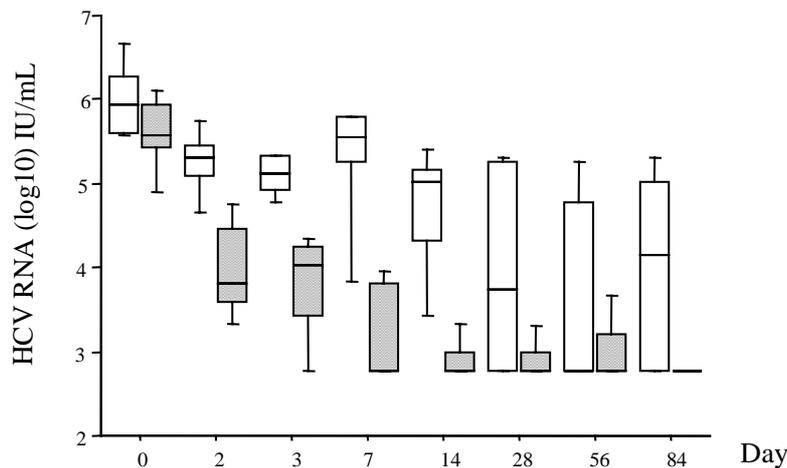


Figure 6b. The median HCV RNA level (bar within the box) during treatment with IFN in patients with genotype non-1. Patients received either standard t.i.w. treatment from the start (open boxes; n = 6) or daily induction therapy for two weeks followed by t.i.w. treatment (filled boxes; n = 5). The boxes show the 25th to 75th percentiles and the lower and upper bars show the 10th and 90th percentiles, respectively.

The more pronounced HCV RNA decline noted initially in the induction group among genotype non-1 patients was lost after day 14 in accordance with other studies (67-69, 132) showing that genotype non-1 (e.g. genotype 2 and 3) can be cleared more readily, both with IFN mono-therapy and by IFN and ribavirin combination therapy. Table I shows the number of patients in the induction and standard treatment groups with undetectable HCV RNA levels day 0 through day 84 during therapy.

Table I. Number of patients (%) in the induction and standard treatment groups with undetectable HCV RNA levels (<600 IU/mL) at days 2, 3, 7, 14, 28, 56 and 84.

Day	2	3	7	14	28	56	84
Induction group (n=10)	0 (0%)	1 (10%)	4 (40%)	6 (60%)	5 (50%)	7 (70%)	8 (80%)
Standard treatment group (n=21)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	2 ^a (10%)	3 ^a (16%)	3 ^a (16%)
p-value	ns	ns	ns	<0.05	=0.06	<0.05	<0.05

^a Two patients in the standard treatment group were withdrawn from therapy after day 14

When the study protocol was prepared, there was a consensus in Sweden to treat patients with chronic HCV infection with low viral load (HCV RNA <1.2 x 10⁶ IU/mL) with IFN mono-therapy and add ribavirin only if the pre-treatment viral load was high. However, during the study, two large international studies were published that showed that most patients benefit from IFN/ribavirin combination treatment independent of pre-treatment viral load (68, 69). Therefore, we found it unethical not to add ribavirin during a further 24 weeks of treatment after the initial study period of 84 days. Of the 9/10 and 15/21 in the induction and standard treatment groups, respectively, that were given combination treatment after the initial 12 weeks of IFN mono-treatment, 7/9 and 4/15, respectively, (p=0.04) achieved a virological sustained response (4/15 vs. 1/12 of genotype 1 infected patients; p=0.02). This seems to indicate that IFN induction treatment prior to combination treatment can increase the number of patients that eventually will achieve a virological sustained response. We conclude that two weeks of IFN induction mono-therapy resulted in a faster and more pronounced decline in HCV RNA levels as compared to standard t.i.w. dosing, most evident for patients infected with genotype 1. Ferenci et al have later confirmed that induction high dose IFN (10 MU) in combination with ribavirin is useful for patients with the difficult to treat genotype 1 infection with high baseline viral load (125).

6.2.2 Paper III

HCV RNA kinetics according to treatment schedule and genotype

At baseline, HCV RNA levels did not differ significantly between the induction and standard treatment groups (1.4×10^6 IU/mL vs. 1.6×10^6 IU/mL respectively). During treatment, the median viral load decreased rapidly in both groups. At day 2 and day 7, the median HCV RNA level in the induction group had decreased to a significantly lower level than in the standard treatment group (0.043×10^6 IU/mL vs. 0.191×10^6 IU/mL; $p=0.05$; and 0.015×10^6 IU/mL vs. 0.102×10^6 IU/mL; $p=0.03$; respectively (Figure 7a), but at all other time points this difference did not reach statistical significance. When patients were separated according to genotype, HCV RNA levels were lower at all time points in the induction group as compared to the standard treatment group in genotype 1 infected patients, but the difference was not statistically significant (Figure 7b). In genotype non-1 patients HCV RNA levels were significantly lower day 2 ($p=0.03$) and 7 ($p=0.05$) in the induction group as compared to the standard treatment group (Figure 7c).

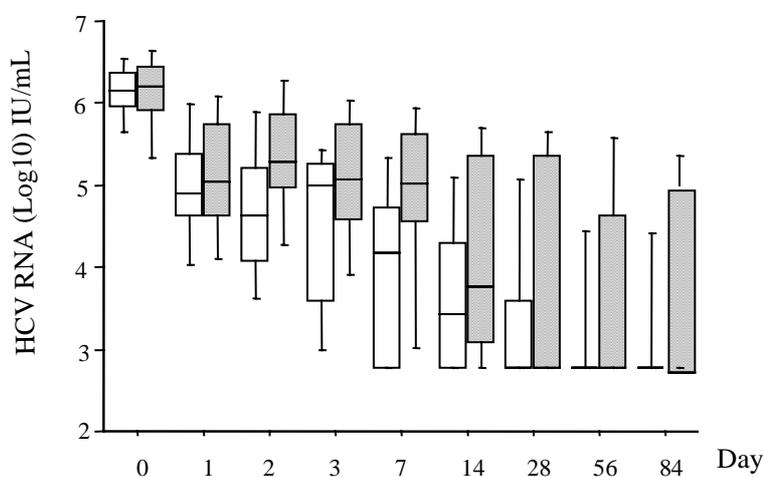


Figure 7a. The median HCV RNA level (bar within the box) for all patients (standard therapy; $n=21$ and induction therapy; $n=10$) during treatment with IFN and ribavirin. Patients received either standard thrice-weekly IFN therapy from start (filled boxes), or daily induction therapy for two weeks followed by thrice weekly treatment (open boxes). Both groups received daily ribavirin treatment. The boxes show the 25th to 75th percentiles and the lower and upper bars show the 10th and 90th percentiles, respectively.

