# STATISTICAL MODELS OF TREATMENT EFFECTS IN CHRONIC HEPATITIS B AND C

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# Statistical Models of Treatment Effects in Chronic Hepatitis B and C

## Statistische modellen van behandelingseffecten in chronische hepatitis B en C

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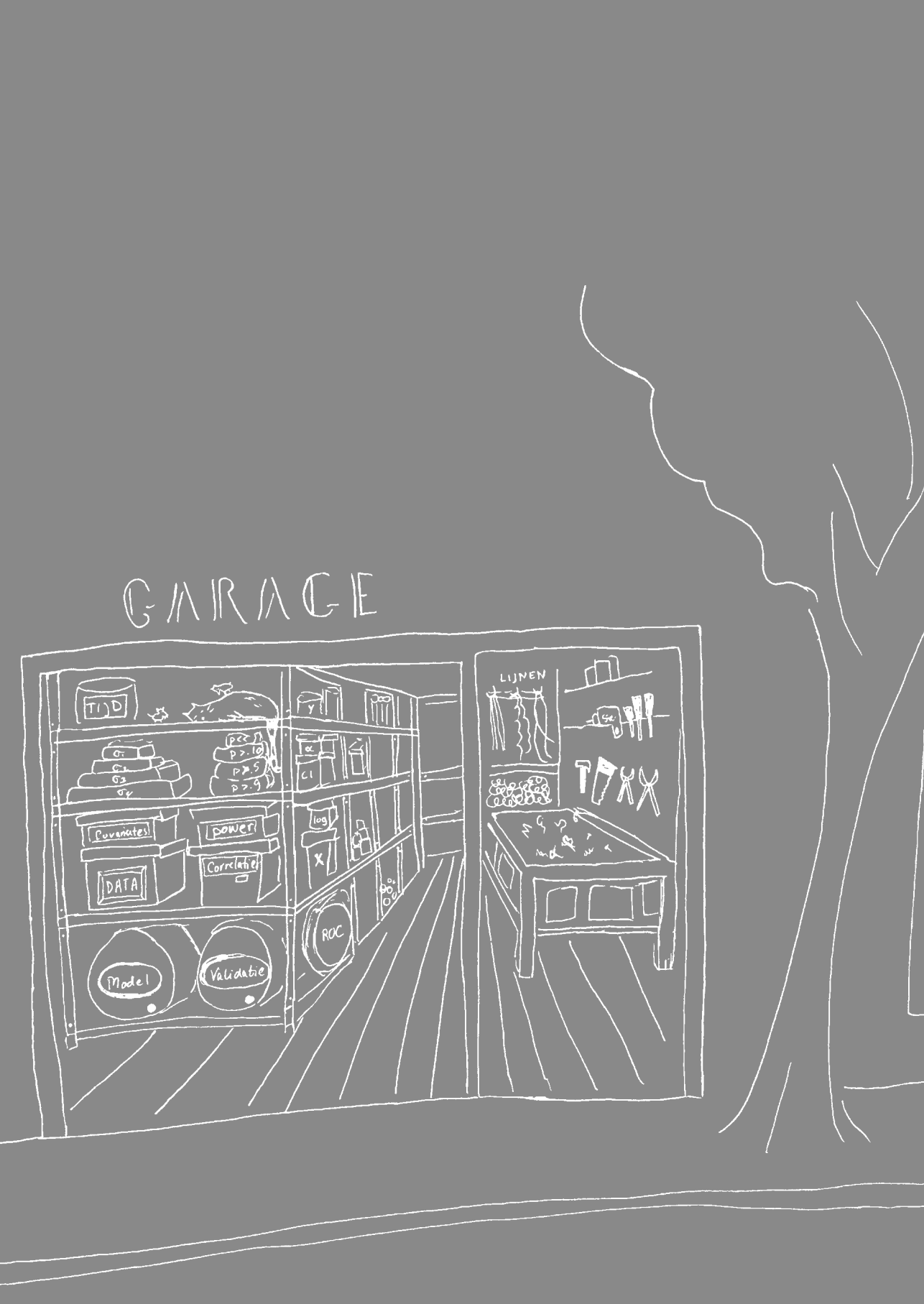
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# CHAPTER 1

Introduction

# INTRODUCTION

This thesis regards treatment effects in patients with chronic hepatitis B and C, and focuses on the tools that are used to analyse these effects. In this introduction the clinical background of hepatitis B and C along with the current treatment options are described. Clinical questions arisen from the effects of treatment are stated and the current state of art of statistical models to study these effects is provided.

# CHRONIC HEPATITIS B

## Hepatitis B virus

Hepatitis B is a viral infection of the liver. The virus was discovered in 1963-1966 in Australian aboriginals.[1-2]

The hepatitis B virus can be transmitted by sexual contact or by contact with infected blood, for example by blood transfusion or intravenous drug abuse. There is a substantial risk of transmission in cases of birth from an infected mother, in obtaining body piercings and tattoos and in sharing tooth brushes or razors with an infected person.[3] The infectiousness of the virus is high which is demonstrated by the fact that the risk of transmission if contact with infected blood is 100 times higher compared to transmission of HIV.[4] Initial symptoms of acute infection with hepatitis B virus are commonly nonspecific flu-like symptoms, more specific symptoms are profound loss of appetite, dark urine, yellowing of the eyes and abdominal discomfort. The virus resolves in 95% of adult cases,[5] contrary to this younger patients become chronic carriers in 30% of cases and in babies born from infected mothers 90% become chronic carriers.[6]

Chronic HBV infection is defined as detectable hepatitis B surface antigen (HBsAg) in patient's serum for at least six months duration. Patients typically present in one of four phases[7] based on the hepatitis B e antigen (HBeAg) status, the amount of viral replication expressed by serum HBV DNA levels and the damage of liver tissue expressed by the serum alanine aminotransferase (ALT) levels: (1) the immune-tolerant phase where HBeAg is detectable (positive), HBV DNA levels are high, while ALT levels are normal (2) the immuno-active phase where ALT levels rise, (3) the immune–control phase defined by low HBV DNA and normalization of ALT, and (4) the final phase where HBeAg has become undetectable. In a considerable number of patients with negative HBeAg, viral replication and damage of liver tissue persist and these patients develop active HBeAg negative chronic hepatitis B.

Chronic hepatitis B infection is a progressive liver disease, which can lead to cirrhosis, liver failure or hepatocellular carcinoma (HCC). The annual incidence of cirrhosis and HCC in patients with chronic hepatitis B is about 6% and 1-2%, respectively.[8]

## Epidemiology

Worldwide 350 million people are affected by chronic hepatitis B and yearly 500,000 people die of HBV-related liver disease, such as cirrhosis and HCC.[4, 9-10] The prevalence and the transmission route of hepatitis B infection vary throughout the world.[10] In the Netherlands the prevalence of chronic hepatitis B is low (<0.5%).[11] In the larger cities like Rotterdam and Amsterdam the prevalence is higher. Risk groups with a high prevalence include immigrants from areas with intermediate or high prevalence of HBV infection (6-8% within the Chinese immigrants[12], 5-6% within the Turkish immigrants[13]), intravenous drug users, males who have sex with males and people with multiple sexual contacts. Although safe and effective vaccine has been available for more than 20 years, HBV infection remains an important health problem. To this date only risk-groups are vaccinated in the Netherlands.

## Antiviral treatment for chronic hepatitis B

The main goal of treatment is to improve survival by preventing progression to cirrhosis, liver failure or HCC. Sustained suppression of the HBV replication is associated with an improved long-term prognosis.[14] The first goal of treatment is therefore to achieve sustained response defined as the suppression of the viral replication. Two therapeutical approaches are available against hepatitis B infection: interferon based therapy in order to induce sustained off-treatment response and nucleoside and nucleotide analogues which aim at maintaining viral suppression during prolonged therapy. At present seven drugs are licensed for the treatment of chronic hepatitis B: interferon alpha, pegylated interferon alpha, lamivudine, adevovir, entecavir, telbuvidine and tenovovir.



# CHRONIC HEPATITIS C

## The hepatitis C virus

Hepatitis C like hepatitis B is a viral infection that attacks the liver. This virus was discovered in 1989 but already earlier recognized as the non-A non-B virus.[15]
Similar to hepatitis B, hepatitis C can be transmitted by contact with infected blood. The risk of transmission of hepatitis C virus is 10x lower than of hepatitis B virus.[16] There is a small risk of transmission in cases of birth from an infected mother, body piercing and tattoos and in sharing tooth brushes or razors with an infected person.[17-19] Sexual transmission of hepatitis C rarely occurs in monogamous heterosexual couples.[20]
The symptoms of an acute infection are often subclinical and only a minority of patients experience severe symptoms such as jaundice and fever. About 60-85% of infected

patients becomes chronic, a much higher rate than after infection with hepatitis B virus in adults. Symptoms are nonspecific, like fatigue and it make take years before patients discover that they are chronically infected with hepatitis C.
Chronic hepatitis C is a slowly progressive liver disease. It has been estimated that 10-20% of patients develop liver cirrhosis within 10-30 years and for those with cirrhosis the yearly risk of developing HCC is about 1-5%.[21]

### Epidemiology

Chronic hepatitis C affects about 170 million people worldwide.[22] The prevalence is high in Egypt(>10%), Asia(5-10%) and southern Europe (1-2.5%).[23] In the Netherlands the prevalence is low (<1 %). Hepatitis C is most prevalent among drug-users, among prison inmates and among haemophiliacs who received blood products before 1991. There is no vaccine against hepatitis C virus.

### Antiviral treatment for chronic hepatitis C

Sustained suppression of the HCV replication is associated with a better long-term prognosis.[24] The first goal of treatment is therefore to achieve sustained viral response. The standard therapy for chronic hepatitis C is a combination of pegylated interferon with ribavirin, with the duration of treatment depending on the HCV genotype. At this moment new agents are evolving fast which creates hope for those patients who do not respond to the standard therapy.

## THE STATISTICAL MODELS

During the last few decades, various statistical models have been used to study treatment results of chronic hepatitis B and C. Treatment options for both hepatitis B and C are expanding and there is thus a growing demand for individual first-line treatment recommendation. This can only be achieved by studying the effects of treatment in detail. Which patients benefit from treatment, in whom could treatment better be stopped and what are the early and long-term effects of treatment? One needs to take in mind that as the options for treatment are changing, the recommendations will change along. New studies with new drugs will therefore always be needed. The tools to study the patient specific effects of treatment are statistical models. In this thesis, advanced statistical models are developed and applied to achieve better insight into how individual patients react to their treatment.

## Prediction models with correction for overfitting

To develop an individual baseline prediction to a dichotomous response of treatment statistical logistic regression techniques are used. The aim of the final model is to identify which patient characteristics or disease specific factors are independently or in combination with each other associated with response. As a result the prediction of response for the individual patient can be assessed.

The design of a good model is a laborious process of comparing different strategies and combinations of covariates using statistical measurements of model fit and performance measurements, such as the Akaike's Information Criteria (AIC) and the Area Under the receiver operating Curve (AUC), in combination with sound statistical knowledge and logical sense, build on knowledge from previous studies and clinical experience.[25-26]

When a well-fitted and stable prediction model has finally been achieved the fitted coefficients needs to be corrected for overfitting. Because the model has been designed on a fixed dataset it is known that the model in general will tend to overfit when applied to a new individual or dataset. As a result the predictive performance will therefore be worse. The best option to solve this issue is to validate the model in an independent and similar dataset. When this option is not available bootstrapping[26] is an established method to study the degree of overfitting. Penalized likelihood estimation[26] can also be used to fit a penalty-score to correct for overfitting.

The final step is to present the prediction model and the method will depend on the audience and the user. The model in general is quite complex, and mathematical formulas can present difficulties in interpretation to some users. Instead nomograms or graphical presentations can be used along with medical decision trees. Another option might be the design of a website which generates a specific predicted probability of response after entering the required patient specific variables.

## Dynamic prediction models

During treatment patients will be monitored regularly. Dynamic update of an individual's prediction of response to treatment based on new information is not routinely implemented in prediction models, but can be of great importance for the individual subject and the further choice of treatment.

Two different statistical approaches are considered: (1) directly modelling the prediction of the outcome variable with the use of logistic regression techniques and (2) indirectly classifying individuals into an outcome category over time using Bayes' theorem.

For the direct approach (1) either the observed marker value or the subject specific pattern of the marker are used as predictors. A generalized estimating equations (GEE)

approach to directly enter the observed marker values in a pooled logistic regression is introduced. In contrast, parameters describing the patterns of the markers on an individual subject can be used as predictors: first a linear mixed regression model is designed, to obtain subject specific patterns of the longitudinal markers and afterwards the estimated random effects are entered in the logistic regression of the clinical outcome, while adjusting for the estimation error of the random effects.[27] For the indirect approach (2) two steps are needed: first the longitudinal profiles of the markers are modelled separately for each outcome group using multivariate linear mixed effect models, hereafter the posterior prediction of response over time is calculated.[28]
The performance of these models varies depending on the clinical question. For comparison of the discriminative ability the area under the receiver operating curves can be used. The models furthermore allow to explore different stopping rules aiming at identifying a stopping time for a subgroup of patients for whom continuation of therapy is meaningless.

## G-estimation and marginal structural mean models with inverse probability of treatment weights.

To study the long-term benefit of treatment on a significant clinical event, like the development of HCC, commonly a cohort follow-up study is performed, since a randomized clinical trial cannot be realized, due to the large numbers of patients or long duration of the study required. In this set-up however, indication of therapy depends on the disease state, measured by a longitudinal surrogate biomarker. If progression of the disease is observed treatment is often started, where after hopefully the state of the disease may improve. If on the other hand the state of the disease stays under control the treatment is not initiated. These relationships are represented in a directed graph (figure 1). Thus the treated group is not comparable to the untreated group and standard methods for survival analysis entering the treatment as a time-dependent covariate, ignoring the time-dependent covariate may therefore produce a biased result.
Two methods are available to estimate the causal effect of a time-dependent treatment in the presence of a time-dependent covariate that is both a confounder and an intermediate variable: the method of G-estimation[29] and the Marginal Structural Models (MSMs).[30-31] The G-estimation and the MSMs allow for appropriate adjustment for confounding. The G-estimation estimates the expansion- or contraction- parameter $\psi$ of the time to event has the treatment never been given. The MSM estimates the hazard rate of exposure through inverse-probability-of-treatment weighting. While the

standard method fails to estimate the true causal effect of treatment on the clinical outcome, both methods offer a sophisticated solution.

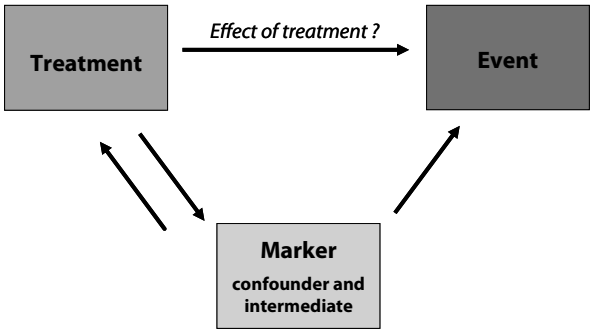


**Figure 1.** Directed graph presenting the model of the effect of treatment on a clinical event in the presence of a maker which is both a confounder as well as an intermediate.

## Pharmacokinetic and pharmacodynamic models

Modelling the immediate biological effect of an antiviral-drug on the virus decay has proven to be an important tool in predicting early response to therapy of chronic viral hepatitis.[32] Estimation of the parameters of the viral decay not only gives insight in the action of the drug but may also suggest a different treatment regimen: prolongation of therapy, more frequent administration or using a higher dose. To describe how the individual patient reacts to treatment often a pharmacokinetic-study is considered, measuring the viral load (the HBV DNA) frequently the first 4 weeks of treatment as well as the weeks after treatment is stopped.

Entecavir and Tenovovir are new nucleoside/nucleotide analogues (NA) against hepatitis B virus. In phase II studies treatment effects during the first 4 weeks are investigated. Next to the evaluation of efficacy and safety in a dose escalating study the pharmacodynamics (PD) is compared between the different doses. An overall picture of the dose effect, however, is only achieved, if also the recurrence of the virus after withdrawal of therapy is investigated in detail.

During treatment with NA's the viral decline of hepatitis B is well described by an exponential bi-phasic model (figure 2): an initial phase of fast elimination of free virus and a slow second phase indicative of the death rate of infected cells. After treatment a rebound is observed, where the mechanism that takes place during viral replication shows a new bi-phasic pattern (figure 3): a fast doubling time followed by a more graduate increase to a more a less a steady state approaching the pre-treatment viral concentration. To describe this relapse-curve the mirror image of the viral dynamic model during therapy was used.

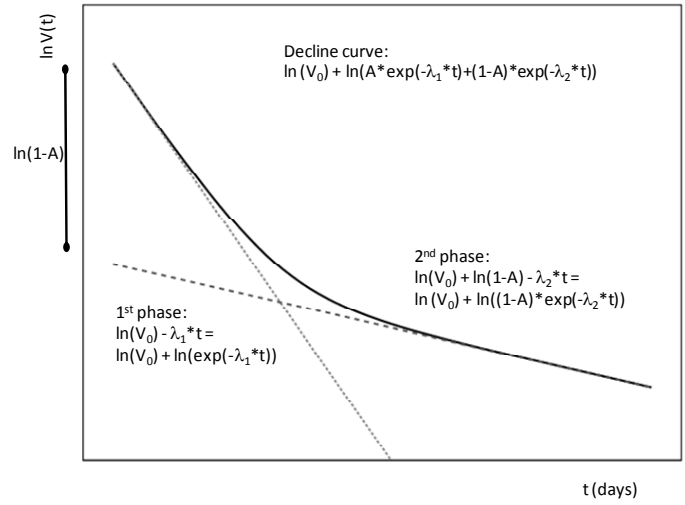


**Figure 2.** During NA treatment: the viral decline is described by a biphasic exponential model. The interpretation of the parameters of the model is: $V_0$ is the initial viral load, $A$ is related to the treatment efficacy, with $ln(1-A)$ ~ total decrease in the first phase $\lambda_1$ is the clearance rate of free virus, $\lambda_2$ is related to the death rate of productively infected cells.

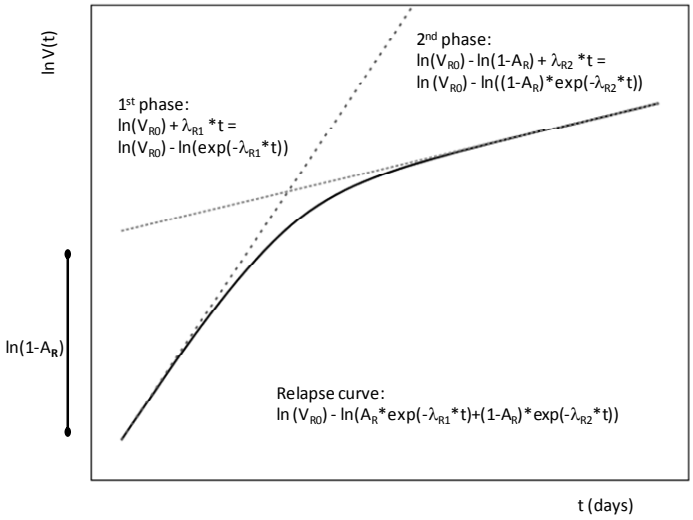


**Figure 3.** The viral rebound after stop of NA treatment is described by a mirror image of the usual biphasic model. The interpretation of the parameters of the model is: $V_{R0}$ is the viral load by end of treatment, $A_R$ is related to the treatment efficacy, with $ln(1-A_R)$ ~ total increase after the first phase, $\lambda_{R1}$ is the growth rate of free virus, $\lambda_{R2}$ is related to the 'birth' rate of productively infected cells, if $\lambda_{R2}$=0 a stable state has been reached.

The extended non-linear PD-models describing HBV DNA during and after therapy can be fitted with available statistical software in two ways: (1) separately for each patient with non-linear regression and (2) combined for all patients with mixed non-linear methods adding random effects to obtain individual patient curves.

Pegylated interferon behaves different than NA's. The drug is standard injected once a week for 48 weeks and the injection of one dose of pegylated interferon $\alpha$-2b (PEG-IFN) results in a decrease of hepatitis B viral load, followed by a slow increase as the drug concentration in the blood declines (see figure). Until now it has been assumed that the effectiveness of the drug stayed constant between injections, but with the observed increase of viral load at the end of the week, new models are necessary to interpret and describe viral kinetics.

When treated with PEG-IFN the biphasic model is not adequate to describe the pattern of the HBV DNA and more complex models are required. First the drug concentration is assessed with a one-compartment model and the results incorporated in the model describing the viral load during the first week (figure 4). As a result, the PEG-IFN concentration, the viral load and also the effectiveness during one week after one injection can be fitted. Assuming that the drug concentration and the viral load pattern after the first injection repeats itself after each injection, the viral and drug kinetics are fitted with a periodical continuation during the first month. The fitted concentration and viral decline allows for comparison of biologically relevant patient characteristics, such as body weight and HBV genotype, which may be important for future treatment protocols. Similar to the analysis of the pharmacokinetic of the NA either non-linear regression analysis per subject or non-linear mixed regression analysis on all subjects with random effects on the parameters can be applied.



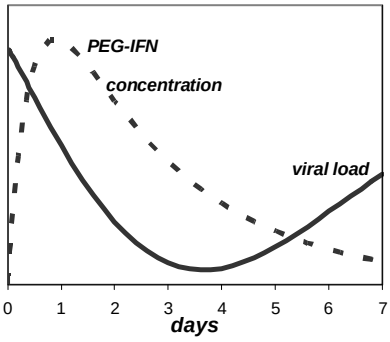


**Figure 4.** The patterns of interferon concentration and viral load after on injection of PEG-IFN.

# AIMS

In this thesis we aim to develop a prediction model of sustained response to PEG-IFN treatment in patients with HBeAg positive chronic hepatitis B. We will introduce new dynamic model methods and improve existing ones to update the prediction of response to treatment when longitudinal markers are available. These methods are applied to chronic hepatitis B patients receiving PEG-IFN therapy. The methods are used as a guide to identify patients, who do not benefit from PEG-IFN, and whom may be advised to discontinue therapy as early as possible in the treatment schedule.

Furthermore, we aim to investigate the long-term effect of glycerrhicin treatment on the development of HCC in chronic hepatitis C patients, who were all non-responders to interferon treatment. A statistical model that corrects for bias when treatment is given depending on the state of the disease is applied.

Finally we aim to describe the early treatment effects of nucleoside/nucleuotide analogous and PEG-IFN in HBeAg positive chronic hepatitis B patients with pharmcokinetic and pharmcodynamic models.