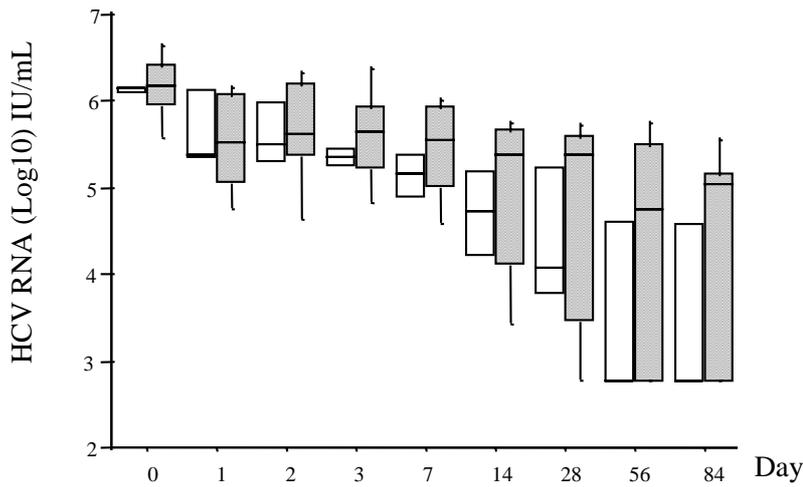


Figure 7b. The median HCV RNA level (bar within the box) for patients infected with genotype 1 (standard therapy; n=10 and induction therapy; n=3) during treatment with IFN and ribavirin. Patients received either standard thrice-weekly IFN therapy from start (filled boxes), or daily induction therapy for two weeks followed by thrice weekly treatment (open boxes). Both groups received daily ribavirin treatment. The boxes show the 25th to 75th percentiles, and the lower and upper bars show the 10th and 90th percentiles, respectively.

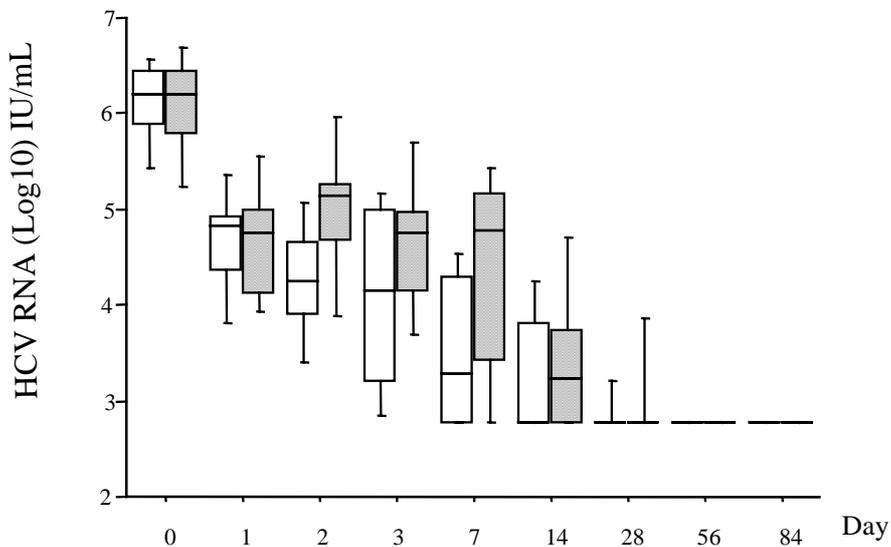


Figure 7c. The median HCV RNA level (bar within the box) for patients infected with genotype non-1 (standard therapy; n=11 and induction therapy; n=7) during treatment with IFN and ribavirin. Patients received either standard thrice-weekly IFN therapy from start (filled boxes), or daily induction therapy for two weeks followed by thrice weekly treatment (open boxes). Both groups received daily ribavirin treatment. The boxes show the 25th to 75th percentiles, and the lower and upper bars show the 10th and 90th percentiles, respectively.

When the overall HCV RNA kinetics according to genotype were analysed in the induction and standard treatment groups together, genotype non-1 infected patients had a more pronounced and faster decline in HCV RNA than genotype 1 infected patients (Figure 8). The difference was statistically significant at every time point during the study period and was translated into a better virological outcome.

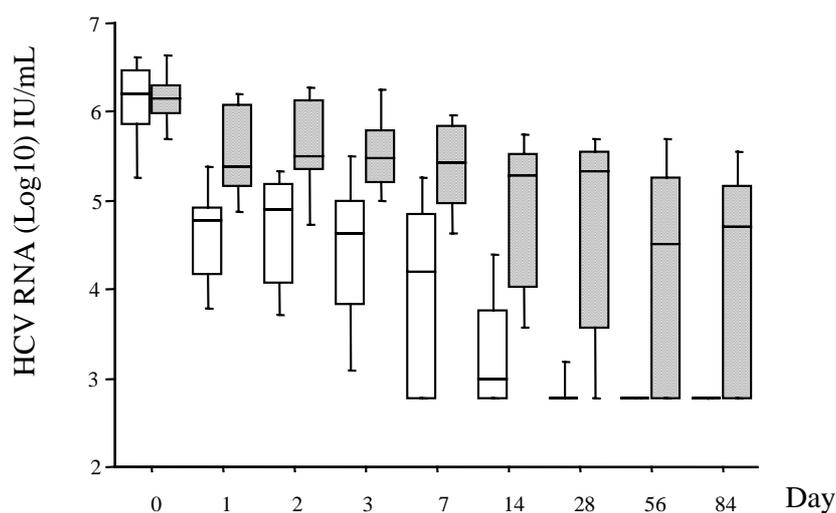


Figure 8. The median HCV RNA level (bar within the box) during treatment with IFN and ribavirin in patients infected with genotype 1 (filled boxes; n=13) or genotype non-1 (open boxes; n=18). The boxes show the 25th to 75th percentiles, and the lower and upper bars show the 10th and 90th percentiles, respectively.

At the end of treatment and at follow-up, genotype non-1 infected patients were HCV RNA negative in a larger proportion than those infected with genotype 1: (17/18 (94%) vs. 5/13 (38%); $p < 0.005$) and (12/18 (67%) vs. 4/13 (31%); $p < 0.05$), respectively (Table II).

Table II. Number of patients (%) in the induction and standard treatment groups with undetectable HCV RNA levels (<600 IU/mL during treatment; <50 IU/mL at end of treatment and at end of follow-up).

Day	1	2	3	7	14	28	56	84	EOT	EOFU
Induction (n=10)	0	0	1 (10)	3 (30)	5 (50)	6 (60)	9 (90)	9 (90)	8 (80)	6 (60)
G 1 (n=3)			0	0	0	0	2 (67)	2 (67)	1 (33)	1 (33)
G non-1 (n=7)			1 (14)	3 (43)	5 (71)	6 (86)	7 (100)	7 (100)	7 (100)	5 (71)
Standard (n=21)	0	0	0	2 (10)	5 (24)	11 (52)	15 (71)	15 (71)	14 (67)	10 (48)
G 1 (n=10)				0	1 (10)	2 (20)	4 (40)	4 (40)	4 (40)	3 (30)
G non-1 (n=11)				2 (18)	4 (36)	9 (82)	11 (100)	11 (100)	10 (91)	7 (64)

The difference between the two treatment groups was not significant at any time-point (Fisher's exact two-tail test)

G 1 = genotype 1

G non-1= genotype non-1

EOT = end of treatment

EOFU = end of follow-up

HCV RNA kinetics in the individual patient

The early viral kinetics in the individual patients differed according to treatment schedule and genotype (Figure 9). Among genotype non-1 patients in the standard treatment group HCV RNA levels rose again between day 1 and 2 after an initial decline (Figure 9a). This was not seen in patients during induction therapy (Figure 9b). Among genotype 1 patients treated with standard therapy, the majority had no or limited virological response (Figure 9c), whereas three patients had an early decline in HCV RNA levels of at least 2 log₁₀, all whom became sustained responders (Figure 9c). Among genotype 1 patients in the induction group, two out of three had a greater than 3 log₁₀ viral load decline at day 56, whereas one patient only dropped 1 log₁₀ (Figure 9d).