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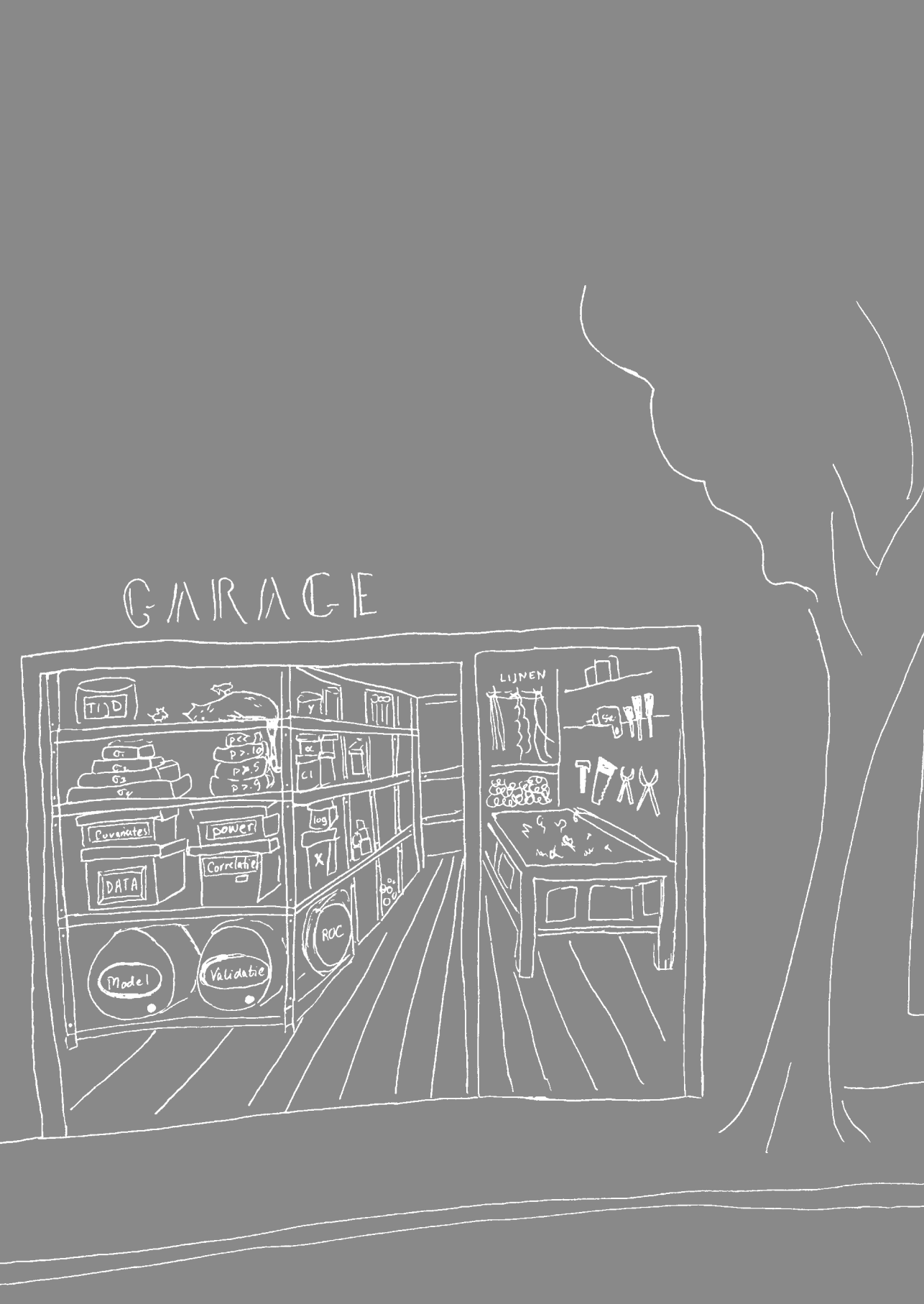
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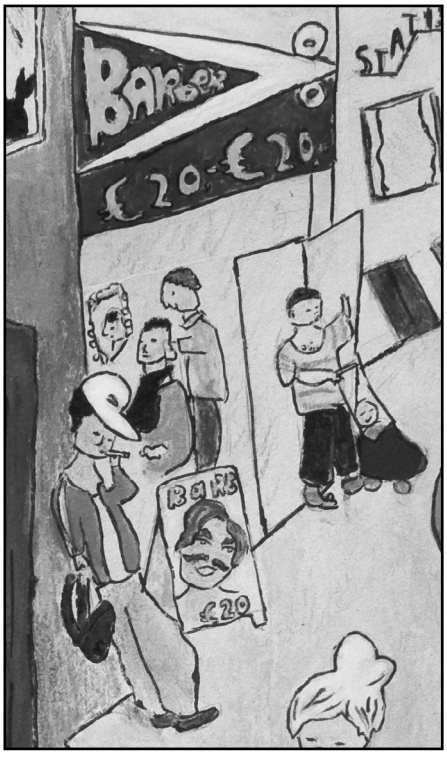
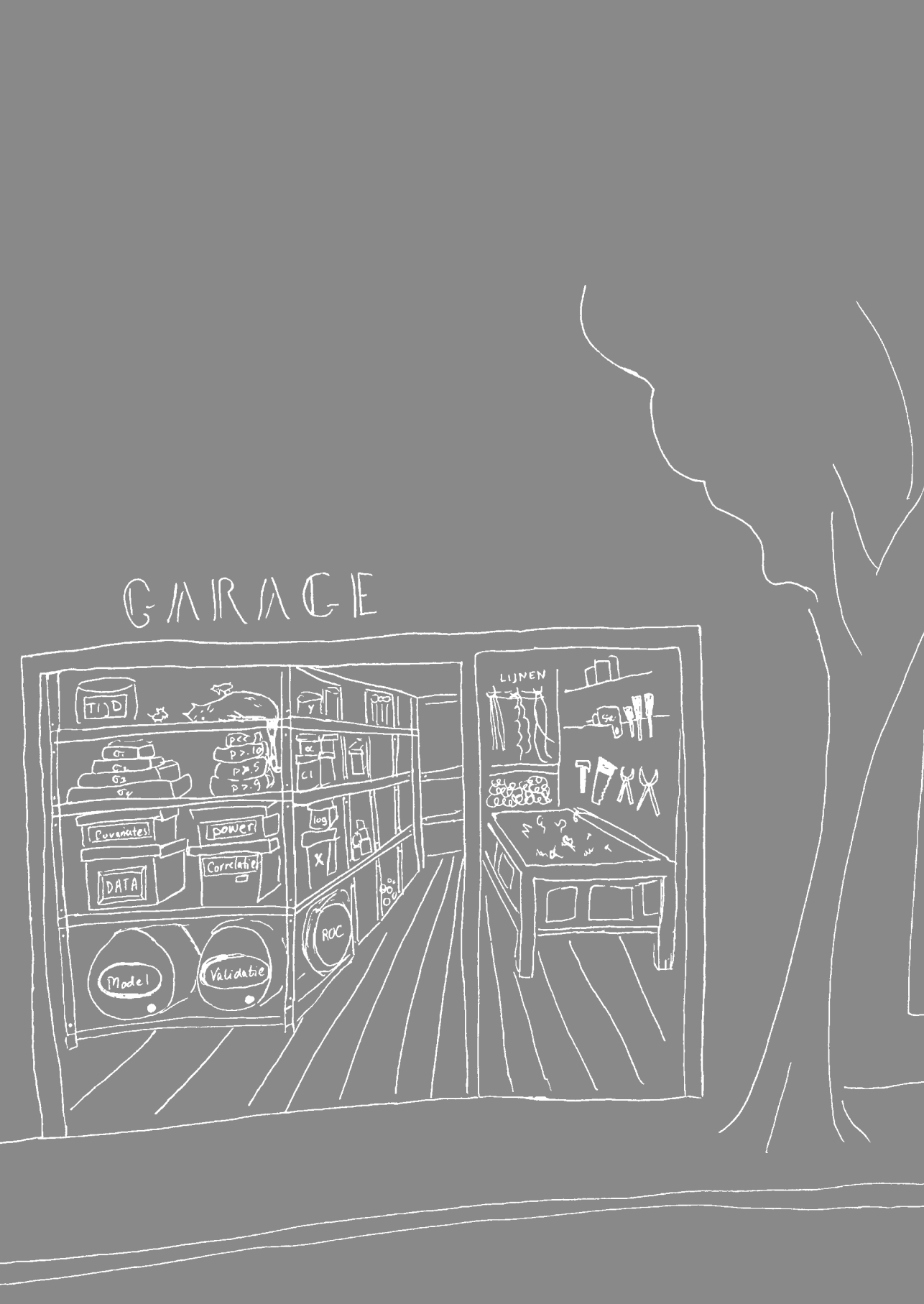
# CHAPTER 2

Prediction of response to treatment

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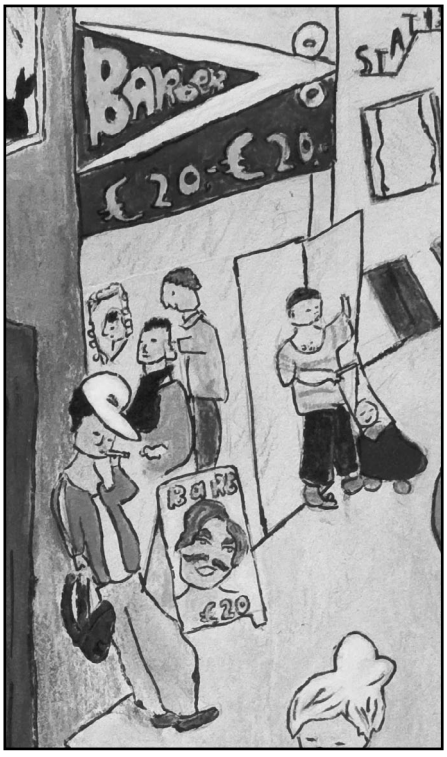
# CHAPTER 2.1

## HBV DNA suppression in HBeAg-positive chronic hepatitis B patients treated with peginterferon or placebo

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*Submitted*

## ABSTRACT

The aim of this study was to compare the decline of HBV DNA during peginterferon (PEG-IFN) therapy with spontaneous fluctuations in viral load in placebo-treated patients with HBeAg-positive chronic hepatitis B.

A total of 136 HBeAg-positive patients who participated in a randomized trial were treated with PEG-IFN alfa-2b for 52 weeks. This group was compared with 167 HBeAg-positive patients who received placebo for 48 weeks using linear mixed regression analysis. Response was defined as negative HBeAg at end of treatment (EOT).

Overall, decline of HBV DNA at EOT was larger in the PEG-IFN group compared with placebo (mean decline 2.3 versus 1.0 log, $p<0.001$) and varied according to HBV genotype. Viral suppression was stronger in the PEG-IFN group compared with placebo starting at week 4 and throughout the entire treatment period ($p<0.001$ adjusted for baseline ALT). The response rate was higher for PEG-IFN than placebo (32% versus 11%; $p<0.001$). Among responders, HBV DNA decline was larger for PEG-IFN than placebo: at week 4 already, the mean difference in HBV DNA decline was 0.7 log ($p=0.001$), which further progressed to 2 log until EOT. ALT levels were significantly related to HBV DNA decline at the next visit and ALT flares (>5 times the upper limit) during PEG-IFN therapy were associated with a stronger HBV DNA decline compared with placebo.

PEG-IFN therapy resulted in a larger HBV DNA decline compared with placebo. Furthermore, the decline of HBV DNA was stronger in HBeAg-positive patients who lost HBeAg or who exhibited an ALT flare during PEG-IFN therapy compared with spontaneous HBeAg loss or flares occurring during placebo therapy.

# INTRODUCTION

More than 350 million people worldwide are chronically infected with the hepatitis B virus (HBV) and are at risk for cirrhosis, liver failure and hepatocellular carcinoma.[1] Hepatitis B e antigen (HBeAg-)positive chronic hepatitis B (CHB) is regarded as the earliest phase of infection.[2] Treatment is generally recommended for patients with HBeAg-positive CHB who do not clear HBeAg spontaneously, have high serum HBV DNA levels and persistently elevated alanine aminotransferase (ALT) levels.[3-4] First-line treatment options consist of third generation nucleos(t)ide analogues (NA), including entecavir (ETV) and tenofovir (TDF), and peginterferon alfa (PEG-IFN).[3-4] ETV and TDF are potent inhibitors of the HBV polymerase activity and are highly effective in maintaining suppression of HBV replication.[5-6] In contrast, PEG-IFN therapy aims at the induction of a sustained off-treatment response through its immunomodulatory effects, but only has a modest direct antiviral effect.[7] Consequently, one year of PEG-IFN therapy in patients with HBeAg-positive CHB resulted in 2.3-4.5 log copies/mL decline of HBV DNA,[8-9] while there was a 6.2-6.9 log drop of HBV DNA after 1 year of therapy with third generation NA.[5-6] In contrast to suppression of HBV DNA, HBeAg loss as a marker of immunological control was observed more often in patients treated with PEG-IFN compared with NA (29-30% versus 21-22%).[5-6, 8-9]

Fluctuations in HBV DNA and alanine aminotransferase (ALT) levels occur spontaneously during the natural course of CHB,[10] and spontaneous HBeAg clearance occurs at an annual rate of 10–15% in adults with elevated ALT levels.[11] Furthermore, the clinical significance of different HBV genotypes for the natural course and treatment response of CHB has become increasingly recognized in recent years.[12-15] Nevertheless, extensive viral kinetics data during PEG-IFN therapy have not been compared to natural occurring fluctuations in viral load during placebo therapy in patients with HBeAg-positive CHB. Therefore, the aim of our study was (1) to compare the pattern of HBV DNA decline between HBeAg-positive patients treated with PEG-IFN alfa-2b and placebo, particularly in patients achieving HBeAg loss and (2) to study the association between HBV genotype and on-treatment ALT levels with HBV DNA kinetics.

# PATIENTS AND METHODS

## Patients

A total of 136 patients treated with PEG-IFN alfa-2b monotherapy and 167 patients who received placebo within two randomized controlled trials were included in this study.[10, 16] The inclusion and exclusion criteria of both trials have been reported in detail previously.[10, 16] In summary, patients were eligible for the original PEG-IFN trial if they had

been hepatitis B surface antigen (HBsAg) positive for >6 months, were HBeAg positive on two occasions within 8 weeks prior to enrolment, had elevated serum ALT levels of 2 - 10 times the upper limit of normal (ULN), and had a serum HBV DNA level >1.0 x $10^5$ copies/mL. Major exclusion criteria were: antiviral therapy within 6 months prior to enrolment, presence of viral co-infections, pre-existing cytopenia or decompensated liver disease. Treatment comprised of PEG-IFN alfa-2b 100 µg weekly (PegIntron, Merck, Whitehouse Station, NJ, USA) for 52 weeks. The dose of PEG-IFN was reduced to 50 µg after 32 weeks of treatment.[16] The 167 patients who received placebo for 48 weeks were eligible for the original study if they had been HBsAg positive for >6 months, had a serum HBV DNA level ≥1 x $10^6$ copies/mL and an ALT level of 1.2 -10 x ULN. Key exclusion criteria were antiviral therapy within 6 months prior to enrolment, presence of viral co-infections or decompensated liver disease.[10]

## Laboratory tests

Serum HBV DNA levels were measured with 4-week intervals. For the PEG-IFN study, an in-house developed TaqMan PCR assay based on the EuroHep standard was used (lower limit of detection 400 copies/mL).[17] For the placebo group, the Roche Amplicor Monitor PCR assay was used.[10] An excellent correlation between these assays has previously been shown.[18] Serum ALT levels were measured using automated techniques and are expressed as values representing a ratio to the upper limit of the normal range (ULN). Determination of HBsAg and HBeAg was performed with the use of commercially available enzyme immunoassays. HBV genotypes were assigned by INNO-LiPa assay (Innogenetics, Gent, Belgium) or by phylogenetic analyses of HBV DNA sequences in the PEG-IFN and placebo group, respectively.[19]

## Statistical analysis

Continuous variables are expressed as means ± standard deviation (SD) or medians with interquartile range (IQR), where appropriate. Comparisons between groups were made using the Chi-square test or Fisher's exact test for categorical variables and the t-test or Mann-Whitney test for continuous variables. Patterns of HBV DNA decline were modelled using linear mixed regression analysis with a heterogeneous autoregressive covariance structure depending on treatment regimen (PEG-IFN versus placebo). Declines of HBV DNA were compared for patients with and without response, defined as HBeAg negativity at the end of treatment (EOT), by extending the model with response and interaction terms with treatment and time. The estimated declines of HBV DNA are

presented as means with 95% confidence intervals (95% CI). ALT levels were categorized according to the WHO grades: <1.25 x ULN (grade 0), 1.25-2 x ULN (grade 1), 2-5 x ULN (grade 2), 5-10 x ULN (grade 3) and >10 x ULN (grade 4). An ALT flare was defined as a serum ALT concentration >5 times ULN (grade ≥3 according to WHO). The association between ALT flares and HBV DNA decline was analysed using linear mixed regression analysis with a heterogeneous compound symmetry covariance structure depending on treatment regimen. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

# RESULTS

## Baseline characteristics

Baseline characteristics of the PEG-IFN (N=136) and the placebo (N=167) group are presented in table 1. The groups were comparable in terms of age and gender. The majority of patients in the PEG-IFN group were of Caucasian origin infected with HBV genotypes A and D, while Asian patients infected with genotypes B and C prevailed in the placebo group (table 1). Nevertheless, a substantial number of genotypes A-D were observed in both groups. Baseline HBV DNA and ALT levels were higher in the PEG-IFN compared with the placebo group (table 1).

**Table 1.** Baseline characteristics

| Characteristics | PEG-IFN (N=136) | Placebo (N=167) | p-value |
|---|---|---|---|
| Mean (SD) age, years | 36 (14) | 37 (12) | 0.53 |
| Male (%) | 107 (78.7) | 119 (71.3) | 0.15 |
| Ethnicity (%) | | | <0.001 |
| Caucasian | 101 (74.3) | 60 (35.9) | |
| Asian | 29 (21.3) | 101 (60.5) | |
| Other | 6 (4.4) | 6 (3.6) | |
| HBV genotype (%) | | | <0.001 |
| A | 47 (34.6) | 45 (26.9) | |
| B | 12 (8.8) | 26 (15.6) | |
| C | 21 (15.4) | 71 (42.5) | |
| D | 51 (37.5) | 19 (11.4) | |
| Other/mixed | 5 (3.7) | 6 (3.6) | |
| Median (IQR) ALT, ULN | 3.2 (2.3-5.2) | 2.3 (1.7-4.0) | <0.001 |
| Mean (SD) HBV DNA, log copies/mL | 9.1 (0.8) | 8.1 (0.9) | <0.001 |

## HBV DNA decline

The mean decline of HBV DNA at week 48 was more pronounced in the PEG-IFN compared with the placebo group (2.3±2.3 versus 1.0±1.3 log, p<0.001; figure 1). Linear mixed regression analysis was used to estimate the effect of PEG-IFN and placebo treatment on HBV DNA suppression. Baseline ALT and HBV DNA levels were included in the model since these baseline factors differed between the two groups. Baseline ALT but not HBV DNA was associated with HBV DNA decline (p<0.001, p=0.21, respectively). Starting at week 4 of treatment and throughout the entire treatment period HBV DNA suppression was stronger in the PEG-IFN compared with the placebo group (p<0.001 with adjustment for baseline ALT).

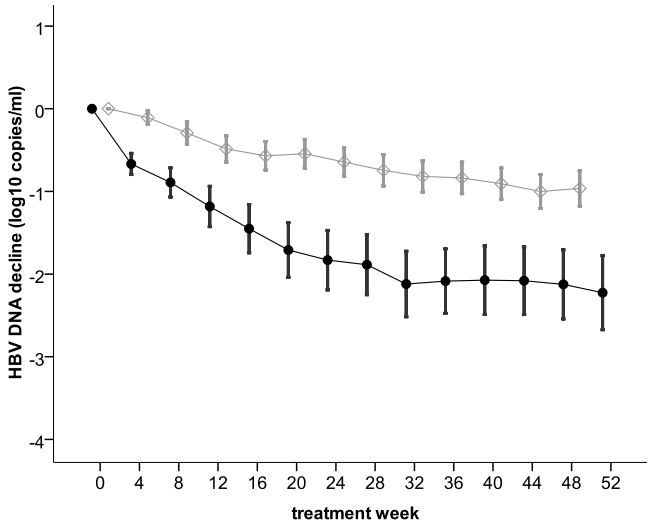



**Figure 1.** Mean decline of HBV DNA for HBeAg-positive patients treated with PEG-IFN (black) and placebo (grey).

## HBV-DNA decline according to HBV genotype

Since the distribution of HBV genotypes was different in the PEG-IFN and placebo group, we repeated the analyses for each genotype separately. HBV DNA levels decreased across all genotypes in both groups, but with different kinetics. The patterns of HBV DNA decline for genotypes A-D are shown in figure 2. After adjustment of baseline ALT and HBV DNA PEG-IFN therapy resulted in a significantly larger HBV DNA decline compared with placebo from week 16 (all p-values <0.01) and 20 (all p-values < 0.05) in patients infected with genotype A and B, in contrast to week 4 (all p-values <0.04) and 12 (all p-values < 0.04) for genotypes C and D (figure 2).

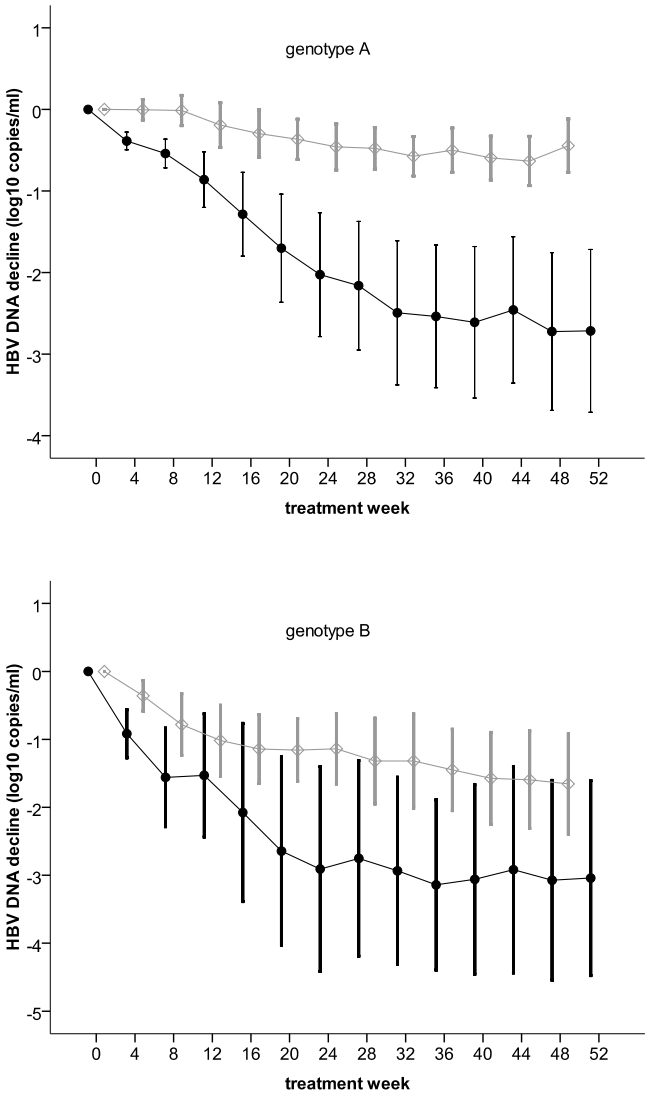
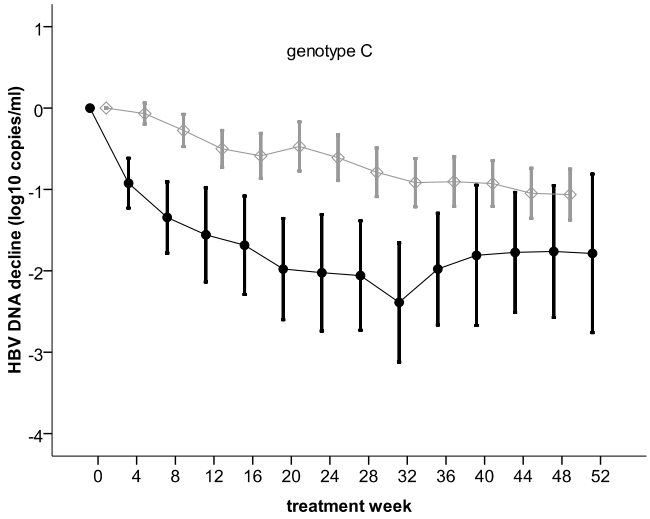



**Figure 2.** Mean decline of HBV DNA for HBeAg-positive patients treated with PEG-IFN (black) and placebo (grey) for genotypes A-D.



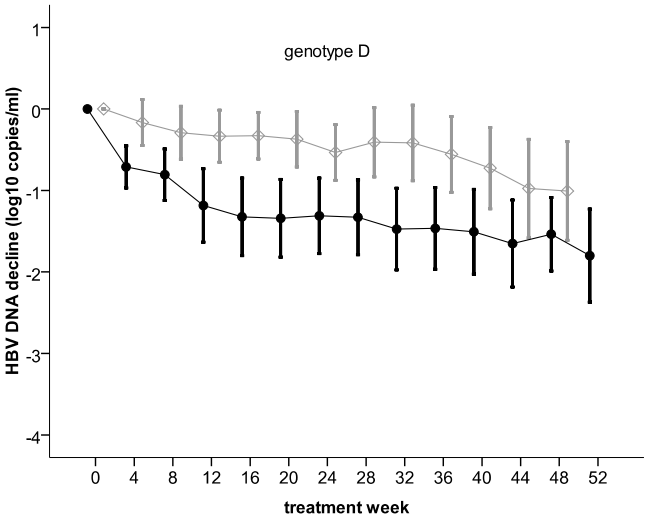




**Figure 2 continued.** Mean decline of HBV DNA for HBeAg-positive patients treated with PEG-IFN (black) and placebo (grey) for genotypes A-D.

## HBV DNA decline according to HBeAg loss

Next, we studied patterns of HBV DNA decline according to the achievement of HBeAg loss at EOT among patients with available HBeAg status. HBeAg negativity at EOT was observed in 40 (32%) of 126 patients in the PEG-IFN group compared with 16

(11%) of 143 patients in the placebo group (p<0.001). Interestingly, patients who lost HBeAg during PEG-IFN therapy exhibited a significantly stronger degree of HBV DNA suppression compared with placebo (figure 3). Already at week 4 a mean difference in HBV DNA decline of 0.60 log (95% CI [0.20; 1.01], p=0.004) was estimated using linear mixed regression analysis, which increased to 1.91 log (95% CI [1.05; 2.76]) at week 20 and remained around 2 log until week 48. Patients treated with PEG-IFN who remained HBeAg positive also had a larger HBV DNA decline compared with placebo (mean difference 0.39 log (95% CI [0.06; 0.71]) at week 48, p=0.02).

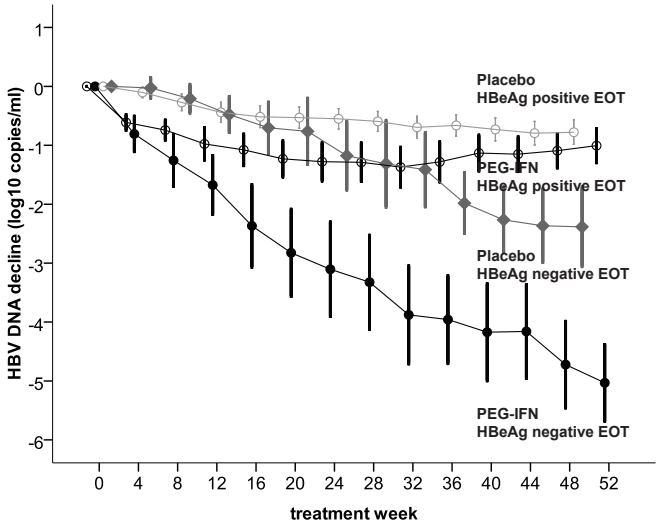



**Figure 3.** Mean decline of HBV DNA for HBeAg-positive patients treated with PEG-IFN and placebo stratified according to HBeAg loss at EOT and treatment regimen (PEG-IFN and placebo).

## Association between ALT flares and HBV DNA decline

The association between on-treatment ALT levels and HBV DNA decline was analysed using linear mixed regression analysis. Figure 4 illustrates that the decline of HBV DNA at the next visit (interval duration 4 weeks) increased with the serum ALT level at the current visit. For the PEG-IFN group, the HBV DNA did not show a decline 0.00 log (95% CI [-0.07; 0.06]) for visits with ALT <1.25 x ULN (grade 0), in contrast to a mean HBV DNA decline 0.97 log; (95% CI [0.74; 1.20] for ALT >10 x ULN (grade 4; p<0.001). The same relation was observed in the placebo group 0.08 log; 95% CI [0.03; 0.13] and 0.40 log; 95% CI [0.28; 0.52] for grades 0 and 4, respectively; p=0.008). However, the degree of viral suppression for visits with ALT grade ≥2 (corresponding with ALT >2 x ULN) in patients who received PEG-IFN was larger compared with placebo (all p-values <0.01, figure 4).

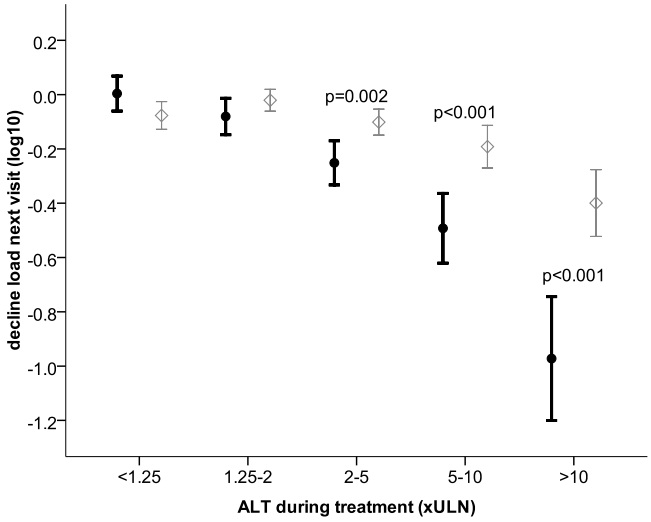



**Figure 4.** Estimated mean decline (with 95% CI) of HBV DNA at the consecutive visit depending on the WHO grade of ALT elevation. *P*-values represent the differences between PEG-IFN (black) and placebo (grey).



A flare (ALT >5 x ULN, grade ≥3) was observed in 164 (10%) of 1588 available visits in the PEG-IFN group and in 230 (13%) of 1755 visits in the placebo group (p=0.01). In the PEG-IFN group, the estimated consecutive decline of HBV DNA was significantly larger for visits accompanied by a flare compared to those without (mean HBV DNA decline 0.54 log (95% CI [0.42; 0.66]) versus 0.08 log (95% CI [0.04; 0.12]), respectively; p<0.001). The same observation was made in the placebo group (mean HBV DNA decline 0.25 log (95% CI [0.18; 0.32]) versus 0.05 log (95% CI [0.03; 0.08]) for visits with a flare versus without, p<0.001). Interestingly, the estimated HBV DNA decline was larger in case of a flare induced by PEG-IFN compared with placebo (p<0.001).

The probability of HBeAg loss at EOT was higher for patients treated with PEG-IFN who exhibited a flare compared to those without, but this difference did not reach the level of significance (21 (36%) of 58 versus 20 (28%) of 72 patients, p=0.30). The proportion of HBeAg loss at EOT was similar for placebo-treated patients with and without flare (11% in both groups).

# DISCUSSION

This study provides a detailed comparison of HBV DNA kinetics between HBeAg-positive patients treated with PEG-IFN and placebo. The results of our study show that

the immunomodulatory agent PEG-IFN induced a larger HBV DNA decline compared with placebo. Furthermore, HBV DNA kinetics was influenced by the HBV genotype. Interestingly, the decline of HBV DNA was faster and stronger in patients with PEG-IFN then placebo-induced HBeAg clearance. Moreover, our data indicate that ALT flares during PEG-IFN therapy were associated with a stronger degree of HBV DNA decline compared with placebo.

IFN-alfa has been used for the treatment of CHB for more than two decades and mainly has an immunomodulatory mode of action, although this cytokine also has a modest direct antiviral effect on HBV replication.[7] The attachment of a polyethyleneglycol (PEG) molecule to IFN improved its pharmacokinetic and pharmacodynamic properties and resulted in stronger suppression of HBV DNA compared with conventional IFN.[20] In our study, one year of PEG-IFN therapy resulted in 2.3 log decline of HBV DNA, which was significantly higher than the spontaneous HBV DNA drop of 1.0 log in placebo-treated patients. Nevertheless, the observed efficacy of PEG-IFN in suppression of HBV replication after 1 year of therapy was markedly inferior compared with third generation NA (6.2-6.9 log).[5-6]

Eight genotypes have been identified (A-H), of whom A-D are most prevalent.[21] Since the four major HBV genotypes (A-D) were represented in both groups, we had the opportunity to show that patterns of HBV DNA decline varied according to HBV genotype. PEG-IFN therapy did not result in a significantly larger HBV DNA decline compared with placebo until week 20 for genotype B and week 16 for genotype A patients. In contrast, PEG-IFN showed a more profound HBV DNA decline already at week 4 and 12 for genotypes C and D, respectively. This finding may appear to be contradictious with the high chance of genotype A patients to respond to PEG-IFN therapy.[13] However, we have previously demonstrated that patients with a delayed HBV DNA decline during PEG-IFN therapy (almost half being genotype A patients) between week 4 and 32 had the highest chance of achieving HBeAg loss and HBsAg loss.[9]

Given their distinct modes of action, different treatment endpoints are applied for the two treatment modalities.[3] While suppression of HBV DNA levels to undetectable levels is the only goal during NA therapy, loss of HBeAg is more frequently used as primary treatment endpoint for patients treated with PEG-IFN. HBeAg loss is a surrogate marker for immunological control over HBV and is associated with increased survival and a reduced risk of developing HCC.[22-23] Spontaneous loss of HBeAg occurred in 11% of placebo-treated patients after 1 year, which is similar to the rate of HBeAg loss reported in natural history studies.[11] The proportion of patients with HBeAg loss was almost three times higher in patients who received PEG-IFN, reflecting its immunomodulatory properties. An important finding of this study was that a markedly larger drop of HBV

DNA was observed in patients who lost HBeAg during PEG-IFN therapy compared with placebo. A significant difference in HBV DNA decline between the two groups was already present at week 4, which progressed to an estimated 2 log at week 48. These findings suggest that HBeAg loss induced by PEG-IFN may be accompanied by a more profound suppression of viral replication compared with spontaneous HBeAg loss.

Treatment induced and spontaneous flares of liver inflammation are often observed in patients with CHB. These flares appear to reflect the activity of the host immune system against the virus. Our study is the first to report that flares induced by PEG-IFN are accompanied with a higher consecutive decline in HBV DNA compared with that observed after a spontaneously occurring flare during placebo therapy. As previously reported, a trend towards a higher chance of HBeAg loss in PEG-IFN treated patients with an on-treatment flare was observed.[24] In contrast, the rate of HBeAg loss was similar in placebo-treated patients with or without flare. The observation that the decline of HBV DNA was stronger in patients with flares, and also in patients with HBeAg loss, during PEG-IFN therapy compared with placebo further underlines the importance of the immunomodulatory effects of PEG-IFN which results in an improved activity of the immune response against HBV.

Although this study is the first to investigate the antiviral efficacy of PEG-IFN compared with placebo, it has limitations. Since the treatment groups were derived from two different randomized controlled trials, their baseline characteristics were not completely comparable at baseline. Baseline ALT and HBV DNA levels were higher in the PEG-IFN group. Therefore, we corrected the linear mixed regression analysis of HBV DNA decline for baseline ALT. Baseline HBV DNA was not associated with the estimated HBV DNA decline. Furthermore, the distribution of HBV genotypes differed between the two treatment arms. However, genotypes A-D were present in substantial numbers across both groups, which allowed us to perform separate analyses of the overall decline of HBV DNA according to genotype.

In conclusion, PEG-IFN therapy resulted in a larger HBV DNA decline compared with placebo. The degree of viral suppression was stronger in HBeAg-positive patients who lost HBeAg or who had an ALT flare during PEG-IFN therapy compared with spontaneous HBeAg loss or flares during placebo therapy.

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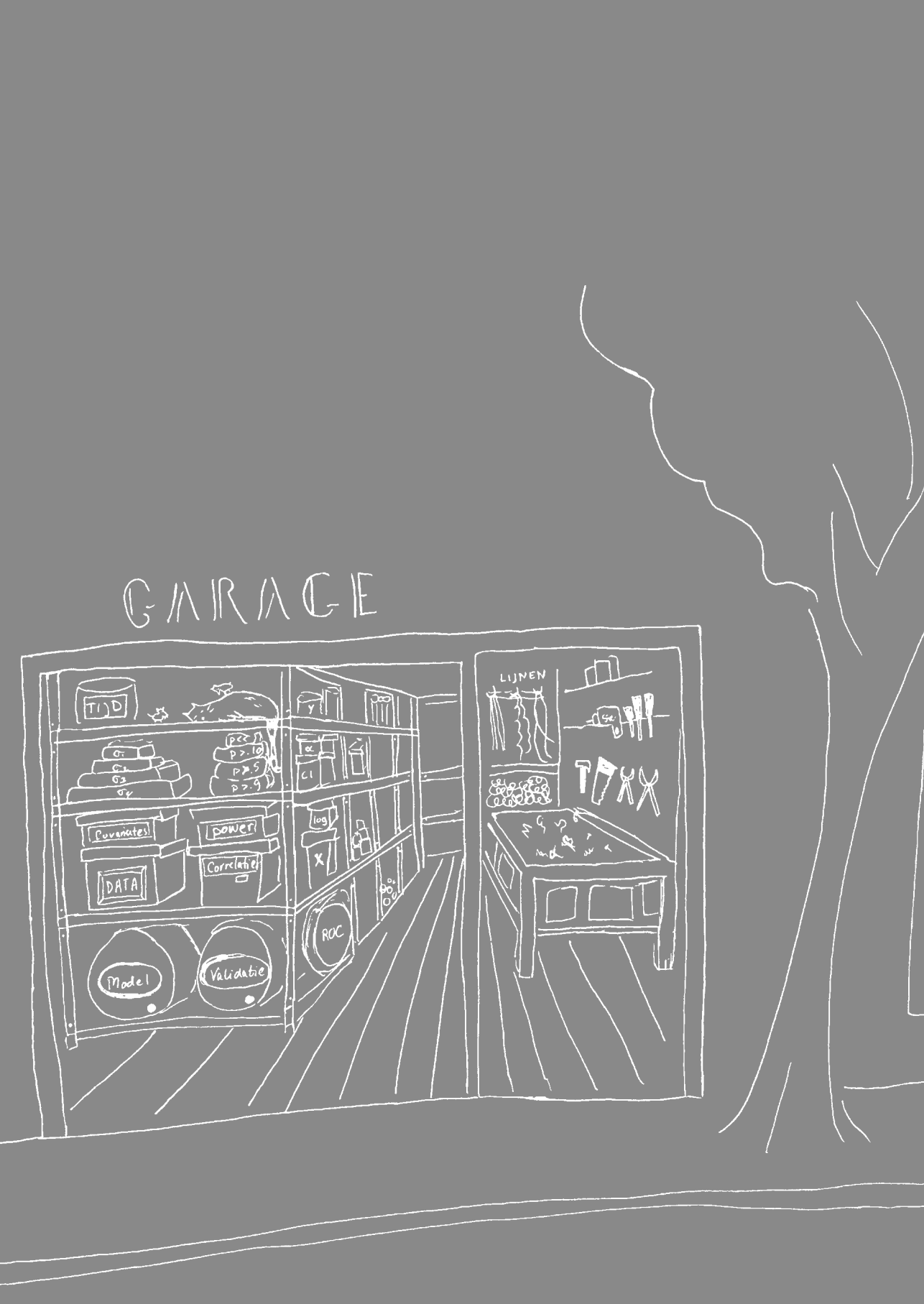
GARAGE
LIJNEN
T1D
DATA
power
ROC
Model
Validatie

# CHAPTER 2.2

## Factors that predict response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa.

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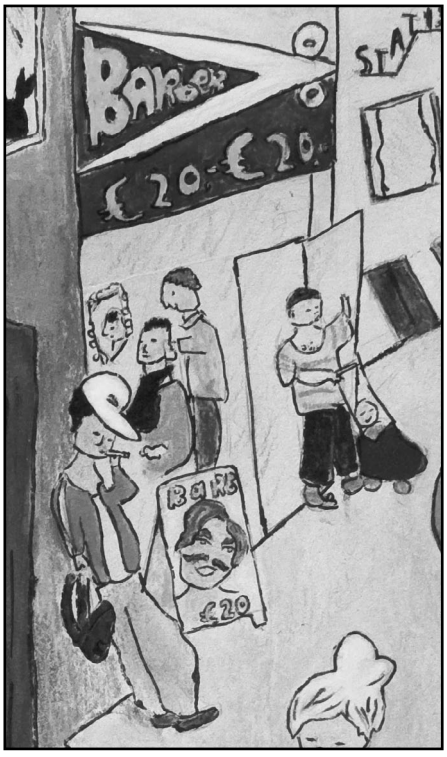

## ABSTRACT

Background: In order to develop a model for the prediction of sustained response to PEG-IFN in all HBV genotypes, individual patient data from the 2 largest global trials in HBeAg-positive chronic hepatitis B were combined.

Methods: Five-hundred-forty-two patients treated with PEG-IFN α-2a (180 µg/week, 48 weeks) and 266 patients treated with PEG-IFN α-2b (100 µg/week, 52 weeks) were included. Sustained response was defined as HBeAg loss and HBV DNA $<2.0x10^4$ IU/ml at 6 months post-treatment. Logistic regression analysis was used to identify predictors of sustained response and a multivariable model was constructed.

Results: Eighty-seven patients were excluded because of missing values or harbouring an HBV genotype other than A-D, leaving 721 patients for analysis. HBV genotype A, high ALT (≥2 x ULN), low HBV DNA ($<2.0x10^8$ IU/ml), female sex, older age, and absence of previous IFN therapy predicted sustained response. Genotype A patients with either high ALT and/or low HBV DNA had a high (>30%) predicted probability of sustained response. High ALT was the strongest predictor in genotype B and low HBV DNA level in genotype C. Genotype D patients had a low chance of sustained response, irrespective of ALT and HBV DNA.

Conclusion: The best candidates (chance of sustained response >30%) are genotype A patients with either high ALT or low HBV DNA, and genotype C patients with both high ALT and low HBV DNA. Genotype D patients have a rather low chance of sustained response. All remaining patients are moderate candidates for PEG-IFN therapy.

# INTRODUCTION

Hepatitis B is a major global health problem. The World Health Organization reported that there are more than 350 million carriers in the world, approximately 75% of whom reside in Asia and the Western Pacific.[1] In this part of the world, HBV infection is usually acquired perinatally or in early childhood. Most patients from these areas typically have HBeAg-positive chronic hepatitis B with high HBV DNA levels and they develop moderate to severe hepatic inflammation with elevated alanine aminotransferase (ALT) levels after 10–30 years of infection.[2] In contrast, patients infected in late childhood, adolescence or adulthood present with elevated aminotransferases after a shorter duration of infection. Although spontaneous HBeAg seroconversion occurs in the majority of HBeAg-positive patients, the duration of hepatic inflammation can be prolonged and severe, and may result in liver cirrhosis. Therefore, antiviral treatment is indicated in patients who remain HBeAg positive with high HBV DNA levels after a 3-6 month period of elevated ALT levels.[3]

Successful treatment of chronic hepatitis B virus (HBV) infection with loss of hepatitis B e antigen (HBeAg), decline in serum HBV DNA and normalization of ALT is associated with favorable long-term outcome, independent of the antiviral drug used.[4, 5] In HBeAg-positive patients, sustained clearance of HBeAg from serum is associated with a higher likelihood of losing hepatitis B surface antigen (HBsAg), reduced incidence of cirrhosis and hepatocellular carcinoma (HCC), and improved survival.[5-8] Of currently available drugs for the treatment of chronic hepatitis B, pegylated interferon (PEG-IFN) still results in the highest rate of off-treatment sustained response after a one-year course of therapy.[9-12] Furthermore, responders to IFN-based therapy have a considerable chance of losing HBsAg, which has been observed in 12-65% of patients within 5 years after HBeAg loss.[7, 8, 13-18] Treatment with PEG-IFN is however often complicated by the occurrence of side-effects such as flu-like symptoms, cytopenia and depression.[19] Nucleos(t)ide analogues such as lamivudine, adefovir, entecavir and tenofovir on the other hand are well tolerated, but because of the modest seroconversion rate and the high risk of post-treatment relapse, prolonged therapy is usually required.[20] Nowadays, maintenance of virological response over prolonged periods is feasible,[21] but may still pose a considerable risk for resistance in the long-term.[22-24]

Since both treatment with PEG-IFN and nucleos(t)ide analogues has proven effective, but also have their advantages and limitations, the question arises what treatment regimen should be used as first-line therapy in which patients. Both the chance of achieving response and specific patient characteristics play a role in the decision on what type of antiviral therapy should be started. Recently performed studies with one year of PEG-IFN in HBeAg-positive patients identified high baseline alanine aminotransferase (ALT),

low baseline HBV DNA, absence of previous IFN therapy, low baseline HBeAg and HBV genotype A or B as predictors of response.[10, 25, 26] Current guidelines do not provide specific recommendations as to which patients should be treated with peginterferon,[27] the above mentioned studies were considered to provide insufficient evidence for such recommendations. The aim of this study therefore was to develop a solid model for the prediction of sustained response to PEG-IFN in individual HBeAg-positive patients, which will allow physicians throughout the globe to choose the optimal candidates for treatment with this drug.

# METHODS

## Patients and study design

Individual data of 542 patients treated with PEG-IFN α-2a 180µg per week for 48 weeks (271 patients with added lamivudine 100 mg daily) and 266 patients treated with PEG-IFN α-2b 100µg per week for 52 weeks (130 patients with added lamivudine 100 mg daily) were analyzed.[10, 11] Post-treatment follow-up lasted six months. Addition of lamivudine did not influence response rates at the end of follow-up (6 months post-treatment) in any way. For the current study, sustained response was defined as clearance of HBeAg from serum and HBV DNA <10,000 copies/ml at six months post-treatment.



The inclusion and exclusion criteria were reported in detail previously and were similar for the two studies.[10, 11] In short, patients were eligible if they had been HBsAg positive for at least 6 months, were HBeAg positive, had elevated serum ALT between one and 10 times the upper limit of normal (ULN), had serum HBV DNA >1.0 x $10^5$ copies/ml (2.0 x $10^4$ IU/ml) and had findings on a liver biopsy within the preceding 12 months that were consistent with the presence of chronic hepatitis B. Exclusion criteria included decompensated liver disease, antiviral therapy within 6 months prior to randomization, viral coinfections (hepatitis C virus, hepatitis delta virus or human immunodeficiency virus), or pre-existent neutropenia or thrombocytopenia.

## Laboratory testing

During therapy and post-treatment follow-up, all patients were monitored monthly by routine physical examination, as well as biochemical and hematological assessments. ALT was assessed locally and therefore expressed as times upper limits of normal (ULN). HBV DNA was assessed monthly using an in-house developed Taqman PCR assay based on the Eurohep standard (lower limit of detection 373 copies/ml) or the Cobas Amplicor HBV Monitor Test (Roche Diagnostics).[28] We compared the two HBV DNA quantification

assays, and found an excellent correlation between the two assays (r=0.930, p<0.001). Plotting the difference against the average of the assays showed no significant correlation (Bland-Altman test; r=0.12, p=0.49), strengthening the conclusion that both assays are comparable in the dynamic range.[6] HBeAg, anti-HBe, HBsAg and anti-HBs were measured with the use of the AxSYM test (Abbott, Abbott Park, IL, USA). HBV genotype analysis was performed by INNO-LiPA Assay (Innogenetics, Gent, Belgium).