The end-of-treatment response rate in the induction and standard treatment groups was 8/10 (80%) and 14/21 (67%), respectively (p=ns); and the sustained response rate was 6/10 (60%) and 10/21 (48%) respectively (p=ns).

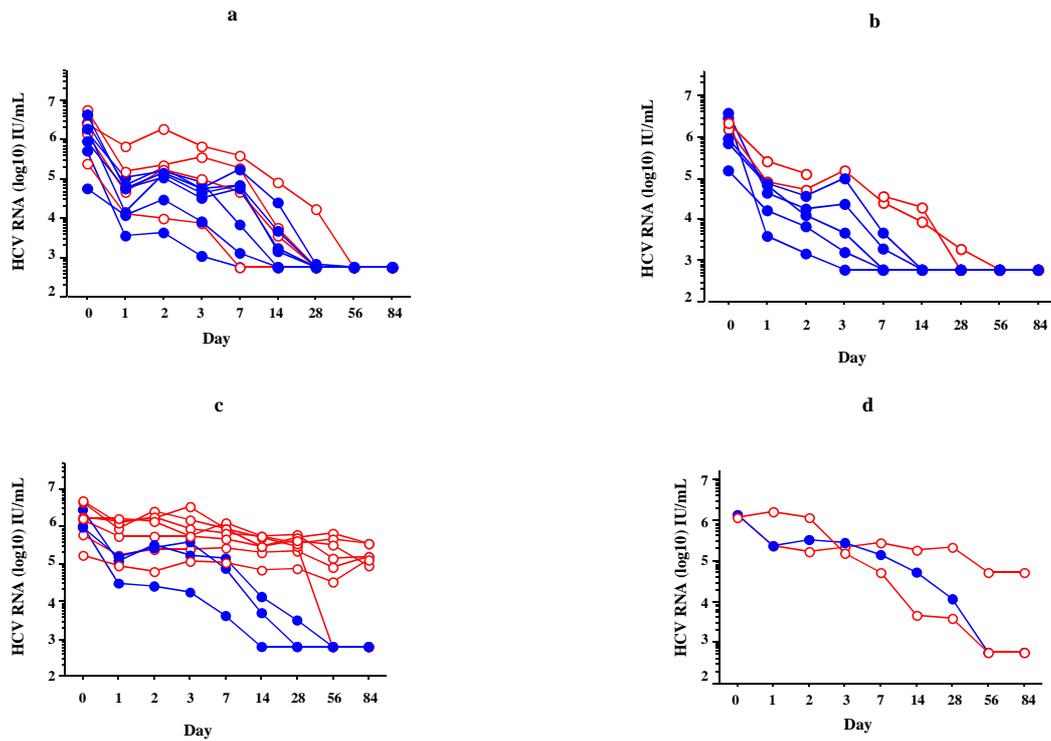


Figure 9. Individual HCV RNA levels during treatment with IFN, administered either daily for two weeks followed by thrice weekly treatment (=induction) or thrice weekly from start (=standard), in combination with ribavirin. Closed and open circles indicate that serum HCV RNA was undetectable or detectable, respectively, at the end of the follow-up period post treatment. **Fig 9a** depicts the viral levels in 11 patients infected with genotype non-1 receiving standard therapy. **Fig 9b** depicts the viral levels in 7 patients infected with genotype non-1 receiving induction therapy. **Fig 9c** depicts the viral levels in 10 patients infected with genotype 1 receiving standard therapy. **Fig 9d** depicts the viral levels in 3 patients infected with genotype 1 receiving induction therapy.

Early HCV RNA kinetics according to final treatment outcome

Totally, 16 patients became sustained responders, six became response/relapsers, seven became non-responders, and two had a virological breakthrough response with undetectable HCV RNA day 56 during treatment but were again positive for HCV RNA at the end of treatment. Four out of 16 patients with a sustained response; one out of six with a response/relapse, and all seven with a non-response, were infected with genotype 1. At baseline, viral levels did not differ between the response and non-response groups, but already at day one after one dose of IFN, the response group had significantly lower HCV RNA levels. The mean decline in viral load from baseline in the sustained response group was 1.4 log₁₀ (range 0.7-2.9 log) or 96% (range 79-99%) day 1 versus only 0.3 log₁₀ (range 0-0.8 log) or 55% (range 0-83%) in the non-

response group. At day 1, the mean viral load decline from baseline in sustained responders infected with genotype 1, was 1.1 log₁₀ (range 0.8-1.5 log) or 91% (range 83-97%). Thus, after the first IFN dose, all sustained responders had a HCV RNA decline of at least 0.7 log₁₀ or 79%. At day 14 and 28, the mean HCV RNA decline from baseline in the sustained response group was 2.4 and 3.0 log₁₀, respectively, and all sustained responders had a HCV RNA decline of at least 1.5 and 2.1 log₁₀, respectively, at these time points. The median HCV RNA levels and mean log₁₀ decline in patients with sustained response, response/relapse and non-response at baseline and days 1, 14 and 28 during treatment, are given in Table III.

Table III. Median HCV RNA levels ($\times 10^6$; IU/mL) and mean HCV RNA decline (log₁₀) from baseline during interferon and ribavirin treatment.

	Baseline	Day 1		Day 14		Day 28	
	HCV RNA (range)	HCV RNA range)	Decline (range)	HCV RNA (range)	Decline (range)	HCV RNA (range)	Decline (range)
SR (n=16)	1.20 (0.06–4.30)	0.06 (0.004-0.24)	1.4 (0.7-2.9)	0.0006 (0.0006-0.05)	2.4 (1.5-neg)	0.0006 (0.0006-0.01)	3.0 (2.1-neg)
RR (n=6)	1.95 (0.25-5.80)	0.12 (0.01-0.81)	1.1 (0.7-1.5)	0.007 (0.0006-0.48)	1.5 (0.9-neg)	0.0006 (0.0006-0.28)	1.7 (1.2-neg)
NR (n=7)	1.40 (0.17-4.50)	0.52 (0.09-1.60)	0.3 (0-0.8)	0.29 (0.07-0.55)	0.7 (0.4-0.9)	0.34 (0.07-0.59)	0.7 (0.4-1.1)
P value*	ns	SR vs NR; p<0.05		SR vs NR; p<0.05 RR vs NR; p<0.05		SR vs NR; p<0.05 RR vs NR; p<0.05	

* Mann Whitney U test
 SR = sustained responders
 RR = response/relapsers
 NR= non-responders

In conclusion, we found that IFN induction treatment in combination with ribavirin during the first two weeks of therapy resulted in a faster HCV RNA decline as compared to standard treatment. The difference in HCV RNA levels, however, was significant only day 2 and day 7 and only in patients infected with genotype non-1. In genotype 1 infected patients, no significant difference was noted between the two treatment schedules. This could however, be due to the low number of patients in the induction group with genotype 1. Overall, patients infected with genotype non-1 had

a more pronounced first- and second-phase viral decline, and a significantly higher sustained response rate, as compared to patients with genotype 1. Finally, we found that by monitoring the viral load decline from baseline a non-response to IFN/ribavirin treatment can be predicted in the individual patient with a high likelihood already after the first IFN dose.