## Statistical analysis

Statistical analysis was performed using the SPSS 15.0 program (SPSS Inc. Chicago, IL) and the R 2.3.1 Project for Statistical Computing (Harrell's Design, HMisc and Foreign libraries). A p-value <0.05 was considered statistically significant (all two-tailed). We performed univariate logistic regression analysis to identify predictors of sustained response among the variables age, sex, HBV genotype (A-D), serum HBV DNA ($log_{10}$ copies/ml), ALT (ln ALT x ULN), treatment allocation (PEG-IFN monotherapy or combination therapy of PEG-IFN and lamivudine), and previous treatment with interferon or lamivudine. We used multivariable logistic regression analysis with backward stepwise selection, using a *p*-value greater than 0.05 for removal of variables, to construct a multivariable linear model that provides a natural logarithm transformed prediction of sustained response. We used restricted cubic spline functions to assess the linearity of the effect of continuous variables. Interactions between variables were explored. Odds ratios (ORs) were calculated with 95% confidence intervals (95%-CI). Since there were interactions between HBV genotype and other factors, ORs for HBV genotype were calculated for 33-year old (mean age), IFN-naïve males with ALT and HBV DNA fixed at 2 x ULN and 1.0 x $10^9$ copies/ml (2.0 x $10^8$ IU/ml), respectively.

Discrimination, which is the ability to distinguish patients who will achieve sustained response from those who will not, was quantified by the area under the receiver-operating characteristic curve (AUC). An AUC of 0.5 indicates no discriminative ability at all, whereas an AUC of 1.0 indicates perfect discrimination. Internal validity was assessed with bootstrap sampling.[29, 30] Two-hundred bootstrap samples were drawn with replacement and with the same size as the original sample. The final prediction model was constructed by applying the penalized maximum likelihood estimation acquired from the bootstrap samples.[31] Nomograms for IFN-naïve patients were constructed based on the logistic regression formulas A nomogram allows for the approximate graphical computation of sustained response with points allocated to each variable based on the logistic regression formula. In order to develop a simple rule for each of the genotypes independent of sex and age, the predicted probability of sustained response in different

patient subgroups was calculated with the logistic regression formulas. Since sex and age also predicted sustained response, but were not included in the flowchart, age was fixed at the respective mean value of each subgroup and the mean predicted probability of sustained response for males and females was calculated. For serum ALT, a low level (<2 x ULN) was considered to be between 1 x ULN and 2 x ULN, and a high level (≥2 x ULN) was considered to be between 2 x ULN and 10 x ULN. For serum HBV DNA, a low level (<copies/ml [<2.0 x $10^8$ IU/ml]) was considered to be between 1.0 x $10^7$ copies/ml (2.0 x $10^6$ IU/ml) and 1.0 x $10^9$ copies/ml (2.0 x $10^8$ IU/ml), and a high level (≥1.0 x $10^9$ copies/ml [≥2.0 x $10^8$ IU/ml]) was considered to be between 1.0 x $10^9$ copies/ml (2.0 x $10^8$ IU/ml) and 1.0 x $10^{11}$ copies/ml (2.0 x $10^{10}$ IU/ml). These cut-offs were chosen because the majority of patients had ALT (95%) and HBV DNA (80%) levels between these values. In addition, these cut-off levels are generally used in clinical practice and recommended by international guidelines for the treatment of chronic hepatitis B.[3, 27, 32, 33]

## RESULTS



Of 808 patients eligible for participation in this study, 87 were excluded because of missing values (n=76) or infection with HBV genotype other than A to D (n=11), leaving 721 patients for analysis. HBeAg loss, HBeAg seroconversion and HBV DNA <10,000 copies/ml (<2.0 x $10^3$ IU/ml) were observed in 254 (35.2%), 232 (32.2%) and 174 (24.1%) of 721 patients, respectively. Sustained response, defined as HBeAg loss and HBV DNA <10,000 copies/ml (<2.0 x $10^3$ IU/ml) at six month post-treatment, was observed in 158 of 721 patients (21.9%). Sustained response was observed in 22.4% of patients treated with PEG-IFN alone and in 21.4% of those treated with PEG-IFN and lamivudine combination therapy (p=0.73). sustained response was observed in 37% of patients with genotype A, 25% with genotype B, 20% with genotype C and 8% with genotype D.

There were no differences in baseline characteristics between patients enrolled and those excluded from participation in this study, except for a lower rate of previous IFN therapy among the participants than the excluded patients (14% vs. 24%, p=0.01). Baseline characteristics of patients with and without sustained response are given in table 1. Patients with sustained response were older, more often were female, had higher baseline ALT and lower HBV DNA levels, and were more likely to have genotype A but less likely to have genotype D infection compared to those without sustained response. The proportion of patients that was previously treated with IFN or lamivudine therapy did not differ between patients with sustained response and those without.

**Table 1.** Baseline characteristics and univariate logistic regression analysis.

| Characteristic | Sustained virological response† (n = 158) | No sustained virological response (n = 563) | Odds Ratio | 95% Confidence Interval Lower | Upper | p |
|---|---|---|---|---|---|---|
| Age | 34.8 ± 11.4 | 32.4 ± 10.6 | 1.02 | 1.00 | 1.04 | 0.01 |
| Female sex | 47 (29.7%) | 120 (21.3%) | 1.56 | 1.05 | 2.32 | 0.03 |
| Serum ALT ( x ULN ) | 4.3 ± 3.0 | 3.9 ± 3.5 | 1.31 | 1.02 | 1.69 | 0.03 |
| HBV DNA ($log_{10}$ copies/ml) | 9.4 ± 1.7 | 9.8 ± 1.8 | 0.85 | 0.77 | 0.95 | 0.003 |
| HBV genotype | | | | | | <0.001 |
| A | 42 (26.6%) | 73 (13.0%) | 1.00 | | | |
| B | 41 (25.9%) | 125 (22.2%) | 0.57 | 0.34 | 0.96 | |
| C | 67 (42.4%) | 266 (47.2%) | 0.44 | 0.28 | 0.70 | |
| D | 8 (5.1%) | 99 (17.6%) | 0.14 | 0.06 | 0.32 | |
| Previous interferon therapy | 22 (13.9%) | 79 (14.0%) | 0.99 | 0.60 | 1.65 | 0.97 |
| Previous lamivudine therapy | 16 (10.1%) | 64 (11.4%) | 0.88 | 0.49 | 1.57 | 0.66 |

†Sustained virological response: HBeAg loss and HBV DNA <10,000 copies/ml at 6 months post-treatment; ULN: upper limit of normal
For age, ALT, GGT and HBV DNA the mean ± SD is given

## Predictors of sustained response

Factors associated with an increased likelihood of sustained response included HBV genotype A infection, high baseline ALT, low baseline HBV DNA, female sex, older age, (table 1). There was no association between sustained response and previous treatment with interferon or lamivudine on univariate analysis. Using multivariate analysis, high baseline ALT was found to be an independent predictor of sustained response (OR 1.57 per 1$log_{10}$ x ULN increase [95%-CI, 1.19 – 2.09], p=0.002). In addition, HBV genotype was associated with sustained response, with higher rates of sustained response in patients with genotype A (OR 1, reference) than B ($OR_{B\ vs.\ A}$ 0.46 [95%-CI, 0.21 – 0.99], p=0.05), C ($OR_{C\ vs.\ A}$ 0.30 [95%-CI, 0.16 – 0.59], p<0.001) or D ($OR_{D\ vs.\ A}$ 0.08 [95%-CI, 0.02 – 0.31], p<0.001). The influence of sex, age, HBV DNA, and previous IFN therapy was significantly different across HBV genotypes (p<0.02 for the interaction between HBV genotype and each of these factors). These variables were therefore also included in the model. We here describe the most important predictive factors. Genotype C and D infected females had a significantly higher chance of sustained response compared to males (OR 2.78 [95%-CI, 1.51 – 5.11] and OR 7.69 [95%-CI, 1.48 – 39.90], p<0.02). Older age was associated with a significantly higher chance of sustained response in genotype A infected patients (OR 1.04 per year increase in age [95%-CI, 1.01 – 1.08], p=0.01). High baseline HBV DNA was associated with a lower likelihood of sustained response in patients with genotype A (OR 0.57 [95%-CI, 0.40 – 0.82], p=0.003) and C (OR 0.77 [95%-CI, 0.65 – 0.91],

p=0.002). Previous IFN therapy resulted in a significantly lower chance of sustained response in patients with genotype A or D (OR 0.21 [0.07 – 0.58], p=0.003). We found no differences in the predictors of response for the two treatment groups.

## Performance of the model

The distribution of the predicted probabilities of sustained response in genotypes A – D is shown in figure 1. The agreement between the predicted probabilities and the observed frequency of sustained response was good (p=0.27 by the Hosmer–Lemeshow goodness-of-fit test). A multivariable model including the variables age, sex, ALT, HBV DNA, HBV genotype and previous interferon therapy had adequate discriminative ability as shown by an area under the receiver-operating characteristic curve (AUC) of 0.72 (95%-CI, 0.67 – 0.77). The AUC was 0.75 (95%-CI, 0.65 – 0.85), 0.65 (95%-CI, 0.55 – 0.75), 0.68 (95%-CI, 0.61 – 0.75) and 0.78 (95%-CI, 0.65 – 0.92) for genotypes A to D, respectively. After bootstrap validation, the area under the ROC curve was 0.69 (95%-CI, 0.60 – 0.77). Since the influence of the predictors was significantly different across genotypes, a validated formula for the prediction of sustained response was generated for each HBV genotype separately. The PEG-IFN HBV Treatment Index is based on these formulas (figure 2). An automated calculator can be found on www.liver-gi.nl/peg-ifn.



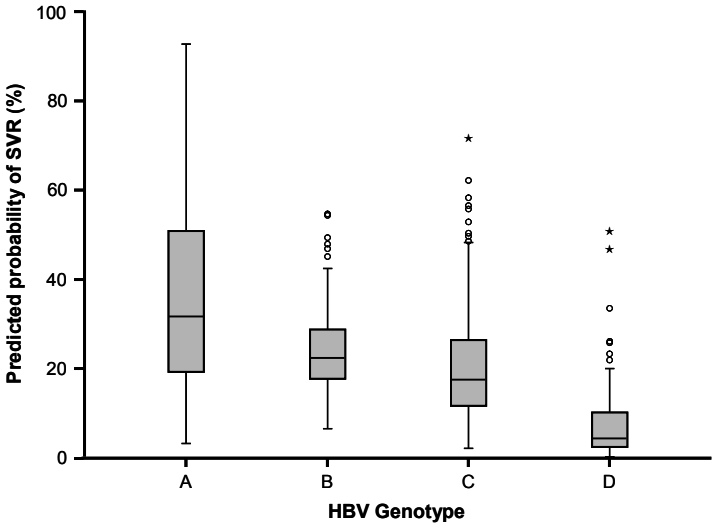


**Figure 1.** Distribution of predicted probabilities of sustained response in patients with HBV genotype A-D. Boxplots show the distribution of the predicted probabilities of sustained response, defined as HBeAg loss and HBV DNA <10,000 copies/ml at six months post-treatment, in patients with genotype A (n=115), B (n=166), C (n=333) or D (n=107).

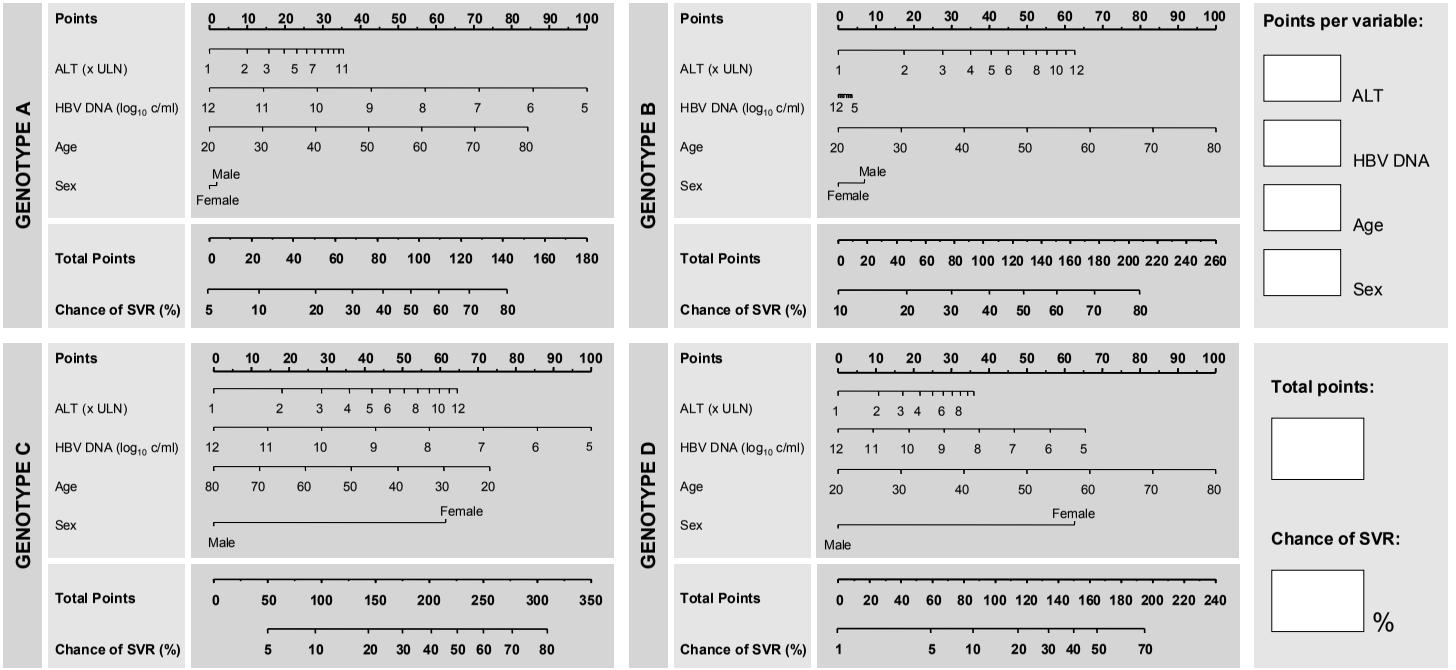


**Figure 2:** PEG-IFN HBV treatment index

These nomograms can be used to obtain a patient-tailored predicted probability of sustained response in IFN-naïve patients with genotype A-D based on ALT, HBV DNA, sex and age. The probability of sustained response is calculated by drawing a vertical line to the top Points axis for each of the four variables ALT, HBV DNA, age and sex. The points for each variable are then summed and located on the Total points axis, and a vertical line is projected from the Total points axis to the bottom scale to get the predicted probability of sustained response. For example: a genotype C infected female (62 points), 25 years old (67 points), with serum ALT of 2.7 x ULN (25 points) and serum HBV DNA of 9.2 $\log_{10}$ copies/ml (40 points) has a total score of 194 points which converts to a probability of sustained response of 37%.

*Logistic regression formulas for HBV genotypes A-D:*

*A: Logit(p)=1.48-0.05(sex=female)+0.04(age)+0.43(lnALT)-0.43(log HBV DNA)-1.46(previous IFN=yes)*

*B: Logit(p)=-2.69-0.12(sex=female)+0.03(age)+0.43(lnALT)+0.01(log HBV DNA)+0.67(previous IFN=yes)*

*C: Logit(p)=0.71+1.02(sex=female)-0.02(age)+0.43(lnALT)-0.23(log HBV DNA)+0.67(previous IFN=yes)*

*D: Logit(p)=-2.38+1.74(sex=female)+0.04(age)+0.43(lnALT)-0.26(log HBV DNA)-1.46(previous IFN=yes)*

## Application of the model in clinical practice

To allow for application of the model in clinical practice, a nomogram for IFN-naïve patients was generated from the validated formula for each of the HBV genotypes separately (figure 2). These nomograms can be used for calculating the probability of sustained response in individual HBeAg-positive patients based on their sex, age, and ALT and HBV DNA level. Average response rates based on the presence of low (<2 x ULN) or high ALT levels (≥2 x ULN), and low (<9$log_{10}$ copies/ml [<2.0 x $10^8$ IU/ml]) or high HBV DNA levels (≥9$log_{10}$ copies/ml [≥2.0 x $10^8$ IU/ml]) are shown in figure 3.

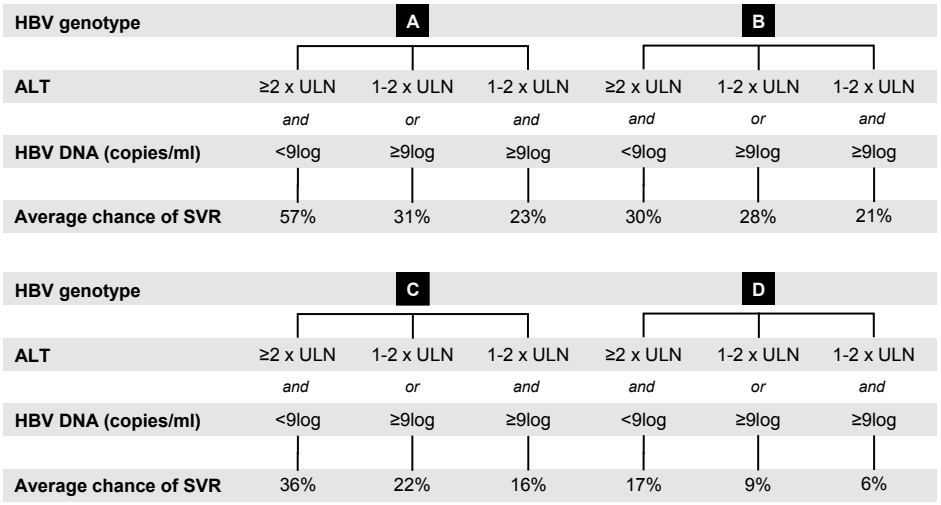


**Figure 3.** Flowcharts to easily obtain average predicted probabilities of sustained response in patients infected with HBV genotype A-D.
These flowcharts show the average predicted probability of sustained response depending on HBV genotype, ALT (above or below 2 x ULN) and HBV DNA (above or below 9$log_{10}$ copies/ml). For a precise estimate of the probability of sustained response in an individual patient, the nomograms in figure 2 can be used.

# DISCUSSION

We combined the data of the two largest studies investigating PEG-IFN in HBeAg-positive chronic hepatitis B in order to develop a model for the prediction of response to PEG-IFN in all HBV genotypes. Although the model is based on the data of patients enrolled in randomized clinical trials with predefined inclusion and exclusion criteria, generalizability of our results is probably good because of the large sample size and wide geographic distribution of the patients. We provided nomograms which can be

used to calculate the predicted probability of response in individual patients. A rapid estimate can be obtained from the provided flowcharts.
We recommend to start PEG-IFN therapy in patients with the highest chance of achieving sustained response (table 2). We arbitrarily chose those with a predicted probability of sustained response of at least 30% to be good candidates for PEG-IFN therapy. About 25% of patients included in this study had a predicted probability of sustained response above this level. This includes all HBV genotype A infected patients except for those with low ALT and high HBV DNA levels. In addition, genotype C infected patients with high ALT and low HBV DNA levels have a high likelihood of response to PEG-IFN. All remaining patients are moderate candidates for PEG-IFN except for those with genotype D, who have a rather low chance of achieving sustained response and are in our view generally not candidates for treatment with PEG-IFN.

**Table 2.** Recommendations for the use of peginterferon (PEG-IFN) as initial antiviral therapy.

| HBV genotype | General recommendations for HBeAg positive chronic hepatitis B patients |
|---|---|
| A | Either high ALT (≥2 x ULN) or low HBV DNA levels (<9log10 copies/ml) |
| B and C | Both high ALT (≥2 x ULN) and low HBV DNA levels (<9log10 copies/ml) |
| D | PEG-IFN therapy is not recommended |

The recommendation to consider PEG-IFN therapy is based on an average predicted probability of SVR of at least 30%. Predicted SVR rates may be higher or lower in selected subgroups of patients. In patients with a predicted probability of SVR <30%, co-factors such as age and co-morbidity can be taken into account when deciding whether or not to start PEG-IFN therapy.

With the licensing of pegylated interferon and an additional five nucleos(t)ide analogues for the treatment of chronic hepatitis B in the last years, choice of antiviral therapy has become more important and more complex at the same time. Since both treatment with IFN-based therapy and nucleos(t)ide analogue therapy have proven effective and can improve long-term outcome, the pros and cons of these drugs as well as patient-specific characteristics should be taken into consideration. All of the major practice guidelines have advocated IFN-based therapy as potential first-line therapy for both HBeAg-positive and HBeAg-negative patients,[3, 27, 32, 33] particularly because sustained response and HBsAg loss seem to occur more often with IFN and PEG-IFN than with the direct antiviral agents.[20] However, the use of PEG-IFN currently accounts for no more than 10% of all prescriptions for the treatment of chronic hepatitis B.[34] The relatively low usage of PEG-IFN may be explained by it's significant side effects and need for administration by injection. Furthermore, recommendations on the use of PEG-IFN in specific subsets of patients who are most likely to have a sustained response and HBsAg seroconversion were lacking. When we are able to identify patients with a high likelihood of response to

PEG-IFN, the proportion of patients achieving sustained response after treatment with this drug can probably be increased.

Most studies investigating IFN-based therapy in HBeAg-positive chronic hepatitis B found that high baseline ALT, low baseline HBV DNA and HBV genotype A or B were associated with of response.[10, 25, 26, 35] In addition to these factors, we identified sex and age as predictors of response to PEG-IFN. It should be mentioned that in both studies more men than women were included. We found that the influence of sex, age, HBV DNA and previous IFN therapy was significantly different across HBV genotypes. HBV genotype thus has great influence on the outcome of PEG-IFN therapy. Therefore, contrary to a statement on this topic in the newest guidelines from the European Association for the Study of the Liver (EASL),[27] we believe that determination of HBV genotype is essential in all patients in whom sustained off-treatment response is pursued. Other potential approaches to tailor PEG-IFN therapy in chronic hepatitis B include quantification of serum HBeAg and HBsAg.[36] These approaches are still being validated and not routinely available to most physicians. Because of limited availability in clinical practice, we also chose not to include liver histology.

Previously we presented a model based on 266 HBeAg-positive patients participating in a single randomised trial.[37] However, the vast majority of these patients were infected with HBV genotype A or D, only a small proportion of patients harboured HBV genotype B or C. To gain a good prediction model for all HBeAg positive patients, we now combined the data of the two largest randomised trials investigating PEG-IFN in HBeAg-positive chronic hepatitis B. We showed that a model based on readily available baseline factors can provide an adequate prediction of sustained response. Ideally, a large confirmatory group would have been used for external validation. Such a group is unfortunately not available. Clinical trials that are currently still ongoing may allow for further validation of the model in the near future.

Since substantial viral replication may persist despite HBeAg loss in some patients, a combined endpoint of HBeAg clearance from serum and low HBV DNA is crucial in HBeAg positive chronic hepatitis B. Particularly patients with HBV genotype non-A infection can develop mutations in the precore or core promoter region and may still be at risk for progressive liver disease despite HBeAg loss.[6, 38] Both clearance of HBeAg and suppression of HBV replication are key events in the natural course and during antiviral therapy in HBeAg positive chronic hepatitis B. HBeAg loss after IFN-based therapy was associated with reduced progression to cirrhosis and HCC, and improved survival.[5, 39] In addition, large population studies have established a clear link between HBV viremia and the risk for HBV-related complications.[40, 41] Serum HBV DNA was the strongest predictor of progression to cirrhosis and HCC, with a significantly higher risk for patients

with HBV DNA above 10,000 copies/ml (2,000 IU/ml) as compared to those with serum HBV DNA <300 copies/ml (relative risk 2.5 [95%-CI 1.6-3.8] and 2.3 [95%-CI 1.1-4.9] for developing cirrhosis and HCC, respectively). Although the proportion of patients with undetectable HBV DNA was relatively low shortly after PEG-IFN therapy,[10] it further increased with prolonged duration of follow-up.[6] Because presence of anti-HBe at 6 months post-treatment was not associated with long-term sustainability of response to PEG-IFN,[6] the combined endpoint of HBeAg loss and low HBV DNA seems optimal.

The parties involved in this study agreed not to perform a direct comparison between the two formulations of PEG-IFN. However, the previously reported results of two studies included in this retrospective analysis were very similar.[10, 11] Unfortunately, we cannot provide recommendations for HBeAg-negative patients, since we only had data of HBeAg-positive patients treated with PEG-IFN. Due to the low sustained response rate in HBeAg-negative patients,[42] PEG-IFN is relatively less often given to HBeAg-negative as compared to HBeAg positive patients. Prediction of response to PEG-IFN therefore seems of greater importance in HBeAg-positive than in HBeAg-negative chronic hepatitis B.

In conclusion, we provide a practical tool to calculate the probability of sustained response to PEG-IFN in individual HBeAg-positive patients, which can be easily used in clinical practice and can thus allow for the selection of the optimal candidates for PEG-IFN therapy. We were unfortunately not able to perform external validation because such a database is not available. Clearly, this should be done when an appropriate patient group is available. We recommend to consider PEG-IFN therapy in all genotype A patients with either high ALT or low HBV DNA levels, in genotype B and C infected patients with both high ALT and low HBV DNA. HBeAg-positive genotype D infected patients are generally not good candidates for treatment with PEG-IFN.

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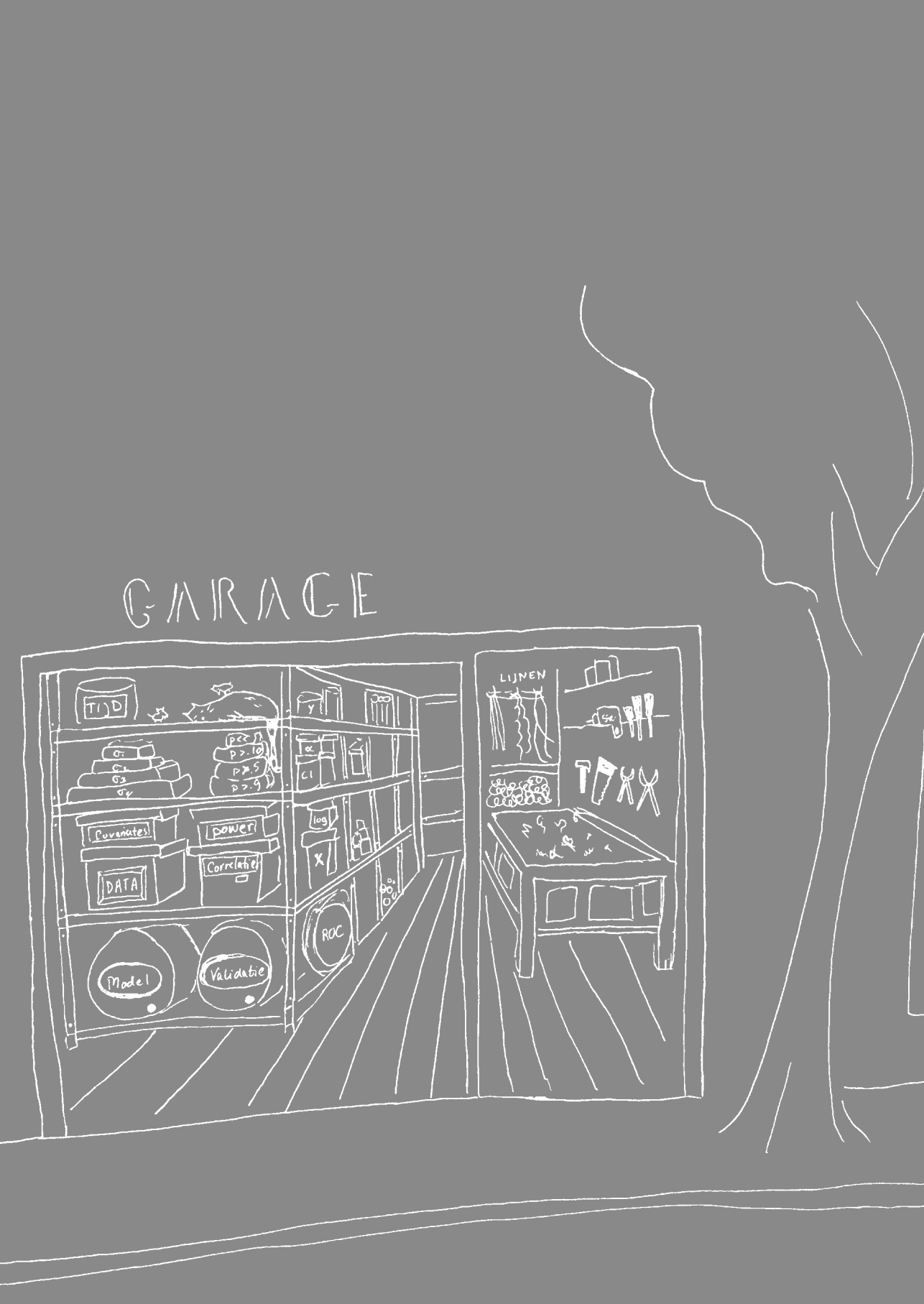
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# CHAPTER 2.3

## Prediction of the response to peginterferon-alfa in patients with HBeAg positive chronic hepatitis B using HBV DNA decline during treatment

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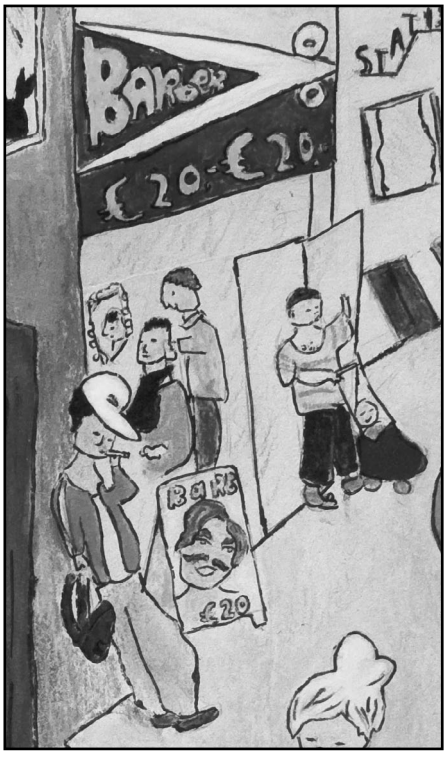

## ABSTRACT

Peginterferon (PEG-IFN) results in HBeAg loss combined with virologic response in only a minority of patients with HBeAg positive chronic hepatitis B. Baseline predictors of response to PEG-IFN include HBV-genotype, pre-treatment HBV DNA levels and ALT. The aims of this study were to develop a model, which improves the baseline prediction of response to PEG-IFN for individual patients by including early HBV DNA measurements during treatment and to establish an early indication for cessation of treatment.

One hundred and thirty six patients treated with PEG-IFN were included in the study. Response was defined as loss of HBeAg and HBV DNA<10,000 copies/ml at 26 weeks post-treatment. Logistic regression analysis techniques were used to develop a dynamic prediction model with HBV DNA during the first 32 weeks of therapy. An early clinically useful rule for dis(continuation) of treatment was identified with a grid of cut-off values of HBV DNA decline during treatment.

Adding HBV DNA decline to baseline prediction increased c-statistics from 0.850 to 0.857, 0.855 and 0.866 at week 4, 12 and 24. A HBV DNA decline of at least 2 $\log_{10}$ within 24 weeks was strongly associated with response when added to the baseline prediction model: OR 6.62 (95%CI, 1.94-22.6; p=0.002).

A dynamic model including HBV DNA decline during treatment provides more accurate predictions of response to PEG-IFN. The model strongly supports individual decision making on treatment (dis)continuation in patients with HBeAg positive chronic hepatitis B. It is recommended to stop PEG-IFN treatment by 24 weeks if HBV DNA declined less than 2log10.

# INTRODUCTION

With the emergence of new antiviral agents, treatment options for chronic hepatitis B virus (HBV) infection have changed considerably. At present, the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) have approved six agents for the treatment of chronic hepatitis B: pegylated interferon (PEG-IFN), lamivudine, adefovir, telbuvidine, entecavir and tenovovir. Current guidelines do not recommend any particular agent as first-line therapy and the preferred initial treatment of individual patients remains controversial.

Response to treatment of chronic hepatitis B patients with positive hepatitis B e antigen (HBeAg positive), defined as HBeAg seroconversion to anti-HBe, suppression of serum HBV DNA and normalization of ALT, is associated with a favourable long-term prognosis, independent of the drug by which the response has been induced.[1-3] PEG-IFN treatment results in HBeAg seroconversion in 30% of treated patients after one year of therapy.[4-5] Response is sustained in over 70% of these patients[6-7] and has shown to prolong survival and to reduce the incidence of hepatolocellar carcinoma.[1] Unfortunately, PEG-IFN therapy is associated with side-effects ranging from flu-like syndromes to neuropsychiatric disorders such as depression in a considerable number of patients.[8] Therefore, patients should be selected for PEG-IFN therapy on the basis of their pre-treatment probability of response, in order to optimize the balance between potentially beneficial effects and harmful side-effects. A prognostic model, that uses readily available baseline variables, has recently been developed for these patients. This model is based on the data from two large trials (n=721) investigating the effect of PEG-IFN in HBeAg positive chronic hepatitis B[4-5] and has shown that response to PEG-IFN depends on HBV-genotype, sex, age, prior treatment with IFN, baseline HBV DNA and ALT levels.[9] The model may be used to select patients at baseline who have a relatively high probability of response. However, for an individual patient uncertainty remains as to whether he or she will actually benefit from this treatment. On-treatment HBV DNA levels may help to refine the prediction of response, and an optimal stopping rule based upon HBV DNA viral load decline during therapy may help to establish an early indication for cessation of treatment.

In the current study the prediction model described by Buster et al[9] was extended by including on-treatment HBV DNA and ALT measurements. This enabled an individual update of the response probability based upon new data acquired after each follow-up visit.

Such a model can be beneficial in guiding and supporting the patient through therapy and may help to identify those patients for whom treatment cessation should be considered. Different models were fitted and compared in order to establish a model that not

only provides the best prediction and discrimination, but also is easy to apply in clinical practice.

# METHODS

## Patients and study design

The data origin from the PEG-IFN monotherapy arm of the HBV 99-01 study,[4] 136 patients received PEG-IFN α-2b 100µg per week for 32 weeks, followed by 20 weeks of PEG-IFN 50µg per week up to a total of 52 weeks. Subjects were subsequently followed up for 26 weeks. Patients randomized to PEG-IFN and lamivudine combination therapy were not included in the current study, because HBV-DNA kinetics during combination therapy showed a different pattern in comparison with PEG-IFN monotherapy; generally a steep decline during therapy followed by a post-treatment relapse.[4] Patients were eligible to participate in the HBV99-01 study if they had been HBsAg positive for at least 6 months, were HBeAg positive, anti-HBe negative, had elevated serum ALT levels 2-10 times the upper limit of normal (ULN), had serum HBV DNA >1.0 x $10^5$ copies/ml and had abnormalities on liver biopsy consistent with the presence of chronic hepatitis B. Exclusion criteria included decompensated liver disease, antiviral therapy within 6 months prior to randomization, viral co-infections (hepatitis C virus, hepatitis delta virus or human immunodeficiency virus), or pre-existent neutropenia or thrombocytopenia.
For the current study sustained response (SR) was defined as the combination of clearance of HBeAg from serum and HBV DNA <10,000 copies/ml at 26 weeks post-treatment as opposed to HBeAg loss alone, since the former offers a higher long-term response sustainability.[6] All patients were monitored every 4 weeks during therapy and follow-up.



## Laboratory testing

HBV DNA was measured using a validated in-house developed Taqman PCR assay based on the Eurohep standard (lower limit of detection 373 copies/ml).[10] To assure comparability with the Cobas Taqman HBV assay (Roche Molecular Systems, Branchburg, NJ) a sample of 40 were recently retested with both assays. The correlation between the two assays was excellent r=0.93.[6] HBeAg, anti-HBe, HBsAg and anti-HBs were measured with the use of the AxSYM test (Abbott, Abbott Park, IL, USA). HBV genotype analysis was assessed by INNO-LiPA Assay (Innogenetics, Gent, Belgium). ALT was assessed locally and therefore expressed as times upper limits of normal (ULN).

## Statistical analysis

In a previous study baseline factors influencing the SR-rate were described in detail and a prediction model (PEG-IFN HBV Treatment Index) was developed which provides a subject specific prediction of SR.[9] Baseline factors associated with SR were: HBV-genotype, age, gender, baseline HBV DNA (copies/ml, $\log_{10}$), ALT ($\log_e$) and previous treatment with IFN.

In the current study the PEG-IFN HBV Treatment Index was first calculated for each subject and was used throughout the analysis as an offset, i.e. a subject specific starting value with the corresponding regression coefficient set to 1. The baseline prediction of SR, $P_{baseline}$, was hereafter updated with data on HBV DNA and ALT during therapy applying logistic regression analysis techniques. For comparison purposes two model approaches were considered: the first was updating the model at each visit with the new information, resulting in 8 models; one for each visit (week 4, 8, 12, 16, 20, 24, 28 and 32), the second an overall generalized estimating equations model[11] using information of all visits and adding the visit time as a continuous factor. The latter model approach allows repeated measurement data and hence reduces the 8 models of the first approach into one overall model. Technically, each visit per patient is treated as an observation and the model then corrects for the fact that each patient contributes with 8 visits. This model is referred to as the dynamic logistic regression model and is sometimes called a pooled logistic regression model.[12-13] The treatment duration (visit time) was added as a linear variable to the model, a restricted cubic spline was used to check the linearity assumption. The effect of the crude HBV-DNA ($\log_{10}$) as well as the effect of HBV DNA $\log_{10}$-decline compared to baseline (= HBV $DNA_{log10}$ at time t – HBV $DNA_{log10}$ at baseline) was studied. ALT was entered in the models as measured and also transformed logarithmically. Interactions between HBV DNA and ALT with treatment duration (visit time) and with HBV-genotype were considered. Changes in HBV DNA and ALT measurements in prior visits were also studied. Discrimination between the different models was quantified by the c-statistics, which is the area under the receiver operating curve. The best model-fit was assessed comparing these (the higher the better) and the Akaike's Information criteria (AIC) or the quasi-likelihood information criteria (QIC) for the generalized estimating equations method (the lower the better). Cross validation, leaving one out, was performed to establish overall performances of the models.

Finally a cut-off value of HBV DNA during treatment was sought to find a clinical useful guiding rule for (dis)continuation of therapy. The optimal cut-off point was established in a multivariable setting[14] including the baseline PEG-IFN HBV Treatment Index: explanatory plots were evaluated, followed by the maximum chi-square approach. Subsequently the cut-off point search was repeated in 500 bootstrap samples for validation of the

optimal cut-off point. The obtained prediction was compared with the baseline prediction model and with the best model achieved with the dynamic logistic regression model. Furthermore the effect of the cut-off value without the PEG-IFN HBV Treatment Index as an offset was used to calculate the negative predicted value (NPV) using only one crude cut-off value.

Statistical analysis was performed using the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA); especially the PROC GENMOD procedure with the REPEATED statement was applied to fit the dynamic logistic regression model. A p-value <0.05 was considered statistically significant for all main effects. In order to minimize over-fitting a p<0.01 was considered statistically significant for interactions and splines

# RESULTS

One hundred and twenty-five of the 136 patients were analyzed. Eleven patients were excluded because of infection with HBV genotype other than A-D. Baseline characteristics are shown in table 1. Figure 1 shows the distribution of the predicted probability of SR at baseline acquired from the PEG-IFN HBV Treatment Index by the observed SR outcome at 24 weeks post-treatment. Observed HBV DNA and ALT levels during the first 32 weeks are displayed in figure 2 for patients with and without SR.



**Table 1.** Baseline characteristics. **Table 1.** Baseline characteristics.

| Characteristic | SR (n = 18) | No SR (n = 107) |
|---|---|---|
| Age | 43.1 ± 15.0 | 34.3 ± 12.9 |
| Female sex | 3 (17%) | 24 (22%) |
| ALT(x ULN) | 4.3 (3.0-6.9) | 3.1 (2.2-4.8) |
| HBVDNA ($log_{10}$) | 9.0 ± 0.7 | 9.1 ± 0.9 |
| HBV genotype A | 12 (67%) | 34 (32%) |
| HBV genotype B | 3 (17%) | 7 (7%) |
| HBV genotype C | 2 (11%) | 19 (18%) |
| HBV genotype D | 1 (6%) | 47 (44%) |

ULN = upper limit of normal; for age, $log_{10}$ HBVDNA (copies/ml) the mean ± SD is given, for ALT the median and inter quartile range is given

## Update of the baseline model by visit

Extending the prediction model by including HBV DNA decline during therapy resulted in an increase of the c-statistics from 0.846 for the baseline prediction to 0.857 at week 4, 0.855 at week 12, and 0.866 at week 24. Including HBV DNA decline from week 20 to 32 significantly improved the predictive capacity of the model (table 2). A similar observation

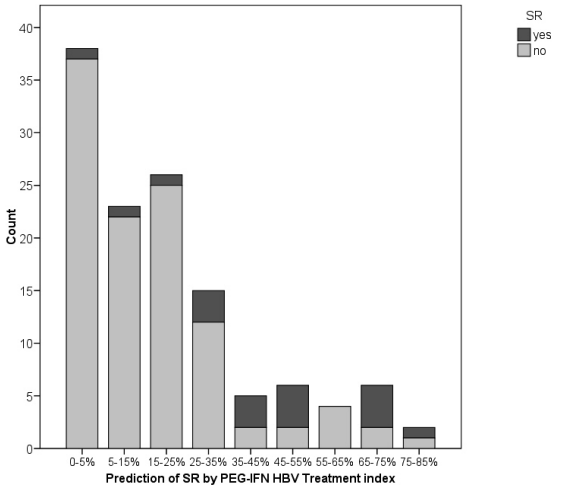


**Figure 1.** Observed sustained response (SR) counts versus predicted probabilities of SR at baseline acquired from the PEG-IFN HBV Treatment Index.

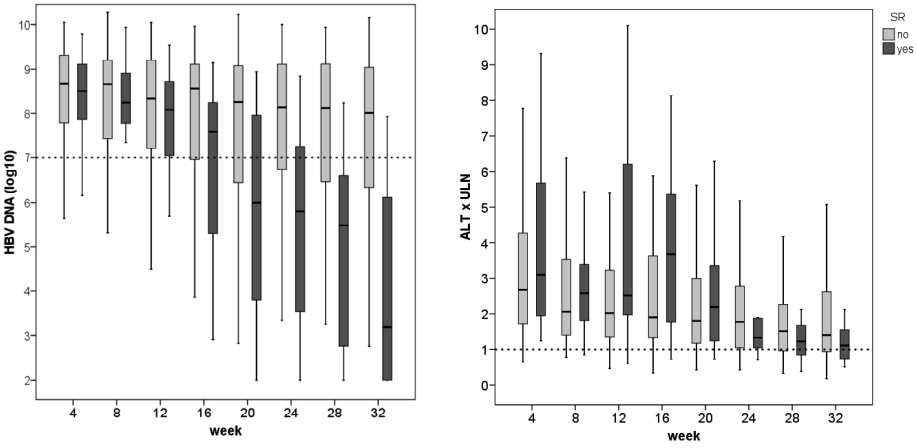


**Figure 2.** HBV DNA levels (a) and ALT levels (b) at week 4-24 of therapy for patients with and without sustained response (SR). The horizontal line in figure 2a suggests a mean HBV DNA decline of 2 $\log_{10}$ compared to baseline.

was made for the inclusion of crude HBV DNA levels (table 2). Cross-validated c-statistics supported these results. On-treatment ALT or ALT change from baseline were not significant at any time (p-values>0.11).

**Table 2.** Logistic regression analysis of sustained response. Extension of the baseline model with on-treatment factors.

| | Week | OR | 95%CI | p-value | c-statistics | cross validated c-statistics |
|---|---|---|---|---|---|---|
| Baseline | 0 | | | | 0.846 | |
| HBV DNA $_{log10}$ | 4 | 1.04 | (0.61 ; 1.78) | 0.881 | 0.854 | 0.827 |
| | 8 | 1.02 | (0.65 ; 1.61) | 0.927 | 0.852 | 0.828 |
| | 12 | 1.07 | (0.74 ; 1.56) | 0.716 | 0.852 | 0.836 |
| | 16 | 1.25 | (0.92 ; 1.69) | 0.147 | 0.856 | 0.842 |
| | 20 | 1.39 | (1.04 ; 1.85) | 0.025 | 0.860 | 0.846 |
| | 24 | 1.39 | (1.06 ; 1.82) | 0.017 | 0.857 | 0.848 |
| | 28 | 1.43 | (1.09 ; 1.89) | 0.010 | 0.864 | 0.852 |
| | 32 | 1.62 | (1.19 ; 2.21) | 0.002 | 0.895 | 0.888 |
| | Overall[#] | 1.33 | (1.05 ; 1.68) | 0.016 | | |
| decline HBV DNA $_{log10}$ | 4 | 1.37 | (0.58 ; 3.27) | 0.477 | 0.857 | 0.839 |
| | 8 | 1.16 | (0.60 ; 2.25) | 0.656 | 0.854 | 0.834 |
| | 12 | 1.18 | (0.74 ; 1.89) | 0.488 | 0.855 | 0.839 |
| | 16 | 1.36 | (0.97 ; 1.90) | 0.076 | 0.857 | 0.845 |
| | 20 | 1.49 | (1.09 ; 2.03) | 0.012 | 0.864 | 0.853 |
| | 24 | 1.47 | (1.10 ; 1.96) | 0.010 | 0.866 | 0.854 |
| | 28 | 1.56 | (1.14 ; 2.13) | 0.005 | 0.870 | 0.859 |
| | 32 | 1.72 | (1.23 ; 2.41) | 0.001 | 0.898 | 0.891 |
| | Overall[#] | 1.47 | (1.13 ; 1.90) | 0.004 | | |

[#]Estimated OR by dynamic logistic regression model for fixed number of treatment weeks




## Dynamic logistic regression model of on-treatment factors

Using the dynamic logistic regression model, significant effects were found of crude HBV DNA levels over time (p=0.016, model A) and of decline in HBV DNA levels over time (p= 0.004, model B); the latter providing better fit and discrimination (model A: QIC = 576.0, mean c-statistics = 0.860; model B: QIC=564.1, mean c-statistics=0.863). In both models duration of treatment (in weeks) was significantly related to response (p=0.027 model A and p=0.011 model B). The effects of ALT levels were not significant (ALT: p=0.406, model C and ln(ALT): p=0.558, model D).