6.3 PAPER IV

Of the 67 patients included in the study, two were withdrawn from treatment after two weeks and excluded from further analyses. The 65 remaining patients with a mean age of 39 years (range 19-66 years) (40 men and 25 women) were treated for at least 12 weeks. Four patients discontinued treatment due to non-response after 12 weeks, and were excluded from the viral end of treatment response analysis. Two further patients with viral end of treatment response were lost before follow-up. At baseline no significant differences were noted between the induction and standard treatment groups concerning demographic data, except for a lower mean age and shorter disease duration in the induction treatment group compared to the standard treatment group (36 vs 43 years, $p < 0.02$; and 9 vs 15 years, $p < 0.02$, respectively). The mean viral load at baseline was 1.26 (0.02-6.1) million IU/mL in the induction group as compared to 0.98 (0.024-5.01) million IU/mL in the standard treatment group (ns). For patients with genotype 1, these figures were 0.92 (0.02-4.0) million IU/mL vs 0.92 (0.024-3.2) million IU/mL (ns) and for genotype non-1, 1.59 (0.079-6.1) million IU/mL vs 1.07 (0.12-5.01) million IU/mL (ns) respectively. At weeks 4, 8, and 12 the mean viral load declined significantly more in genotype non-1 patients as compared to genotype 1 (Figure 10a) ($p < 0.0001$ at all time points).

The mean viral load decline was greater in the induction group than in the standard treatment group at weeks 4, 8, and 12 (Figure 10b) ($p < 0.02$, 0.054, and 0.01, respectively). Among genotype 1 infected patients, induction treatment resulted in a significant greater decline in HCV RNA levels at weeks 4 and 8 ($p < 0.05$), but not at week 12 ($p = 0.06$) (Figure 10c). For genotype non-1 infected patients no significant difference between the treatment groups was noted at any time point.

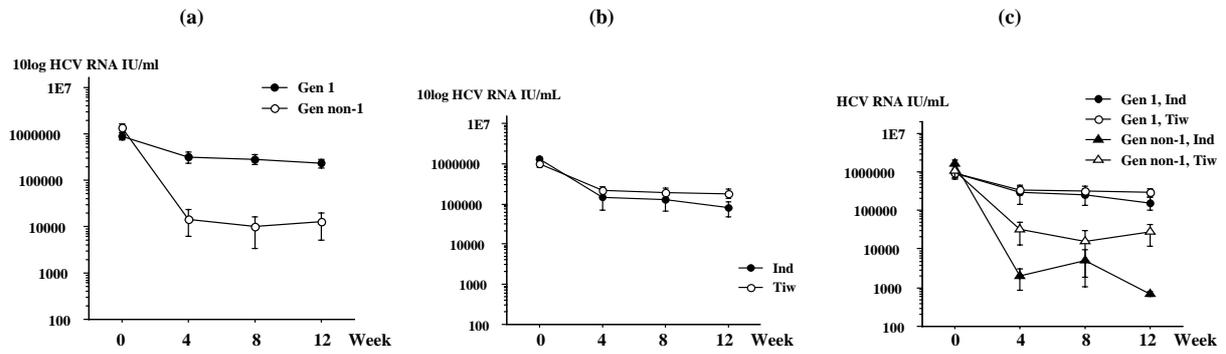


Figure 10. Hepatitis C virus (HCV) RNA levels (mean±SE) according to: (a) genotype 1 or non-1; (b) treatment group (Ind, induction treatment group; T.i.w., standard treatment group); (c) genotype and treatment group at baseline and weeks 4, 8, and 12 during the initial interferon monotherapy.

HCV RNA kinetics in the individual patients according to genotype and treatment

Figure 11 shows individual HCV RNA curves during the first 12 weeks of treatment for sustained virological responders and non-responders with genotype 1 (11a and b) and non-1 (11c and d) respectively, according to initial induction or standard treatment.

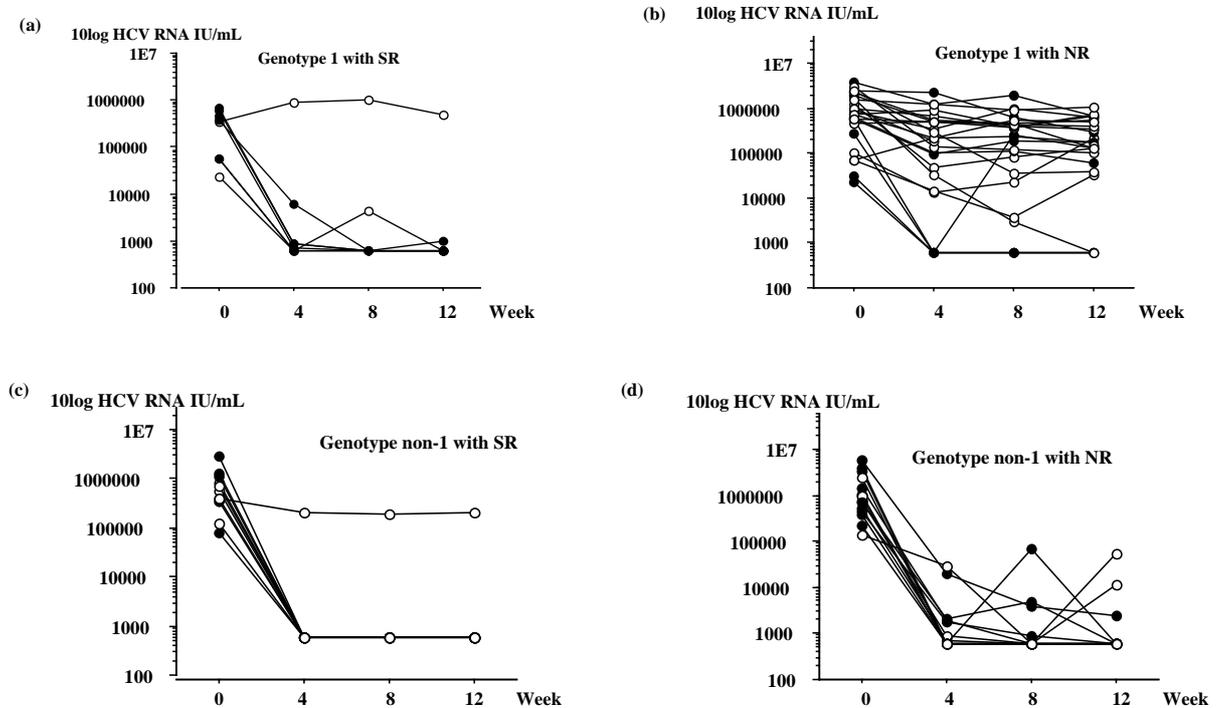


Figure 11. Sustained virological response or non-response at follow-up (a negative or positive qualitative hepatitis C virus (HCV) RNA test, respectively, at follow-up) according to (a, b) genotype 1 or (c, d) non-1, and according to initial induction (filled circles) or standard treatment (open circles). The individual HCV RNA levels during the initial 12 weeks of interferon monotherapy are shown.

All sustained responders had a steep HCV RNA decline by week 4 except two patients (Figure 11a and c) on standard treatment (one with genotype 1 and one non-1) with almost a flat response during the first 12 weeks of treatment. These two patients had ribavirin added during week 12-36, which reverted their initial virological non-response to a sustained response.

Five of the 8 patients with genotype 1 and sustained virological response (Figure 11a), had ribavirin added from week 12, and four of these 5 had standard treatment from start. Two other patients had initial high-dose induction treatment followed by high dose IFN standard treatment for 24 weeks in total. Only one patient, a female with the lowest HCV RNA level at baseline, received standard IFN mono-therapy during the entire 48 weeks of treatment.