The dynamic logistic regression equation of prediction of SR at a specific week, $P_{SR, week}$, is estimated by the crude observed HBV DNA, model A:

$$logit(P_{SR,\,week}) = 2.041 - 0.026 * week - 0.286 * HBV\,DNA_{log10} + PEG\text{-}IFN\ HBV\ Treatment\ Index,$$

or by the $log_{10}$ decline in HBV DNA compared to HBV DNA baseline, model B:

$$logit(P_{SR,\,week}) = -0.529 - 0.034 * week - 0.382 * (HBV\,DNA_{log10} - HBV\,DNA_{log10}(baseline)) + PEG\text{-}IFN\ HBV\ Treatment\ Index.$$

The overall estimated OR for HBV DNA levels and HBV DNA decline calculated by the dynamic logistic regression are presented in table 2, for weeks of therapy held fixed. In table 3, the dynamic logistic regression models were used to estimate the effects of a 1 $log_{10}$ decline of HBV DNA or 1xULN increase in ALT after 4 weeks of treatment for the individual patient. The effects of HBV DNA decline at 4, 12 and 24 weeks are plotted in figure 3. The application of non-linear functions (splines) of time, HBV DNA or ALT did not improve the fit of the model nor did adding interaction terms with HBV-genotype or time. However, a trend was observed of an increasing effect of HBV DNA decline per week: p=0.018 for the interaction term week*HBV $DNA_{log10}$, when added to model A

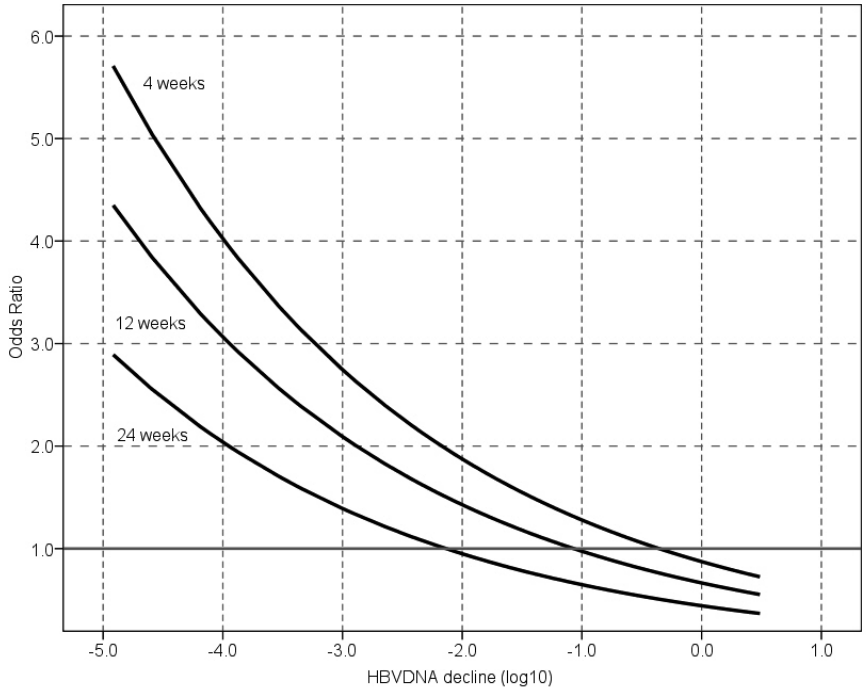


**Figure 3.** Results of the dynamic logistic regression model. Estimated OR for sustained response according to HBV DNA decline after 4, 12 and 24 weeks of treatment compared to baseline.

and p=0.056 for the interaction term week*decline HBV $DNA_{log10}$ when added to model B. These findings corroborate the increasing OR presented in table 2.

**Table 3.** Dynamic logistic regression analysis of sustained response. Extension of the baseline model with on-treatment factors.

| Model | On-treatment factors | Comparison | OR | 95%CI | p-value |
|---|---|---|---|---|---|
| A | Rx week<br>+ HBV DNA during Rx | 1 $log_{10}$ (copies/ml)<br>decrease / 4 wk | 1.20 | (1.03 ; 1.40) | 0.017 |
| B | Rx week<br>+ decline HBV DNA during Rx | 1 $log_{10}$ (copies/ml)<br>decrease / 4 wk | 1.28 | (1.08 ; 1.51) | 0.004 |
| C | Rx week<br>+ ALT during Rx | 1 xULN (IU/l)<br>increase / 4 wk | 1.06 | (0.95 ; 1.19) | 0.289 |
| D | Rx week<br>+ ALT during Rx | 1 log-e xULN (IU/l)<br>increase / 4 wk | 1.18 | (0.70 ; 2.00) | 0.525 |

*Model A, B, C and D use as an offset the baseline subject specific PEG-IFN HBV treatment index*

## Application of the model in clinical practice

Figure 2 suggests that a cut-off at 2-$log_{10}$ decline in HBV DNA levels provides optimal discrimination. The maximal chi-square approach of a grid of cut-off points of HBV DNA decline (table 4) supported that a 2 $log_{10}$ decline in HBV DNA within 24 weeks of therapy resulted in the best prediction. To validate the findings 500 bootstrap samples were drawn and the cut-off point search was repeated; in 89% of cases the maximal cut point was 2 $log_{10}$ decline, in 11% it was 2.5 or 3 $log_{10}$ decline. The discriminative ability of 2 $log_{10}$ decline compared well with the dynamic logistic regression of HBV DNA decline (the c-statistics of 0.867 versus 0.863) and was higher than the baseline model (c-statistic 0.846). When the 2 $log_{10}$ decline of HBV DNA was used as predictor of response alone, i.e. without the baseline prediction, a NPV of 94% was obtained. The optimized predicted probability of SR, dependent on the occurrence or absence of a 2 $log_{10}$ decline in HBV DNA within 24 weeks of therapy, is shown in figure 4. This optimized probability of SR can be used to decide whether or not to continue PEG-IFN therapy. For example a patient with a 30% prediction of SR at baseline with more than a 2 $log_{10}$ HBV DNA drop during the first 24 weeks of treatment will have a higher SR prediction rate of 40%, whereas the same patient without a 2 $log_{10}$ drop before week 24 will have an updated SR rate of less than 10%, or a negative predicted value of 90%.

Table 1 shows that the vast majority of patients in this study were infected with genotype A and D, and only a small proportion of patients harboured HBV genotype B and C. To test whether the 2 $log_{10}$ rule applies for all genotypes the maximal chi-square approach of a grid of cut-off points was repeated including the interaction term with genotype. In none of the models the interaction term was significant (p>0.71) and the lowest

**Table 4.** Logistic regression analysis of sustained response by HBV DNA decline within 24 weeks.

| | | OR | 95%CI | | p-value | c-statistics |
|---|---|---|---|---|---|---|
| baseline model | | | | | | |
| + | | | | | | |
| decline | 0.5 log | 7.6 | (0.8 | ; 68) | 0.071 | 0.864 |
| HBV DNA | 1 log | 5.4 | (1.5 | ; 19) | 0.006 | 0.859 |
| | 1.5 log | 5.2 | (1.5 | ; 18) | 0.006 | 0.865 |
| | 2 log | 5.7 | (1.7 | ; 20) | 0.004 | 0.867 |
| | 2.5 log | 3.2 | (1.0 | ; 10) | 0.051 | 0.847 |
| | 3 log | 2.7 | (0.8 | ; 8.8) | 0.104 | 0.844 |

*A 0.5,…, 3 $log_{10}$ decline within 24 weeks of therapy is observed when HBV DNA drops 0.5, …, 3 $log_{10}$ or more compared to baseline HBV $DNA_{log10}$, or if HBV DNA is undetectable at any visit prior or at week 24.*

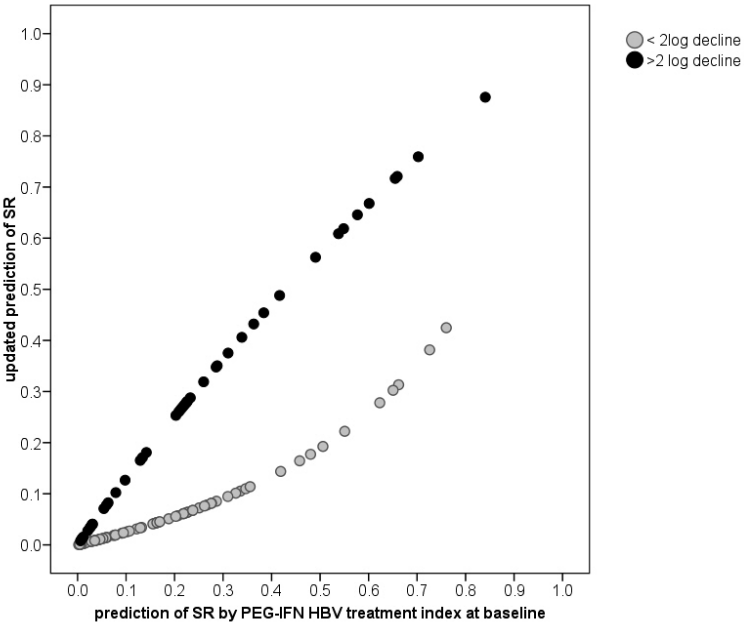


**Figure 4.** Updated predicted probability of SR acquired at baseline by the PEG-IFN HBV Treatment Index depending on the presence or absence a 2 $log_{10}$ decline in HBV DNA within 24 weeks of therapy.

chi-square was again achieved for the 2 $log_{10}$ decline. When the 2 $log_{10}$ decline rule of HBV DNA was used as a univariate predictor of response per HBV genotype, NPV's of 91%, 80%, 100% and 97% for genotype A, B, C and D were obtained, respectively. For HBV genotype specific cut-off values this implies that the stopping rule works well for genotype A, C and D, but needs to be confirmed for genotype B.

## DISCUSSION

Recently a model was presented that predicts sustained response (SR) of HBeAg positive chronic hepatitis B patients to PEG-IFN therapy, using baseline characteristics.[9] In the present study a simple statistical method was introduced for the assessment of dynamic prediction using single or repeated measurements applying contemporary methods and readily available programming procedures.[15] It enabled individual updates of prediction of SR by adding on-treatment data on HBV DNA decline. Patients without a 2 $\log_{10}$ HBV DNA decline within 24 weeks of treatment were identified as having a lower probability of response, and discontinuation should be considered in these patients.

The total number of patients who achieved SR, defined as HBeAg loss and HBV DNA below 10.000 copies/ml was only 18/125 = 14% in our study, thus, in order to minimize overfitting, a simplified model was reached using only duration of treatment and HBV DNA decline as additional variables. Ideally, the model fit should be confirmed using an independent and preferably larger dataset. Since such a dataset was not available, internal cross-validation and bootstrap methods were applied to verify the findings. Absolute HBV DNA levels and HBV DNA decline were equally good predictors of (non-) response, but a model fitted with HBV DNA decline provided better performance. Adding ALT levels or interaction terms did not significantly improve the models. These findings confirm the importance of frequent HBV DNA monitoring during therapy as proposed in recent guidelines.[16]



Different patterns of HBV DNA decline during PEG-IFN treatment have been described in relation to response in the literature, but no solid stopping rule has yet been identified. In a previous study three response profiles during treatment were described; early HBV DNA decline within the first four weeks, delayed decline between weeks 4 through 32, and late decline after week 32.[17] There was an association between HBV DNA levels and HBeAg and HBsAg loss at the end of follow-up, but a stopping rule was only suggested for patients with genotype A. Absence of a 1 $\log_{10}$ decline at week 32 was a good predictor of non-response in those patients. In another study HBV DNA kinetics during the first 4 weeks of treatment were analyzed in detail,[18] but no association between the kinetic parameters and HBeAg loss was found. Using patients treated with nucleos(t)ide analogous or PEG-IFN Dahari et al[19] described 5 HBV DNA decline profiles: a classic biphasic decline, a flat-responder profile, a rebounder profile, a triphasic decline and a stepwise decline. These observations confirm that the relationship between HBV DNA levels during PEG-IFN therapy and HBeAg loss is complex. The HBV DNA patterns as predictor of response are stronger in this study because the combined endpoint, HBeAg loss and HBV DNA below 10.000 copies/ml (2,000 IU/l), was chosen. As pointed out

previously[9] the combined endpoint is more sustainable and associated with a better long term prognosis than HBeAg loss alone[6, 20] and therefore seems an optimal choice. In a recent study of HBeAg positive chronic hepatitis B patients the association between HBeAg seroconversion and quantitative HBeAg decline and HBV DNA decline during the first 24 weeks of PEG-IFN were studied.[21] Fried et al reported that an HBeAg (PEIU/ml) >100 at week 24 was the best predictor for non-response with a NPV of 96%, while an HBV DNA level (copies/ml) of >9 $\log_{10}$ at week 24 only provided a NPV of 86%. Quantitative HBeAg was not measured in this study since no standardized and approved test has yet been developed. Thus these measures are not available for clinical practice. When the cut-off level of >9 $\log_{10}$ HBV DNA copies/ml at week 24 was applied in our cohort and used to predict a combined response of HBeAg loss and HBV DNA<10,000 copies/ml, it was observed that 23% of patients had a HBV DNA> 9 $\log_{10}$ at week 24, and none of these patients achieved SR. This is consistent with the findings presented by Fried et al.

Unlike the treatment of chronic hepatitis C where early stopping rules at week 4 or week 12 are used, the results of the present study and those of others[21] suggest that in the treatment of HBeAg-positive chronic hepatitis B a minimum period of 24 weeks is necessary before cessation of PEG-IFN therapy is considered. In recently published guidelines of the European Association for the Study of the Liver[16] absence of 1 $\log_{10}$ HBV DNA decline at week 12 is advised as a stopping rule, although it has never been justified in clinical studies. When this rule is applied on the population in this manuscript, 59% of patients should stop treatment and a NPV of 88% is observed. Even more worrying is the low sensitivity of 50%; i.e. 50% of patients responding to therapy would have to discontinue it. Zoulim et al[2] suggest this early stopping rule at week 12 for patients treated with nucleos(t)ide analogous, but as recently reported this rule is not applicable for patients treated with PEG-IFN.[22]

This study has several limitations. The dose of PEG-IFN alfa-2b was reduced to 50 µg/wk after 32 weeks, which is not common practice. However, it is unlikely that this has influenced the results of the study. The response rates of the main study were comparable with those reported by Lau et al. investigating the efficacy of PEG-IFN alfa-2a for the treatment of HBeAg-positive chronic hepatitis B.[5] Although PEG-IFN alfa-2a and PEG-IFN alfa-2b are not fully comparable, it has previously been shown that higher doses of PEG-IFN alfa-2a do not result in higher response rates.[23] Furthermore, the baseline prediction model used here was constructed on the combined data of the two trials of Janssen and Lau, to some extent adjusting for this difference.[9] Another limitation of this study is that the HBV DNA measurements were assessed with an in-house developed

Taqman PCR. A reliability study with the Cobas Taqman was performed which proved that the two assays are comparable in the dynamic range.[6]

In conclusion, a dynamic prediction model adding HBV DNA decline during therapy can provide a more accurate prediction of PEG-IFN induced SR than a prediction model based solely on baseline factors. The models presented here provide valuable information that can be used in individual decision making on treatment (dis)continuation in patients with HBeAg positive chronic hepatitis B during PEG-IFN therapy. Clinicians should consider discontinuing PEG-IFN treatment in patients who have not experienced a 2 $\log_{10}$ decline in HBV DNA levels before week 24.

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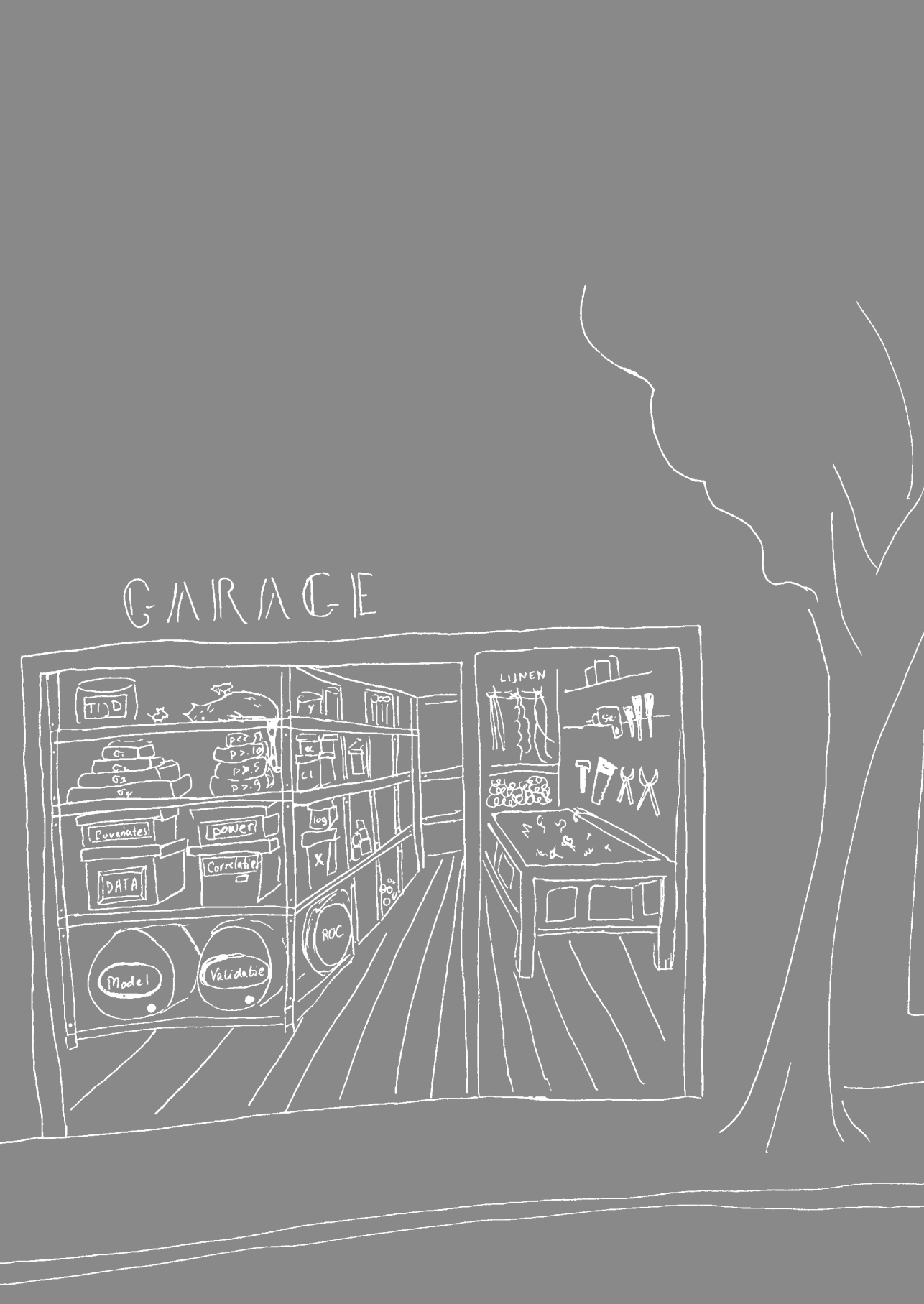
GARAGE
LIJNEN
T1D
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power
ROC
Model
Validatie

# CHAPTER 2.4

## Dynamic prediction of response to HBV-treatment using multivariate longitudinal profiles

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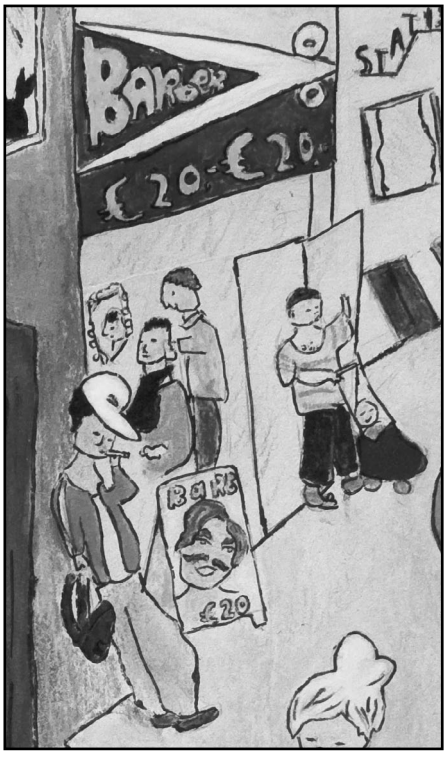

# ABSTRACT

Dynamic updating of the prediction of a significant clinical event is essential for the individual patient when new information becomes available. If the patterns of longitudinal markers change during follow-up along changes the clinical prognosis. Our aim is to incorporate these longitudinal profiles in a dynamic way to repeatedly update the individual prediction of the event. The general concept is presented specifically in the logistic regression setup when the clinical event is a binary outcome. We introduce a newly developed method and elaborate on existing ones. A new direct approach is proposed extending the usual logistic regression of baseline variables with the observed repeated measurements of the markers. The model is designed to update the prognosis of the outcome each time new information becomes available. An other direct approach using the behavior of the markers over time is discussed. Proceeding in this way first linear mixed modeling is applied to fit the subject specific patterns of the markers and afterwards the random effects are entered in the logistic regression while adjusting for the estimation error of the random effects. We finally apply an indirect prediction method using multivariate mixed effects models. The patterns of the markers are allowed to vary depending on the outcome variable. Thereafter, the empirical Bayes estimates are used to obtain posterior probabilities that are subsequently used to update the probability of the outcome variable each time new information becomes available. The different methods are illustrated with data on treatment of chronic hepatitis B patients and an extensive comparison of the performance of the different methods is made.

We conclude that the prediction of response obtained at baseline can be significantly improved with the above mentioned methods and may be useful tools to update the prognosis for the individual patient. The direct approach is easy in use and furthermore performed best in our application.

# INTRODUCTION

Dynamic updating of an individuals prediction of a specific outcome based on new gathered information is not routinely implemented in prediction models, but can be of great importance for the individual patient, for the clinician and the further choice of treatment. We shall introduce the general problem in the setting of therapeutic treatment of chronic hepatitis B.

In the last years treatment options for chronic hepatitis B have largely been extended. Still the virus is very difficult to eliminate and only 10-36% of the treated patients remains in remission after therapy.[1,2] Peg-interferon (PEG-IFN) has proven effective, but also has its limitations regarding multiple and possible serious side-effects.[3] However, it has been demonstrated that the response to a course of interferon is durable and leads to both improved survival and reduction of the incidence of hepatocellular carcinoma.[3,4] Therefore, prediction of response to PEG-IFN is of great importance. During therapy the patient is monitored at frequently scheduled follow-up visits and several markers are measured to anticipate continuation. Different patterns of these markers have been described, ter Borg et al.[5], and also flares (sudden increase) of markers have been identified as possible predictors of response, Flink et al.[6] Up to now however, these measurements have not been implemented routinely to update the individual prediction of response. These may even proof helpful in guiding and supporting the patient through the long treatment and identify patients who will have little benefit by continuation of treatment. The above sketched situation is the inspiration of this paper.

Our aim is to develop a dynamic prediction model to update the prognosis of the individual patient dependent on the new information that becomes available after each follow-up visit. The statistical challenge is to find a powerful tool to make use of the multivariate longitudinal profiles to predict the binary outcome variable. We introduce a new method and elaborate on existing methods. The performance of the different methods are furthermore extensively compared. Two different views are considered: (1) directly modeling the prediction of the outcome variable with the use of logistic regression techniques with repeated updates and (2) indirectly classifying individuals into an outcome category over time using Bayes' theorem.

For the direct approach (1) either the observed marker value or the subject specific pattern of the marker is used as predictor. We suggest a GEE solution directly entering the observed marker values.[7] In contrast, Maruyama et al.[8] first fitted a linear mixed effect model to obtain the subject specific patterns of the longitudinal markers and then entered the estimated random effects in a logistic regression. Since the estimated random effects have measurement errors an adjustment of the

prediction is necessary. For the indirect approach (2) two steps are needed: first the longitudinal profiles of the markers are modeled separately for each outcome group using a multivariate linear mixed effect model, whereafter the posterior probability of each response category over time is calculated. This approach was proposed by Brant[9] and further extended by Morrell et al.[10]

Below the general concept of the approaches is introduced and the two methods are presented. These are illustrated with data from the HBV9901-study on chronic hepatitis B patients. The performance of the methods are compared and different treatment strategies and stopping rules are designed. Finally the methods are discussed in view of their computational effort, predictive performance and their clinical applicability.

# DYNAMIC PREDICTION MODELS

Let us first introduce some notation: Denote by $\boldsymbol{Y}_{i,j} = (Y_{1,i,j}, \ldots, Y_{r,i,j})$ a random vector of $l$ continuous markers obtained at visit $j$ for subject $i$ and let $\boldsymbol{y}_{i,j} = (y_{1,i,j}, \ldots, y_{r,i,j})$ be the corresponding observed values; $i = 1, \ldots, N$ and $j = 1, \ldots, m$, $m =$ maximum number of visits over all subjects. Define by $\mathcal{Y}_{i,k} = \{\boldsymbol{y}_{i,j},\ j \leq k\}$ the history of the observed markers until visit $k$. The observed visit time for the $j$th visit for the $i$th subject is given by $t_{i,j}$. Let $R_i$ be the binary outcome variable for subject $i$, in this paper consider $R_i$ as the response to therapy (0=no response/ 1=response). For individual $i$ define with $p_{i,j}$, $0 \leq p_{i,j} \leq 1$, the probability of $R_i = 1$ at $t_{i,j}$. Especially let $p_0$ be the overall baseline probability of response, $R_i = 1$. Our interest is to estimate $p_{i,j}$ based on the longitudinal history of the observed markers $\mathcal{Y}_{i,j}$ . For the covariates measured at baseline (visit 0), let $\boldsymbol{X}_i$ be the corresponding design matrix for subject $i$.



## Cross sectional prediction

Consider the most simple situation of only one visit and one binary covariate Y (0 or 1) measured per subject. The direct approach to analyse the prediction of response, $R = 1$ given $Y = y$ is with a logistic regression model

$$\text{logit } Pr(R = 1 | Y = y) = \alpha + \beta y.$$

For the indirect approach we rewrite $Pr(R = 1|Y = y)$ as

$$Pr(R = 1|Y = y) = \frac{p\,SE}{p\,SE + (1-p)\,(1-SP)}$$

using Bayes' theorem. Here $p = Pr(R = 1)$ is the overall fraction of responders (the 'prior probability') and $Pr(Y = 1|R = 1)$ is the behavior of Y in the group of responders, i.e. the sensitivity (SE) of Y and $Pr(Y = 1|R = 0)$ is the behavior of Y in the group of non-responders, corresponding to 1-specificity (1-SP). Bayes' theorem then states that the left hand side is the 'posterior probability' of response given $Y = y$. In this simple situation the direct and the indirect methods are similar and $\alpha = \log((1-SE)/SP) + \log(p/(1-p))$ and $\beta = \log(SE \cdot SP/((1-SE)(1-SP)))$.
Suppose Y is continuous, then the indirect approach is written

$$Pr(R = 1|Y = y) = \frac{p\,f_1(\boldsymbol{y})}{p\,f_1(\boldsymbol{y}) + (1-p)\,f_0(\boldsymbol{y})}$$

where $f_1(\boldsymbol{y})$ represents the probability density function of Y at the point y in the presence of response (R=1), and $f_0(\boldsymbol{y})$ the probability density function of Y at y in the absence of response (R=0), illustrated in Figure 1a. The direct method and the indirect method are the same when Y is normal distributed in both populations of responders and non-responders with mean $\mu_1$ and $\mu_0$, respectively, and common variance $\sigma^2$. In this case $\alpha = -\frac{\mu_1^2-\mu_0^2}{2\sigma^2}$ and $\beta = \frac{\mu_1-\mu_0}{\sigma^2}$. Both methods are easily generalized to handle a multivariate normal vector Y of covariates with common covariance matrix for the responders and non-responders. For the indirect approach the requirement of a common covariance matrix for responders and non-responders is ignored in the longitudinal prediction.
In the following the two approaches are extended to handle repeated measurements of Y over time.

## Longitudinal prediction with the direct approach

### The observed makers as predictors

Suppose a baseline prediction at t=0 exist and at visit $j$ the markers $\boldsymbol{Y}_{i,j}$ are observed. A simple update of the baseline prediction model is to extend the prediction at t=0, $p_{i,0}$ with the new information observed at visit $j$, as suggested by Steyerberg(chapter 20),[11] i.e.

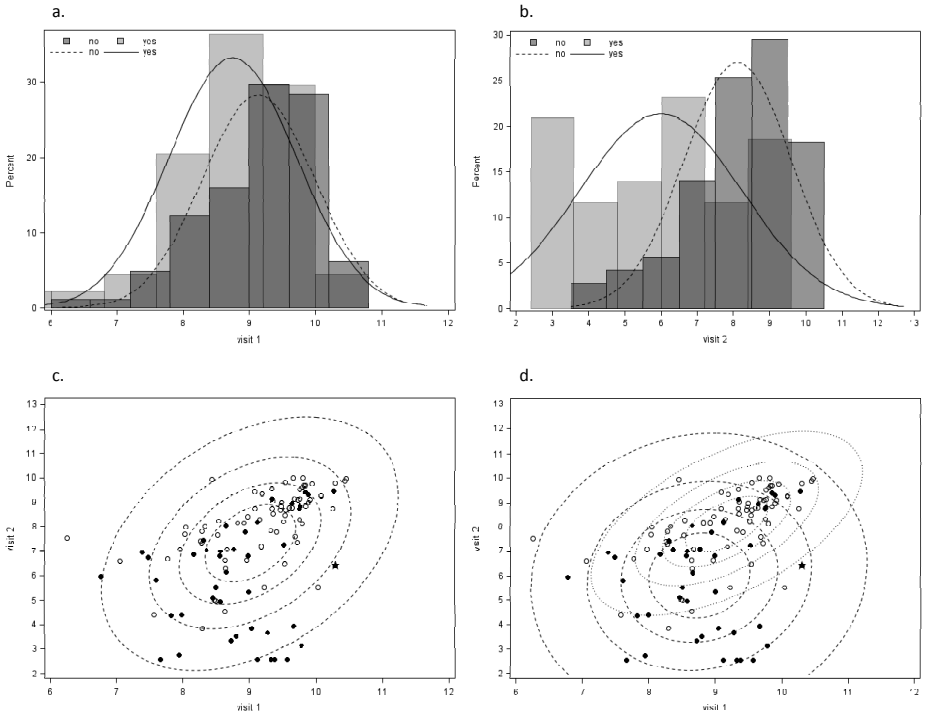


**Figure 1.The** distribution of a marker at a. visit 1 and b. visit 2 by response and the bivariate distribution of the marker c. overall and d. by response. For the subject highlighted by * the marker lies as an outlier at visit 1 but not at visit 2, and stays an outlier in the bivariate distribution in c. as well as in d.

$$\begin{aligned}\text{logit } p_{i,j} &= \text{logit } Pr(R_i = 1|\text{ visit} = j, \boldsymbol{y}_{i,j}, p_{i,0}) \\ &= \alpha_j + \beta_j \boldsymbol{y}_{i,j} + PI_{i,0},\end{aligned}$$

where $PI_{i,0} = logitp_{i,0}$ the baseline log odds of response is used as offset. This results in $j = 1, \ldots, m$ (the number of follow-up visits) logistic regression models with each an update of the baseline prediction. Alternatively we suggest to combine these models in a GEE manner: a pooled logistic regression analysis treating each visit as an observation and in the analysis correct for the fact that a patient is present with more visits. This idea is borrowed from Hernan and Robins,[12] who used it in their set up of the Marginal Structural Mean Model. With the inclusion of the visit time as an explanatory variable and an interaction term with the markers to estimate changes in effect of the markers over time the full GEE model, with an independent working correlation matrix, is written:

$$\begin{aligned}\text{logit } p_{i,j} &= \text{logit } Pr(R_i = 1|\text{visit} = j, \boldsymbol{y}_{i,j}, p_{i,0}) \\ &= \alpha + \beta_T t_{i,j} + \beta_Y \boldsymbol{y}_{i,j} + \beta_{Y,T} \boldsymbol{y}_{i,j} t_{i,j} + PI_{i,0}\end{aligned}$$

where $\alpha_j = \alpha + \beta_T t_{i,j}$ and $\beta_j = \beta_Y \boldsymbol{y}_{i,j} + \beta_{Y,T} \boldsymbol{y}_{i,j} t_{i,j}$.
If the effect of the markers does not change significantly over time the interaction terms may be dropped and the model simplifies to:

$$\begin{aligned}\text{logit } p_{i,j} &= \text{logit } Pr(R_i = 1|\text{visit} = j, \boldsymbol{y}_{i,j} + p_{i,0}) \\ &= \alpha + \beta_Y \boldsymbol{y}_{i,j} + \beta_T t_{i,j} + PI_{i,0}\end{aligned}$$

Here time $t_{i,j}$ is entered as a linear term, alternatively a smooth function of time can be used. The model can further be defined to include baseline covariates $X_i$, the history of markers $\mathcal{Y}_{i,k}$ or include interactions over time with baseline covariates. Also changes of effect of baseline variables over time and even changes of effect of markers by baseline variables as well as covariates measured during the visits could be studied. In case of irregular time intervals between visits it is advisable to extend the model with the distance between visits to study this effect on the outcome. The model results in a set of new updated predictions depending on visit j and measurement $Y_{i,j}$.

**The patterns of the makers as predictors**

Above the observed values of the markers were included directly as predictors of $R_i = 1$. Instead the behavior of the markers over time, for example the increase or decrease over time or some other summary measures, may be better predictors of the clinical outcome. Maruyama et al.[8] applied this idea to data on smoking cessation. We shall adapt their method in a dynamic way. Suppose the markers are observed until time T then the model is fitted in two steps. First a multivariate linear mixed model is fitted to the markers $\boldsymbol{Y}_i = (\boldsymbol{Y}_{i,1}, \ldots, \boldsymbol{Y}_{i,m})$ observed in the interval [0, T], i.e. $t_{i,m} < T$:

$$\boldsymbol{Y}_i = \boldsymbol{X}_i \boldsymbol{\beta} + \boldsymbol{Z}_i \boldsymbol{b}_i + \boldsymbol{\epsilon}_i \tag{1}$$

In this expression $\boldsymbol{\beta} = (\beta_1, \ldots, \beta_p)'$ is a vector of fixed effects, $\boldsymbol{b}_i = (b_{i,1}, \ldots, b_{i,q})'$ is a vector of random effects, $\boldsymbol{X}_i$ and $\boldsymbol{Z}_i$ are design matrices for fixed and random effects, respectively. These design matrices may contain time, time squared or as

suggested above a smooth function of time. The random effects are assumed to be normally distributed $\boldsymbol{b_i} \sim N(0, \boldsymbol{D})$ independent across patients with a covariance matrix $\boldsymbol{D}$. The errors are assumed independent normally distributed $\boldsymbol{\epsilon_i} \sim N(0, \Sigma_i)$, where $\Sigma_i$ is a block-diagonal matrix with diagonals $diag(\sigma_1^2, \ldots, \sigma_r^2)$. The errors are further assumed to be independent of the random effects $\boldsymbol{b_i}$. The Empirical Bayes (EB) estimates of the random effects are given by $\hat{\boldsymbol{b}}_i = E(\boldsymbol{b}_i|\boldsymbol{Y} = \boldsymbol{y}_i) = \hat{\boldsymbol{D}}\boldsymbol{Z}_i'\hat{\boldsymbol{V}}_i^{-1}(\boldsymbol{y}_i - \hat{\boldsymbol{X}}_i\hat{\boldsymbol{\beta}})$ and the variance of the predictions $\hat{\boldsymbol{b}}_i - \boldsymbol{b}_i$ is $var(\hat{\boldsymbol{b}}_i - \boldsymbol{b}_i) = \hat{\boldsymbol{D}} - var(\hat{\boldsymbol{b}}_i)$ with $var(\hat{\boldsymbol{b}}_i) = \hat{\boldsymbol{D}}\boldsymbol{Z}_i'\{\hat{\boldsymbol{V}}_i^{-1} - \hat{\boldsymbol{V}}_i^{-1}\hat{\boldsymbol{X}}_i(\sum_1^N \hat{\boldsymbol{X}}_i'\hat{\boldsymbol{V}}_i^{-1}\hat{\boldsymbol{X}}_i)^{-1}\hat{\boldsymbol{X}}_i'\hat{\boldsymbol{V}}_i^{-1}\}\boldsymbol{Z}_i\hat{\boldsymbol{D}}$ where $\boldsymbol{V}_i = \boldsymbol{Z}_i\boldsymbol{D}\boldsymbol{Z}_i' + \Sigma_i$.
In the second step a logistic regression of the clinical outcome with the EB estimates as predictors are fitted:

$$\begin{aligned}\text{logit } p_{i,j} &= \text{logit } Pr(R_i = 1|\boldsymbol{X}_i, \mathcal{Y}_{i,j}, t_{i,j} \leq T) \\ &= \boldsymbol{\gamma}_0 + \boldsymbol{\gamma}_1\boldsymbol{X}_i + \boldsymbol{\gamma}_2\hat{\boldsymbol{b}}_i\end{aligned}$$

Since $\hat{\boldsymbol{b}}_i$ is estimated with error Maruyama[8] suggest to adjust the predictions using the following normal approximation of the standard logistic distribution achieved by the delta method:

$$\text{logit } p_{i,j}^{adjusted} = \frac{\boldsymbol{\gamma}_0 + \boldsymbol{\gamma}_1\boldsymbol{X}_i + \boldsymbol{\gamma}_2\hat{\boldsymbol{b}}_i}{\sqrt{1 + (\boldsymbol{\gamma}_2' var(\hat{\boldsymbol{b}}_i - \boldsymbol{b}_i)\boldsymbol{\gamma}_2)/c^2}}$$

where $c = 15 * \pi/16\sqrt{3}$.
This approach results in estimates of the prediction of response using the longitudinal profile of the markers observed until time T. The process may sequentially be repeated when new visits are scheduled and new measurements of the markers are reported. Hereby a dynamic update of the prediction of response is obtained at each new visit. For a future *new* patient a possible dynamic updating strategy for prediction is: Suppose the markers are observed until visit time $T$ for all subjects. For the *new* subject observed until visit $k$ the prediction of response $p_{new,k}$ at $t_{new,k}$ could be estimated as follows: the observed values of the markers are added to the total database and the multivariate linear mixed model is fitted. Borrowing information of all subjects observed in the time interval $[0, T]$ the subject specific random effects are achieved for the *new* subject. The prediction of response at visit $k$, $p_{new,k}$ are now estimated with the logistic regression, and adjusted as described above. For the next visits of the *new* subject the updated predictions of response are obtained sequentially repeating the steps above for each visit.

## Longitudinal prediction with the indirect approach

Brant and Morrell[10] present a prediction process classifying a future subject into the outcome groups, responder and non-responder sequentially one observation at a time. First assume a training dataset exists and consider a future *new* subject. Let this subject enter both the subgroup of responders and the subgroup of non-responders. By the indirect approach first the multivariate linear mixed effects model of the longitudinal markers, model (1), is fitted but now separately for the subgroup of responders and non-responders resulting in two sets of estimates indicated with the index $r = 0$ or 1. As a result the future subject is characterized by a predictive density $f_r(y_{new})$, which will be introduced below.
Given the prior probability $p_0$ of response and the estimation result of the longitu-

$$Pr(R_{new} = 1|\boldsymbol{Y}_{new} = \boldsymbol{y}_{new}) = \frac{p_0 f_1(\boldsymbol{y}_{new})}{(1-p_0)f_0(\boldsymbol{y}_{new}) + p_0 f_1(\boldsymbol{y}_{new})}$$

Brant and Morrell[10] propose three estimation approaches to compute the posterior probabilities, namely the marginal approach, the conditional approach and the random approach:
The **marginal approach** uses the marginal distribution of $\boldsymbol{Y}_{new}$ conditioning on $R_{new} = r$, $r = 0, 1$ determined by model (1):

$$[\boldsymbol{Y}_{new}|R_{new} = r] \sim N(\boldsymbol{X}_{r,new}\beta_r, \boldsymbol{V}_{r,new}) \tag{2}$$

where $\boldsymbol{V}_{r,new} = \boldsymbol{Z}_{r,new}\boldsymbol{D}_r\boldsymbol{Z}'_{r,new} + \Sigma_{r,new}$.
For the *new* subject it then follows that the longitudinal marker has a marginal density function $f_r(\boldsymbol{y}_{new})$ given by the multivariate normal probability density function with mean $\boldsymbol{X}_{r,new}\beta_r$ and variance $\boldsymbol{V}_{r,new}$, $r = 0, 1$. This marginal density function can now be used to calculate the posterior probability of response.
The **conditional approach** uses the conditional distribution of $\boldsymbol{Y}_{\boldsymbol{r,new}}$ given $r = 0, 1$ and a vector of individual random effects $\boldsymbol{b}_{r,new}$ derived from model (1):

$$[\boldsymbol{Y}_{\boldsymbol{new}}|R_{new} = r, \boldsymbol{b}_{r,new}] \sim N(\boldsymbol{X}_{r,new}\beta_r + \boldsymbol{Z}_{r,new}\boldsymbol{b}_{r,new}, \Sigma_{r,new}) \tag{3}$$

For a new subject, individual random effects are estimated using the EB estimates as given previously and hereafter inserted in (3). The density function, $f_r(\boldsymbol{y}_{new}|\boldsymbol{b}_{r,new})$ of the conditional distribution of $(\boldsymbol{Y}|R_{new} = r, \boldsymbol{b}_{r,new})$, $r = 0, 1$ is now used to estimate the posterior probabilities.

Finally in the **random approach** the distribution of the individual random effects $b_{r,new} \sim N(0, \boldsymbol{D_r})$ is used to compute the posterior probabilities. The density function $f_r(\boldsymbol{y}_{r,new})$ is equal to the density of $\boldsymbol{b}_{r,new}$ evaluated at the estimated value of the random effect $\hat{b}_{r,new}$, $r = 0, 1$, at each stage of the prediction process.
For each of the three approaches the prediction process proceeds sequentially one observation at a time for the new subject. First the markers of the first visit are considered and the $\boldsymbol{X}_{r,new}$ and the $\boldsymbol{Z}_{r,new}$ design matrices are constructed for each outcome non-responder and responder, $r = 0, 1$. The marginal means and the EBs are calculated for each outcome and finally depending on the approach the considered and the prediction process continues: the $\boldsymbol{X}_{r,new}$ and the $\boldsymbol{Z}_{r,new}$ design matrices are constructed for each outcome group, the marginal means and then the posterior probabilities are calculated etc. The prediction process is completed for all observations, sequentially extending with the markers of the next visit.
The influence of three approaches on the posterior probabilities depends on how well the mixed linear model describes the markers. If the patterns of the markers of the new subject follow the patterns of the population mean well of either the response or non-response group the random effects are either close to zero or far away from zero. If close to zero this indicates that the data is more likely to come from this particular group. If far away from zero this indicates that the subject is unlikely to come from this group. For the marginal and the conditional approach this results in posterior probabilities close to one or zero. The random effect approach depends only on the distribution of the random effects and this maybe explains the more moderate posterior probabilities (see application). The impact of a subject with patterns of the markers which do not follow the mean pattern of neither the response nor the non-response group may be illustrated with the following simple example. Consider the case where Y is observed at two visits: (Y1, Y2), figure 1. In figure 1.a and 1.b the density of the observations Y1 and Y2 are given by response yes or no. In figure 1.c the density of (Y1, Y2) is plotted and in 1.d by response yes or no. In the specific case where Y1 is an outlier of the distribution, but at visit 2 at the center of the distribution in the multivariate normal distribution the case remains an outlier. The influence on the marginal and the conditional approach is enormous once a prediction in the wrong direction is made and it is difficult to get back on track. In the application an example of this situation is given.
Summarizing the indirect method of Morrell and Brant,[10] three approaches are considered each giving a new posterior prediction of response depending on the longitudinal profile of the observed markers. In the following all three approaches are applied.

# CLINICAL DATA ON HEPATITIS B PATIENTS

The data reported here are from the international HBV9901-trial on chronic hepatitis B, HBeAg positive patients, Janssen et al.[1] Two-hundred and sixty-six patients were randomized to receive either peg-interferon mono-therapy for 52 weeks or PEG-IFN in combination with lamivudine. The final overall response, defined as HBeAg negativity 24 weeks after treatment, was achieved in 36%, there was no significant difference between treatment arms (36% vs 35%, p=0.91). During treatment the two markers: the viral load (HBV DNA (copies/ml)) and the disease activity (ALT (U/ml)) were measured every 4th week until the end of follow-up (week 76). Since the HBV DNA decline under lamivudine showed a total different pattern in comparison with PEG-IFN monotherapy, with in general a steep decline during therapy followed by a relapse post-treatment, only the subset of patients with peg-interferon monotherapy (n=136) will be studied here. Furthermore patients with other HBV genotypes than A, B, C, and D (n=11) are excluded.

For this study response to therapy, $R = 1$ is defined as loss of HBeAg at end of follow-up, week 78 and $R = 0$ otherwise. The time unit is weeks with $Y_{1,i,j}$ the load, i.e. $\log_{10}$ value of the viral load (HBV DNA) and $Y_{2,i,j}$ the $\log_e$ value of the ALT. Our main clinical interest is to estimate $p_{i,j}$ in the first time-interval from week 4 to week 32 to identify a possible stopping rule as early as possible in the treatment schedule.

In a previous study[13,14] we described in detail baseline factors influencing the response rate and developed a prediction model which provides a subject specific prediction of response. The baseline factors associated with response were: HBV-genotype, age, gender, load = baseline HBV DNA (copies/ml, $\log_{10}$), ALT ($\log_e$) and previous treatment with IFN. The effect of the baseline covariates is summarized by the prognostic index $PI_{i,0}$ of subject $i$. In general define for the explanatory baseline variables: HBV-genotype, binary variables $geno_{i,G}$, where G = A, B, C or D (genotype A is set as the reference) , $age_i$ (in years), $sex_i$ (0 is male, 1 is female), $PrRx$ (0 for not treated before, and 1 for previous treated) and baseline $ALT_0$ and $load_0$.

# RESULTS

The observed evolution and variability of the two markers: HBV DNA and the ALT in the group of non-responders and responders are shown in figure 2. For the responders an early decline of the load is observed while the non-responders show

more or less no change from baseline load. Differences in ALT-behavior are less pronounced.