Eleven genotype non-1 infected patients were virological sustained responders and all of them had a rapid decline in HCV RNA levels except one (Figure 11c) that lacked such a response but later achieved a sustained virological response after ribavirin was added weeks 12-36. The 13 non-responders with genotype non-1 infection who had an initial 2-3 log decline in HCV RNA levels at week 4, but later did not achieve a virological sustained response, did not receive ribavirin, with one exception (Figure 11d).

This confirms that genotype 2 and 3 infected patients have a more rapid and pronounced decline in HCV RNA levels during IFN mono-therapy as compared to genotype 1 infected. Induction therapy did not induce a more pronounced decline in HCV RNA levels in patients with genotype 2 and 3 at the measured time-points. However, in Paper II this could be seen temporarily during the first 14 days of treatment in this patient category. Daily induction therapy seems to induce a more lasting and pronounced decline in HCV RNA levels in genotype 1 infected patients compared to standard dosing t.i.w. This difference is less conspicuous when IFN is given in combination with ribavirin (Paper III). The more pronounced decay in HCV RNA levels induced by initial IFN induction therapy seemed to improve the virological sustained response rates when ribavirin was added to IFN mono-therapy after the initial 12 weeks of treatment in Paper II. Other studies have shown benefit for genotype 2 and 3 when IFN induction therapy is used in combination with ribavirin (121) but not for genotype 1 infected patients (123). As earlier mentioned one study showed benefit with induction treatment for genotype 1 infected patients when high doses (10 MU) of IFN was used (125). Ribavirin is thought to exert its effect mainly by reducing the relapse rate after treatment stop (122). In this study,

however, an initial virological non-response to IFN mono-treatment reverted to response after ribavirin was added.

In conclusion, in paper IV we found that genotype non-1 infected patients have a more pronounced HCV RNA decline during the first 12 weeks of IFN mono-therapy as compared to genotype 1 infected. Induction therapy did not induce a more pronounced HCV RNA decline at the time points measured. The vast majority of patients that accomplished a virological sustained response had a steep fall in HCV RNA levels by week 4, in most cases by 3 log₁₀. The few patients that achieved a sustained response despite lacking a viral load decline had their treatment changed and supplemented with ribavirin.

6.4 PAPER V

Of 60 patients recruited for the study, 53 were included in the final analysis. No significant difference in baseline characteristics was noted between genotype 1 and genotype non-1 infected patients except for baseline mean ALT levels, which were higher in genotype non-1 infected (p=0.009).

Overall virological outcome

Thirty-four of the 53 patients achieved sustained virological response. Eight of 19 (42%) genotype 1 infected patients versus 26/34 (76%) genotype non-1 (genotypes 2 or 3) infected patients achieved a sustained virological response (p=0.0127). Among genotype 1 infected, 2/9 of those treated 24 weeks, and 6/10 of those treated 48 weeks achieved a virological sustained response (p=n.s.).

HCV RNA decline in the individual patients

In genotype 1 infected patients with sustained response, the HCV RNA decline was greater at week 1 and week 4 compared to patients with non-response (week 1; p=0.0018 and week 4; p=0.0026) (Figure 12a). Also response/relapsers had a greater HCV RNA decline at week 1 and week 4 compared to non-responders (week 1; p=0.0367 and week 4; p=0.0167) (Figure 12a).

In genotype non-1 infected patients, sustained responders had a more pronounced HCV RNA decline compared to the only non-responder at week 1 and week 4, however not significant (Figure 12b).

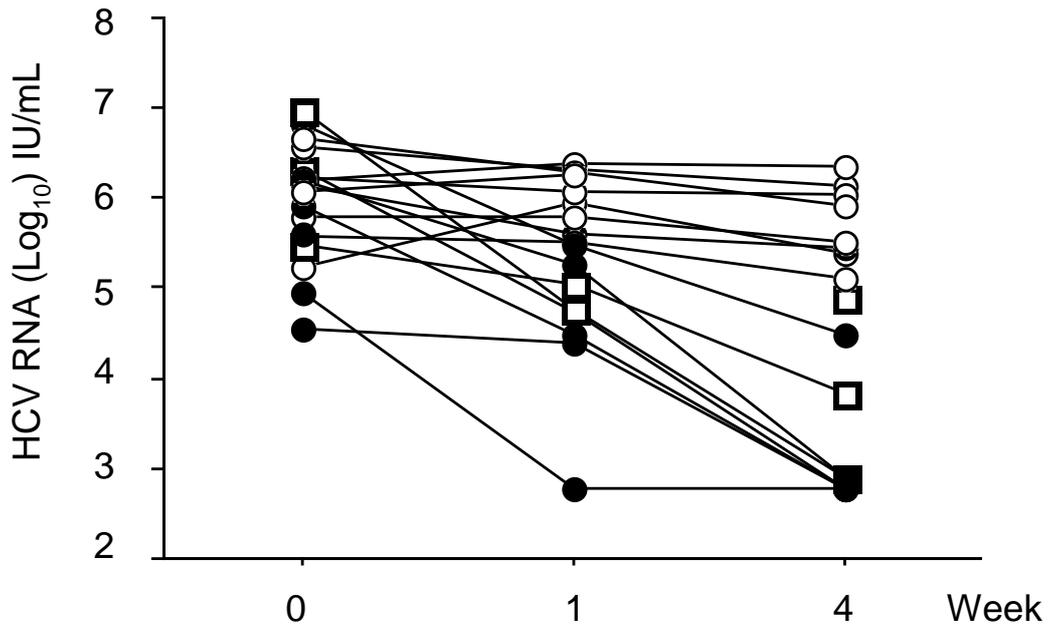


Figure 12a. Individual HCV RNA levels in patients with genotype 1 infection during treatment with pegylated interferon alpha-2a and ribavirin. Filled circles = sustained virological responders; open circles = non-responders; open squares = responder/relapsers.

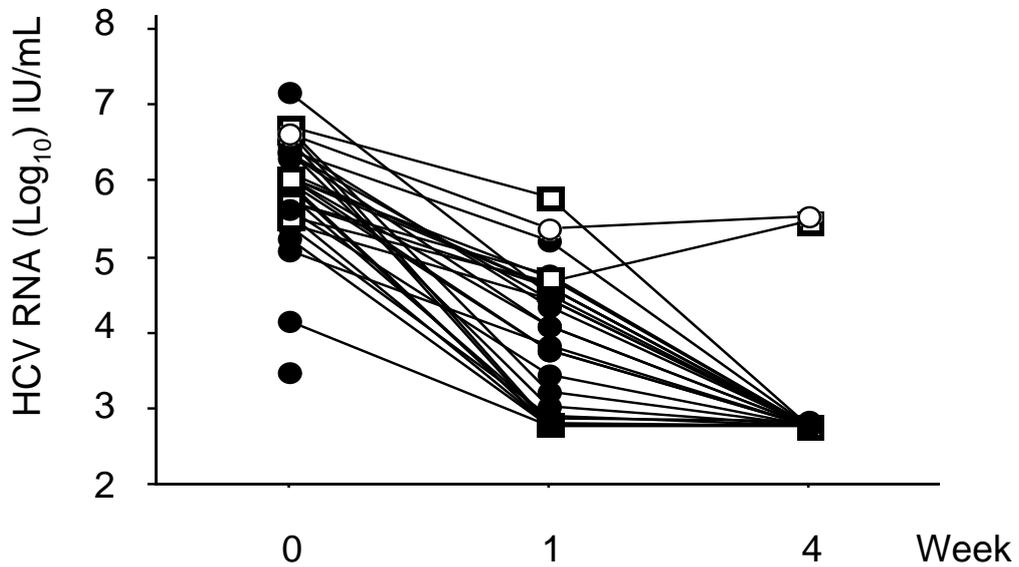


Figure 12b. Individual HCV RNA levels in patients with genotype non-1 infection during treatment with pegylated interferon alpha-2a and ribavirin. Filled circles = sustained virological responders; open circles = non-responders; open squares = responder/relapsers.

Sub-group analysis of HCV RNA kinetics at day 1, week 1, and week 4.

In the 15 patients analysed also day 1, no significant difference was noted in HCV RNA levels between sustained responders and the combined group of responder/relapsers and non-responders at day 1, whereas it was noted week 1 ($p=0.0265$) and week 4 ($p=0.0293$) when sustained responders had a more pronounced HCV RNA decline. This difference at week 1 and week 4 was noted also for the sub-group of patients infected with genotype 1 (0.12×10^6 IU/mL vs 1.8×10^6 IU/mL; $p=0.0201$, and 0.0008×10^6 IU/mL vs 0.81×10^6 IU/mL; $p=0.0455$, respectively) (Figure 13a). All patients with genotype non-1 ($n=6$) in this subgroup became sustained responders (Figure 13b).

When the HCV RNA \log_{10} decline from baseline to day 1 in genotype 1 and genotype non-1 infected was compared, no significant difference was noted. The decline from baseline to week 1, before the second dose of peg-IFN was administered, however, was significantly more pronounced in genotype non-1 infected patients as compared to genotype 1 infected ($p=0.0292$).

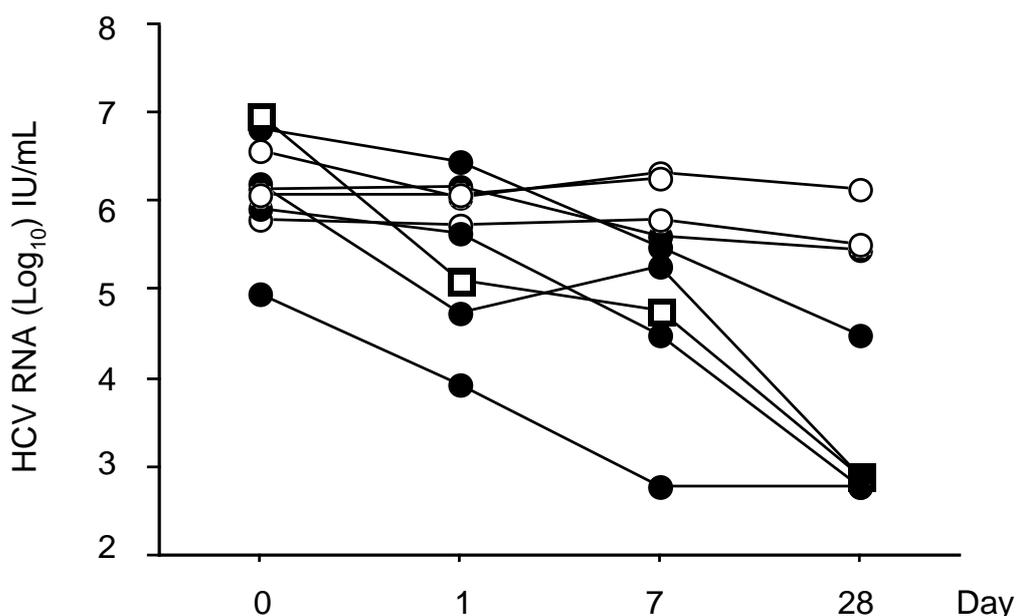


Figure 13a. Individual HCV RNA levels in patients with genotype 1 infection day 1, 7, and 28 during treatment with pegylated interferon alpha-2a and ribavirin. Filled circles = sustained virological responders; open circles = non-responders; open squares = responder/relapsers.

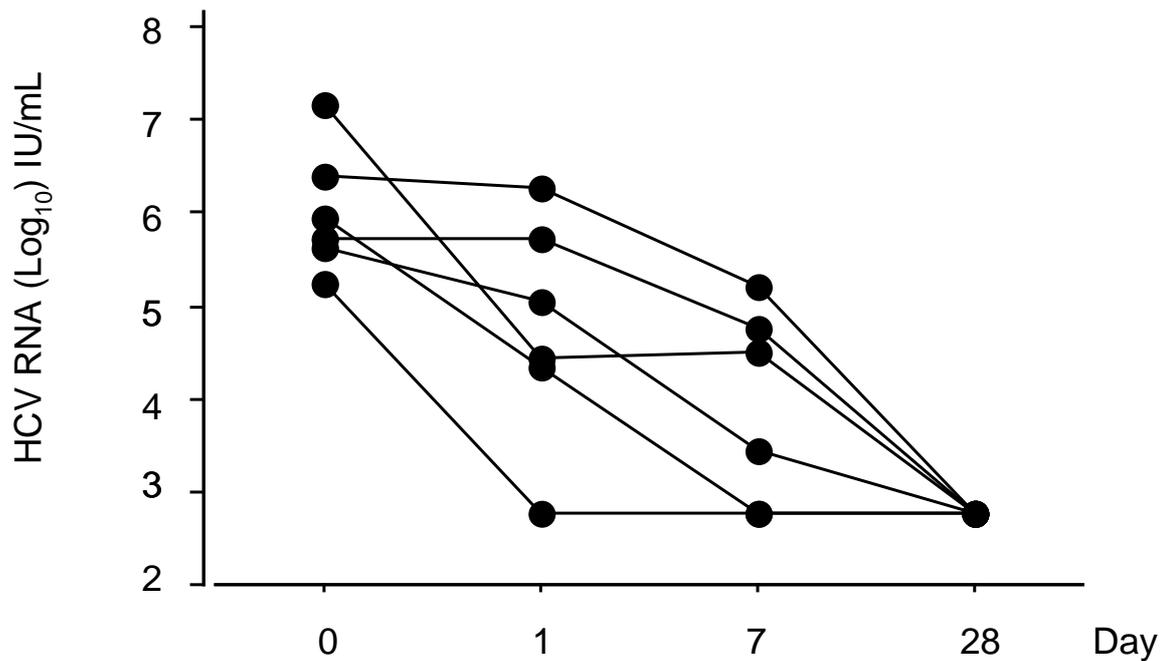


Figure 13b. Individual HCV RNA levels in patients with genotype non-1 infection day 1, 7, and 28 during treatment with pegylated interferon alpha-2a and ribavirin. SR = sustained virological responders.

HCV RNA kinetics week 1 and week 4 among all patients

Overall, sustained responders had significantly lower serum HCV RNA levels week 1 and week 4 compared to the combined group of responder/relapsers and non-responders (week 1; $p < 0.0001$, and week 4; $p = 0.0004$) (Table IV). Furthermore, sustained responders also had a more pronounced HCV RNA decline as compared to the combined group of response/relapsers and non-responders at week 1 and week 4 ($p = 0.0003$, and $p = 0.0159$, respectively) (Table IV). The decline in HCV RNA levels from week 1 (before the second dose of peg-IFN) to week 4 was greater in sustained responders compared to the combined group of response/relapsers and non-responders ($p = 0.0528$), and non-responders separately ($p = 0.0455$).

Table IV. Median HCV RNA levels and ranges ($\times 10^6$ IU/mL) and mean \log_{10} HCV RNA decline from baseline during peg-interferon alpha 2a and ribavirin treatment in 53 patients with chronic hepatitis C virus infection.

	Baseline	Week 1	Decline (range)	Week 4	Decline (range)
	HCV RNA (range)	HCV RNA (range)		HCV RNA (range)	
SR Total (n=34) ^a	0.93 (0.0029-1.47)	0.0067 (0.000599-0.39)	1.99 (+0.25-3.78)	0.000599 (0.000599-0.28)	2.89 (0.66-4.39)
Genotype non-1 (n=26) ^b	0.93 (0.0029-1.47)	0.0042 (0.000599-0.16)	2.24 (+0.25-3.78)	0.000599 (0.000599-0.000700)	3.03 (0.69-4.39)
Genotype 1 (n=8) ^c	1.06 (0.03-6.7)	0.0547 (0.000599-0.39)	1.16 (0.15-2.18)	0.000599 (0.000599-0.28)	2.46 (0.66-3.53)
Non-SR Total (n=19) ^d	1.5 (0.16-9.1)	0.2805 (0.000645-2.30)	0.85 (+0.72-3.86)	0.10 (0.000599-2.20s)	1.72 (+0.17-4.06)
Genotype non-1 (n=8)	1.45 (0.33-5.3)	0.043650 (0.000645-0.62)	1.61 (0.85-3.86)	0.000599 (0.000599-0.34)	2.75 (0.06-3.95)
Genotype 1 (n=11) ^e	1.5 (0.16-9.1)	1.029 (0.058000-2.30)	0.24 (+0.72-2.2)	0.29 (0.000800-2.20)	0.89 (+0.17-4.06)
SR vs non-SR*	p=n.s.	p<0.0001	p=0.0003	p=0.0004	p=0.0159
GT1 SR vs Non-SR*	p=n.s.	p=0.0063	p=0.0168	p=0.0039	p=0.010
GTnon-1 SR vs Non-SR*	p=n.s.	p=0.010	p=0.0583	p=n.s.	p=n.s.

*Mann-Whitney U test

^a Week 1: n=31

^b Week 1: n=24

^c Week 1: n=7

^d Week 1 + 4: n=18

^e Week 1 + 4: n=10

SR = sustained responders; Non-SR = response/relapsers and non-responders; GT1=genotype 1; GT-non1=genotype-non1

Log₁₀ HCV RNA decline among all patients according to final outcome

Week 1

Eleven of the 34 sustained responders (32.3%) had $>2 \log_{10}$ drop in HCV RNA at week 1 compared to only 1/21 (4.8%) in the combined group of responder/ relapsers and non-responders, $p = 0.0161$, and no non-responder reached a comparable decline (Table IV).

Week 4

Among sustained responders, the HCV RNA decline to week 4 continued and at that time point only 4/34 sustained responders had HCV RNA levels above 600 IU/mL, the detection limit of the test (Figure 12a and 12b). At week 4, 36 patients had a $>2 \log_{10}$ drop in HCV RNA levels. Of these, 29 had genotype non-1 infection (23 sustained responders and 6 response/relapsers), and 7 genotype 1 (6 sustained responders and 1 response/relapser).

Week 12

Only 1/34 (2.9%) sustained responders was positive for HCV RNA at week 12 versus 11/19 (58%) of those who did not achieve a sustained response ($p < 0.0001$). This sustained responder was infected with HCV genotype 1b strain and had a minimal HCV RNA decline at week 1 and week 4.

Predictability of final outcome according to viral decline weeks 1, 4, and 12.

The positive predictive value for a later virological sustained response was 92%, 77%, and 81% at week 1, 4, and 12 respectively, when a $>2 \log_{10}$ drop in viral load week 1, or week 4, or a negative HCV RNA test at week 12 was used as criteria for response (Table V). The negative predictive value for a later non-sustained response (i.e. response/relapse or a non-response) was 51%, 61% and 92% at week 1, 4 and 12 respectively, when the aforementioned criteria was not met (Table V).

Table V. Predictability of sustained response and non-sustained response to treatment with peg-interferon alpha 2a and ribavirin when using $>2 \log_{10}$ drop in viral load from baseline at week 1 or 4 or a $>2 \log_{10}$ drop and/or a negative HCV RNA test at week 12.

	SR	Non-SR	Total
Week 1 ($>2\log_{10}$ drop)			
Yes	11	1	12
No	20	19	39
Total	31	20	51
Week 4 ($>2 \log_{10}$ drop)			
Yes	27	8	35
No	7	11	18
Total	34	19	53
Week 12 (HCV RNA negative)			
Yes	33	8	41
No	1	11	12
Total	34	20	53

Abbreviations: SR = sustained responders; Non-SR = non-responders and response/relapsers; NR = non-responders; PCR = polymerase chain reaction; PPV = positive predictive value; NPV = negative predictive value.

Week 1: PPV = $11/12=92\%$; NPV for non-SR = $19/39=51\%$

Week 4: PPV = $27/35=77\%$; NPV for non-SR = $11/18=61\%$

Week12: PPV = $33/41=81\%$; NPV for non-SR = $11/12=92\%$

An early prediction of a later response or non-response to treatment is warranted to avoid unnecessary costs and adverse events. Treatment week 24 was initially considered to be the best time point for prediction of a non-response, but presently treatment week 12 is considered to be the most appropriate time point for prediction of a later non-response if a $>2 \log_{10}$ drop in viral load or a negative HCV RNA test has not been achieved (126, 133). Prediction of non-response has been claimed possible already at day 1 during treatment with standard IFN by us, and by others in a subset of patients (120, 134), but in this small study this was not possible. By week 1 however, before the second peg-IFN dose had been given, a significant difference

was noted between these groups ($p=0.02$), which was seen also week 4 ($p=0.0293$) (Figure 13a).

The HCV RNA \log_{10} decline was greater in patients with genotype non-1 infection already at week 1 compared to genotype 1 infected ($p=0.0292$). In patients who achieved a $>2 \log_{10}$ drop in HCV RNA levels already week 1 or 4 during treatment a positive predictive value (PPV) of 92% and 77% respectively could be made for a sustained response but a low negative predictive value (NPV) of 51% and 61% respectively when such a response was not achieved (Table V).

Since peg-IFN has slower absorption compared to standard IFN (78 hours versus 10 hours) resulting in later maximum serum concentration (74), we might have missed an early difference in HCV RNA decline by analysing HCV RNA levels already day 1. The HCV RNA decline day 7, still after only one single dose of peg-IFN had been given in combination with ribavirin, however, as mentioned earlier offered a better chance to predict a later sustained virological response.

In conclusion, in paper V we found that during treatment with peg-IFN-alpha 2a in combination with ribavirin, analysis of HCV RNA levels already day 1 was not useful for prediction of treatment outcome in contrast to findings during treatment with standard IFN and ribavirin. Week 1, on the other hand, can be used with a better precision for predicting sustained virological response in the subgroup of patients who have achieved a $2 \log_{10}$ drop in HCV RNA levels, and week 12 for predicting a non-response if such a decline has not been achieved confirming the presently used guidelines.

7 GENERAL DISCUSSION AND CONCLUSIONS

Chronic HCV infection is a widespread disease with a global prevalence of at least 3%. Of infected subjects 20-30% will develop end stage liver disease with cirrhosis within 20-30 years of whom 2-4% will develop HCC per year (5). End stage liver disease due to chronic HCV infection is the most prevalent indication for liver transplantation in the Western world. There is a need for improved therapy in patients with chronic HCV in particular those infected with genotype 1. Treatment for chronic HCV infection has improved considerably during the last decade, but still almost 50% of genotype 1 infected patients will not clear their infection with the presently used treatment combination (68, 69, 76, 78, 135). Since genotype 1 is the most prevalent genotype a large proportion of patients will thus not achieve a sustained virological response with the presently used therapy. Whether persons with non-response to therapy have a primary resistant virus, or if resistance is induced during therapy is not known. Suboptimal initial therapy, however, is likely to promote development of resistance by inducing emergence of escape mutants in particular if periods of low or no serum interferon levels are present during treatment.

During recent years the knowledge of HCV has increased including the knowledge on the viral kinetics during antiviral therapy. The HCV RNA decline in serum has been shown to be at least biphasic during treatment. The first rapid phase lasts for 24-48 hours when a substantial drop in viral load up to 3 log₁₀ can be observed. The second phase is believed to reflect clearance of infected hepatocytes and is probably immune mediated and lasts for weeks - months in most responders. In true non-responders no decline in viral load at all can be observed. In order to avoid unnecessary treatment with adverse events for the individual, and high costs for the society, it is desirable to predict the final outcome as early as possible. The early HCV kinetics during different antiviral treatment regimens can be used to characterise, individualize, and optimize treatment for chronic hepatitis C.

With the purpose to determine and further characterise the dynamics of the HCV RNA levels during the early phases of different antiviral treatment regimens, and to determine the impact that genotype has on the kinetics, and to predict and possibly further optimise the evaluation of treatment response, we studied: 1 the HCV kinetics during triple therapy with IFN, ribavirin, and amantadine in previous non-responders and response/relapsers to standard IFN/ribavirin treatment; 2 the HCV RNA kinetics

in patients with low baseline viral load during treatment with standard IFN given as initial induction dosing (daily dosing) followed by standard t.i.w. dosing or standard t.i.w. dosing from onset; 3 the HCV RNA kinetics during combination therapy with IFN and ribavirin; 4 the HCV RNA kinetics during IFN induction dosing during the initial 10-14 days followed by standard dosing, compared to standard dosing from onset; 5 the HCV RNA kinetics during combination therapy with peg-IFN-alpha 2a and ribavirin.

In **Paper I** we found that addition of amantadine to standard combination therapy had little if any beneficial effect. Data from two recently published pilot studies had indicated that amantadine was effective against chronic HCV infection (108, 109). Thirteen non-responders and 10 response/relapsers to a previous course of peg-IFN and ribavirin were given triple therapy including amantadine 200 mg/day orally during 24 weeks. At the end of treatment, one previous non-responder and five previous response/relapsers had become HCV RNA negative, but only one previous response/relapser achieved a final sustained virological response. The limited effect of amantadine when added to standard combination therapy has later been confirmed in a meta-analysis of several trials (84).

In **Paper II** we studied the HCV RNA levels during the first 84 days of IFN induction (daily dosing during the first 14 days and thereafter t.i.w.) and standard t.i.w. treatment in 31 treatment naive patients with low pre-treatment HCV RNA levels ($<1.2 \times 10^6$ IU/mL). Already at day 2, the median HCV RNA level was significantly lower in the induction group as compared to the standard treatment group. This difference persisted during the study period for patients infected with genotype 1, but was not maintained from day 14 and onwards for patients infected with genotype non-1. Eighty percent in the induction group versus 16% in the standard t.i.w. group achieved undetectable HCV RNA levels (<600 IU/mL) at day 84 ($p<0.05$).

In **Paper III**, we studied the HCV RNA levels during the first 84 days of IFN induction and standard t.i.w. treatment with the addition of ribavirin in 32 treatment naive patients. Treatment duration was 24 weeks in patients with genotype non-1 or genotype 1 with low baseline viral load ($<1.2 \times 10^6$ IU/mL), and 48 weeks for patients with genotype 1 and high baseline viral load. The initial HCV RNA decline was more pronounced in the induction group. Overall, and in contrast to what was found in Paper II, this difference was small and significant only at day 2 and day 7 in patients infected with genotype non-1.

Furthermore, already at day 1 the mean HCV RNA decline from baseline was significantly greater in the group of patients who became sustained virological responders as compared to non-responders; and all sustained responders had a viral load decline of minimum 0.7 log₁₀ (79%) after the first dose of IFN. Thus lack of early decline in viral load seems to predict non-response. This can be used for a decision to stop or continue therapy in the individual patient.

In **Paper IV** pooled data from the study in Paper II and from a Lithuanian study were used in 65 patients to study the HCV RNA kinetics week 4, 8, and 12 during IFN induction and standard treatment. A total of 34 patients received induction treatment and 31 standard treatment. Patients infected with genotypes 2 and 3 had a significantly steeper decline in HCV RNA levels at all time points ($p < 0.001$) as compared to genotype 1 infected. Induction therapy resulted in a more pronounced HCV RNA decline at weeks 4, 8, and 12 ($p < 0.02$, 0.054, and 0.01, respectively) as compared to standard scheme. With few exceptions, patients with a sustained viral response had a 3 log₁₀ decline in viral levels at week 4. Two patients (one genotype 1 and one non-1) with an initial viral non-response during the first 12 weeks of treatment, responded with viral clearance after addition of ribavirin.

In **Paper V** we studied serum HCV RNA levels in 53 treatment naive patients with chronic HCV infection at week 1, week 4, and week 12 during treatment with peg-IFN-alpha 2a and ribavirin and the usefulness of the early HCV RNA kinetics to predict the final virological treatment outcome. In a sub-group of 15 patients HCV RNA levels were in addition analysed also at day 1. Patients who achieved sustained virological response had a significant more pronounced decline in HCV RNA levels at week 1 and week 4 compared to the combined group of response/relapsers and non-responders ($p = 0.0003$, and $p = 0.0159$, respectively). This was also noted in genotype 1 infected patients with sustained response versus non-response ($p = 0.0018$, and $p = 0.0026$, respectively).

The HCV RNA decline to day 1, however, was not useful for prediction of treatment response. The decline week 1, on the other hand, could be used for prediction of a later virological response with a positive predictive value of 92% in the subgroup of patients who had achieved a 2 log₁₀ drop in HCV RNA levels, and week 12 for a later non-response in patients who lacked such a response with a negative predictive value of 92%.

In an untreated patient the serum HCV RNA levels are fairly stable over time and an equilibrium between the amount of virus produced by infected hepatocytes and the

amount cleared by the immune system is established. When antiviral treatment is given, serum HCV RNA levels decline if the viral strain is sensitive to the therapy given. We have shown that the viral decay differs between individuals according to genotype, and that a difference in viral decay is noted early during treatment between patients who will finally clear the virus and patients who will not.

When peg-IFN-alpha 2a was used in combination with ribavirin, no difference, however, was noted in HCV RNA levels between responders and non-responders already after the first treatment day. At week 1 and week 4, however, sustained responders had a significantly more pronounced drop in HCV RNA levels as compared to those who did not achieve a sustained response. We also found that a positive predictive value of 92% was noted for a final virological sustained response in the subgroup of patients who had achieved a 2 log₁₀ drop in HCV RNA levels after the first week of treatment with peg-IFN-alfa 2a in combination with ribavirin, and that the best time point for prediction of a non-response was week 12 when a negative predictive value of 92% was noted in patients who did not achieve this decline in viral levels.

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10 APPENDIX: PAPERS I, II, III, IV, AND V