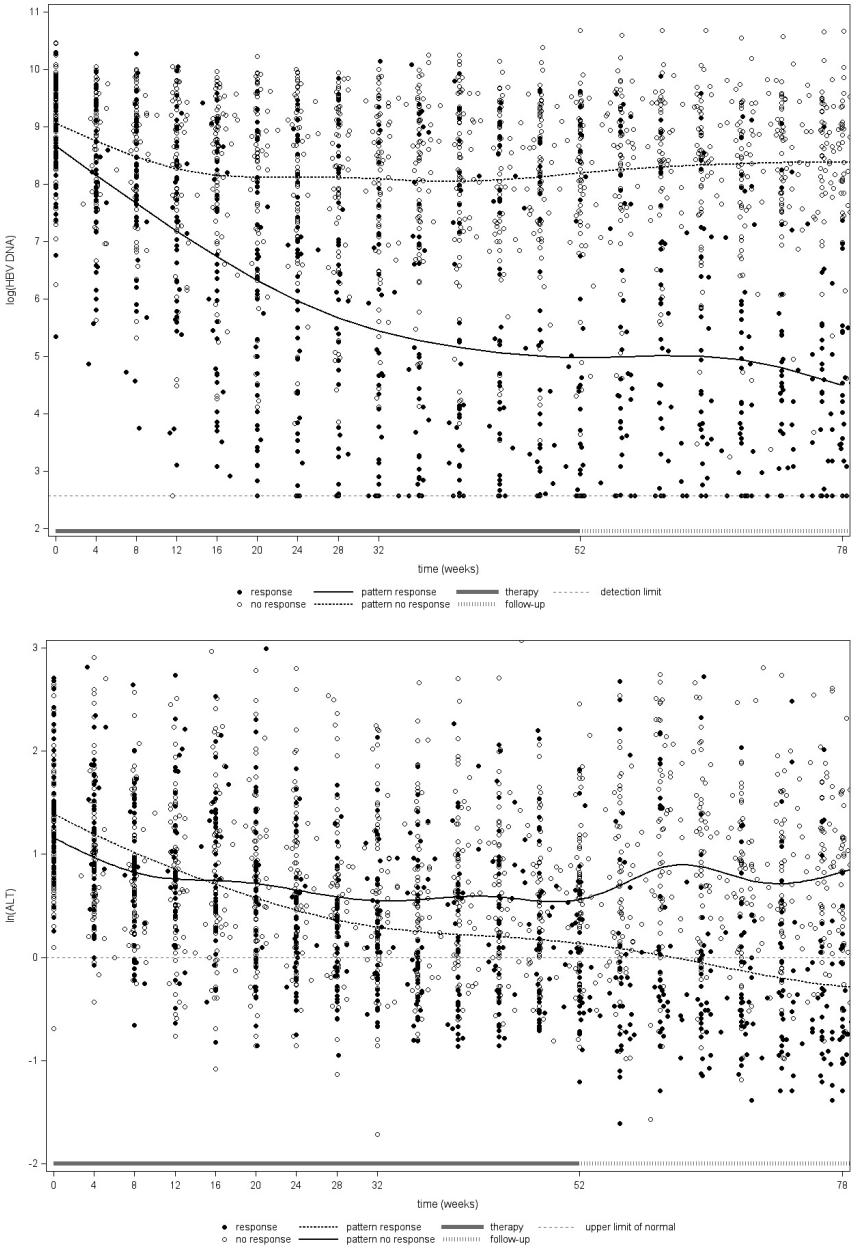


**Figure 2.** Observed HBV DNA and ALT during treatment (week 0-52) and during follow-up (week 52-78) by response, together with the marginal predicted means.

## Results of the longitudinal prediction with the direct approach

First the result of the logistic regression method is presented. In a previous study[13,14] of the response to therapy a prognostic model of baseline factors were designed and validated, and a alongside prognostic indexes ($PI_0$) were calculated (as described in Section 3). In this updating pooled logistic regression model the subject specific PI's are used as offsets. The final model to predict response using the data of all patients, n=125 and the repeated measurements of the load and the ALT the first 32 weeks of therapy is

$$\begin{aligned}\text{logit } p_{i,j} &= \text{logit } Pr(R_i = 1|\text{visit week} = t_{i,j}, \text{load}_{i,j}, ln(\text{alt})_{i,j}, \text{offset} = PI_{i,0}) \\ &= \alpha + \beta_T t_{i,j} + \beta_{\text{load}}\text{load}_{i,j} + \beta_{\text{alt}} ln(\text{alt}_{i,j}) + PI_{i,0}\end{aligned}$$

This model was obtained by comparing Akaike's Information Criterium (AIC's), the log likelihood and the Area Under the receiver operating Curve (AUC); i.e. the c-statistic of different models, Steyerberg, chapter 11 and 16.[11] The model did not improve by adding B-splines (Steyerberg, chapter 12)[11] of load, of ALT or of time, neither by adding interaction terms with baseline variables nor by extending with the load or the ALT of the previous visit.

For individual prediction of the response for subject $i$, the model above was refitted leaving out subject $i$. This gives a cross-validated prediction for each patient. To illustrate the results of the fitted model the individual prediction of 3 typical subjects are plotted in figure 3. Figure 3.a is a responder with an early drop of HBV DNA around week 16 and a steady decline of ALT to normal-levels, the baseline prediction of response was 62%. The dynamic prediction of response changes to around 90% after week 16. Two non-responders are plotted in 3.b and 3.c. The subject in 3.b had at baseline already a low prediction of response around 10%, the load declines 0.5 log the first 20 weeks and only from week 24 a decline is seen, the prediction of response changes little over time and stays around 10%. In 3.c the subject with genotype C experiences a sudden drop of HBV DNA at week 12 just to return to baseline levels at the next visit. This pattern is not often seen (in this specific case probably an error occurred at the laboratory, however this could not be confirmed) but may illustrate what happens in the case where a dose-reduction is anticipated or the subject does not adhere to treatment. However the direct prediction approach with the observed markers do not seem to suffer from this; the prediction of response gave an increase of response at week 12 and a decrease afterwards.

The individual predictions of response over time for all subjects are plotted in figure 4 separate for non-responders and responders.

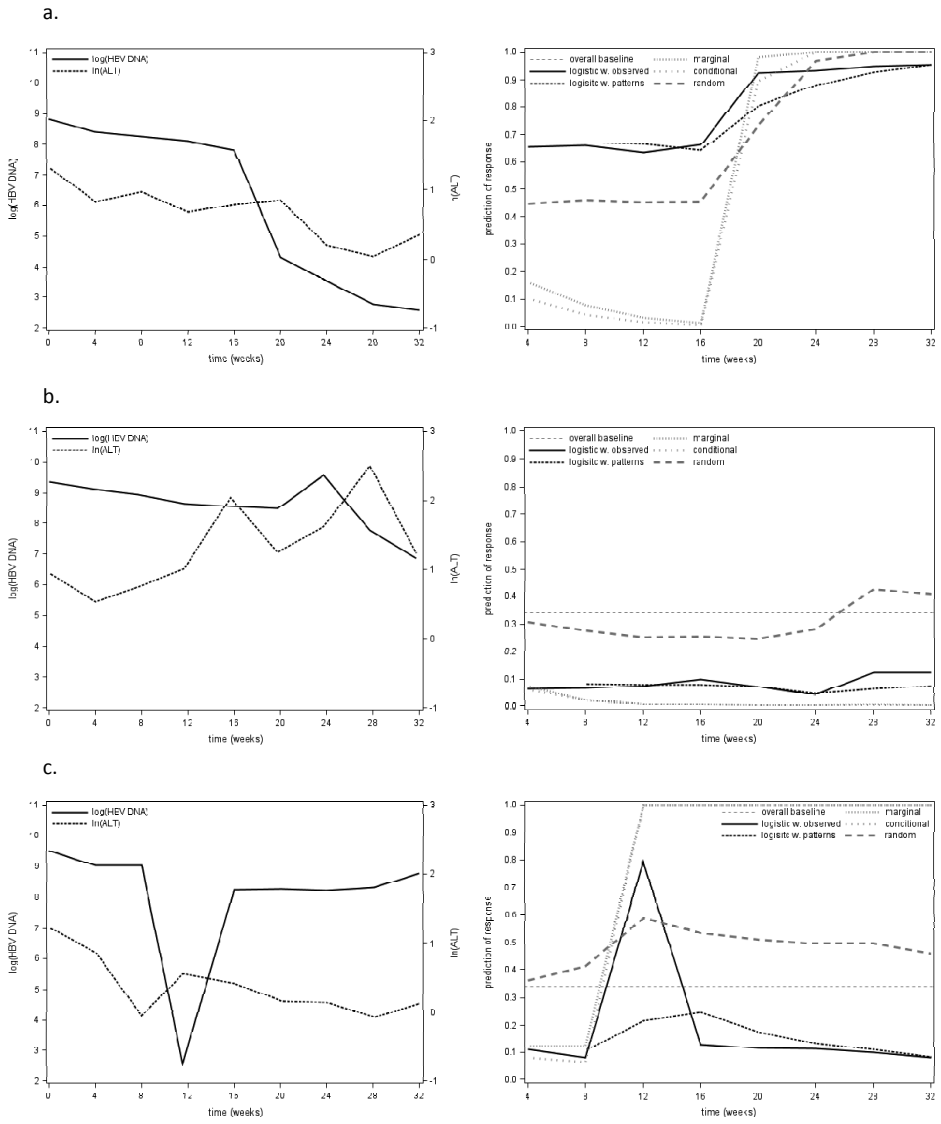


**Figure 3.** Behavior and prediction of tree typical subjects: a. subject with a response at week 78, b. subject without response at week 78 and c. a subject without response at week 78, but with a sudden dip in HBV DNA at week 12. In the left panel the observed individual behavior of HBV DNA and ALT the first 32 weeks is given. In the right panel the estimated dynamic prediction of response at each new visit with the different methods

Applying the logistic regression method of the patterns of the markers the following results were obtained. A simple linear mixed model without baseline covariates with random intercepts and random slopes was first designed to estimate the subject specific patterns of the load during the first 32 weeks:

$$\boldsymbol{Y}_{i,j} = \beta_1 + \beta_2 t_{i,j} + b_{1,i} + b_{2,i} t_{i,j} + \epsilon_{i,j}$$

where $(b_{1,i}, b_{2,i})'$ are i.i.d. normally distributed random effects with covariance matrix $\boldsymbol{D}$. Further, $\epsilon_{i,j}$ are i.i.d. normally distributed error terms with zero mean and variance $\sigma^2$ and $\beta_1$ and $\beta_2$ are the fixed effects.
For each subject $i$ the model was first fitted omitting data after visit 3 (week 8) of subject i. (Omitting also visit 3 brings us back to a simple logistic regression model with extension of one observation, which therefore is not considered here.) The logistic regression of response, $R = 1$, was subsequently fitted including the PI as an offset, similar to the previous method, and then adding the random effects as predictors:

$$\begin{aligned}\operatorname{logit}(p_{i,3}) &= \operatorname{logit}(R_i = 1 | \hat{b}_{1,i}, \hat{b}_{2,i}, \text{offset} = PI_{i,0}) \\ &= \gamma_0 + \gamma_1 \hat{b}_{1,i} + \gamma_2 \hat{b}_{2,i} + PI_{i,0}\end{aligned}$$

With the covariance matrix of the estimated random intercept and random slope the prediction of response was afterwards adjusted as described in section 2.2.
Subsequently, the next visits of subject $i$ were added one at a time to the total data and the models (first the linear mixed model and then the logistic regression) were refitted.
We extended the linear mixed model with baseline covariates and repeated the process described above. As could be expected this did not change the predictions since the logistic regression also includes these variables via the PI's. Next we designed a bivariate linear mixed model of the load and the ALT. At all visits the random intercept and random slope of ALT were never significant (p's>0.85) and even the c-statistics declined reflecting overfitting. We therefore chose to present the simple model above.
The results are displayed in figure 3 for three typical subjects and in figure 4 the overall plots of the prediction of response separate for subject with and without an observed response. The prediction of response looks very similar to the previous approach for subject a and b while for subject c the estimates are more smooth and without sudden jumps. This observation is also seen in figure 4.

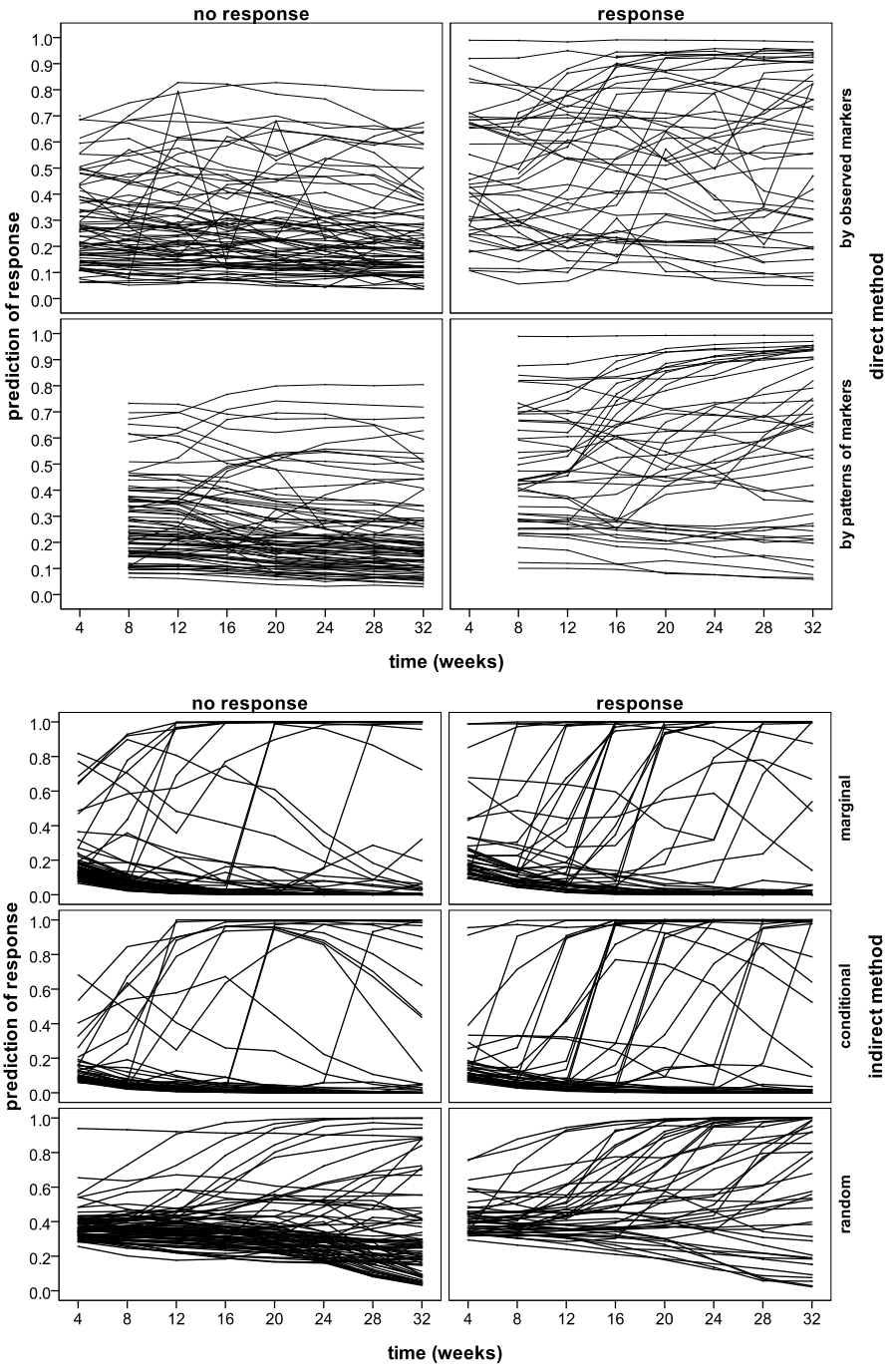


**Figure 4.** Dynamic prediction of response by the different methods separate for the group with and without response at week 78

## Results of the longitudinal prediction with the indirect approach

A bivariate linear mixed effects model was designed to model = $load$ and $ln(ALT)$ simultaneous. To catch the behavior of the load and ALT over time B-splines of time with 5 inner knots and 3 degrees were included as fixed effects while for the individual fit a B-spline with no inner knots and 2 degrees was included as random effects:

$$\begin{aligned} Y_{1,i,j} = load_{i,j} = \beta_1^1 + \beta_2^1 \text{sex}_i + \beta_3^1 \text{age}_i + \beta_4^1 \text{PrRx}_i \\ + \beta_5^1 \text{geno}_{i,B} + \beta_6^1 \text{geno}_{i,C} + \beta_7^1 \text{geno}_{i,D} \\ + B_{3,5}^{1,fixed}(t_{i,j}) \\ + b_{0,i}^1 + B_{2,0,i}^{1,random}(t_{i,j}) + \varepsilon_{i,j}^1 \end{aligned}$$

$$\begin{aligned} Y_{2,i,j} = ln(alt)_{i,j} = \beta_1^2 + \beta_2^2 \text{sex}_i + \beta_3^2 \text{age}_i + \beta_4^2 \text{PrRx}_i \\ + \beta_5^2 \text{geno}_{i,B} + \beta_6^2 \text{geno}_{i,C} + \beta_7^2 \text{geno}_{i,D} \\ + B_{3,5}^{2,fixed}(t_{i,j}) \\ + b_{0,i}^2 + B_{2,0,i}^{2,random}(t_{i,j}) + \varepsilon_{i,j}^2 \end{aligned}$$

where $B_{2,0,i}^{l,random}$ is the B-spline depending on 2 random effects $b_{1,i}^l, b_{2,i}^l$, $l = 1$ for load and $l = 2$ for ln(ALT). That is, the random effect vector for load is $\boldsymbol{b}^1{}_i = (b_{0,i}^1,\ b_{2,i}^1,\ b_{3,i}^1)'$, i.i.d. normally distributed random effects with zero mean and a general covariance matrix $\boldsymbol{D}^1$ . Further, $\varepsilon_{i,j}^l$ are i.i.d. normally distributed error terms with zero mean and a variance $\sigma_l^2$. Finally, $\beta_1^l, \ldots, \beta_7^l$ along with $B_{3,5}^{l,fixed}$ are fixed effects for load, $l = 1$ and ln(ALT), $l = 2$. Convergence was not achieved when adding a B-spline with a higher degree to the random effect.

For each subject $i$ the model above was fitted separately to the group of responders and non-responders, omitting the data of subject $i$, similar to the cross-validated approach of the pooled logistic regression above. With the two sets of fit per subject - as a responder and as a non-responder - the posterior probability of response was then calculated one visit at a time with the marginal approach, the conditional approach or the random effect approach. The process is illustrated with the subject specific plots in figure 3. The pattern of the observed load of the subject in 3a is best described by the marginal fit of the group of responders (figure 2). The prediction of response for the marginal and the conditional approach is already emerging to 100% at week 16. For the random effect approach the prediction remains at the

overall baseline prediction of response until week 16 thereafter it too increases to 100%. In Figure 3b the pattern of the load behaves like the marginal fit of the non-responders (figure 2). The prediction of response declines quickly to below 10% for the marginal and conditional approach, while the random effect approach stays around 36%. The pattern of the load of the subject with a sudden drop in HBV DNA, figure 3.c, behaves overall as a non-responder however because of the drop the prediction process emerges to 1 at the time of the drop and does not recover when the load increases at the next visit. This problem is observed both for the marginal and the conditional approach, while the random approach dwells around 30-50%.

The overall fit of the posterior probability of response per approach are given in figure 4 separately for observed responders and non-responders. As well as for the marginal as the conditional approach a clear separation of the posterior prediction of response to either almost 0 or almost 1 is observed, while the random approach gives a slower increase or decrease of the prediction of response. The separation is best for the observed non-responders while for the responders some subjects have a low prediction of response.



## Comparison

First some general comments on the estimated predictions (figure 4) and on our experience working with the methods. For the indirect approach the marginal and conditional methods behave similar and either predict close to 0% or 100% (figure 4) this reflects poor calibration with overconfident prediction. The marginal approach seems to detect the direction of response earlier, but both approaches suffer from the fact that once a direction is predicted it cannot return and an absorbing state is achieved. We illustrated this phenomena in figure 1. In practice this indeed makes them less useful. The effect of treatment of hepatitis B is difficult to predict, since the virus can mutate or the virus suddenly may respond to therapy. Keeping this in mind the use of the overall clinical history of the markers in the marginal and conditional approach attribute to the problems with these methods.

The indirect random approach and the direct approaches behave more flexible over time (figure 4) and therefore also allow for a better calibrated prediction, keeping the possibility open for changing direction. The logistic regression has the advantage that it can actually change direction. The model is moreover constructed in such a way that it only uses information of the last visit and not the whole clinical history, since this did not contribute to the model-fit. The baseline logistic results is easy

to use in clinical practice and to extend the logistic regression with the dynamic model fit can easily be incorporated. No extra statistical computational work has to be done, but inserting the new observed markers in the estimated prediction model. For this reason the method has a strong clinical applicability.

To compare the predictive performance of the different approaches the aspects of discrimination and calibration are studied.[11] The c-statistics for each week and for each method is plotted in figure 5 together with the results of the baseline model using only PI. Past week 16 an increase of the c-statistics is observed, suggesting that beyond baseline prediction at least 16 weeks of treatment is necessary before an early update of prediction of response is sensible. The best discriminative ability for this data was observed with the direct approaches. The calibration slope[11] for each week are depicted in figure 6. The indirect methods all have suffers from severe calibration problems. The predictors behave more like classifiers and are overconfident. The direct methods perform well with a calibration slope close to one, with a little overfitting for the direct approach with the observed markers mainly before week 16.

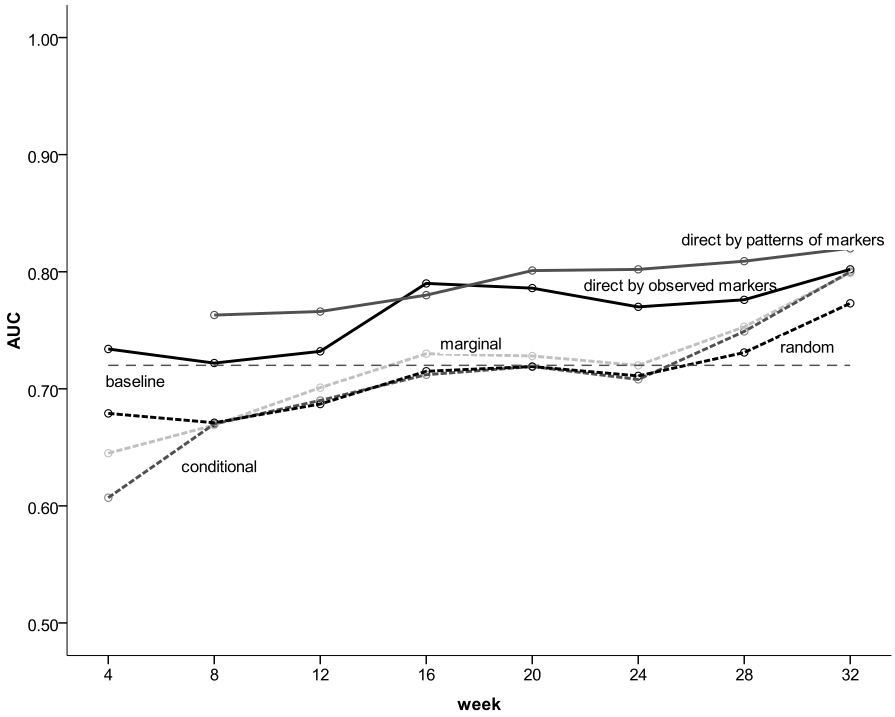


**Figure 5.** The AUC (c-statistics) for the different methods by visit week.

Finally some comparison of classification outcomes. In table 1 several stopping rules were applied with different cut-points. Our aim for a clinical useful stopping rule are to stop as many subjects without a true response as possible(i.e. not to stop a possible responder); therefore a high negative predictive value (NPV) and a high sensitivity (SENS) are important while the specificity (SPEC) and the positive predicted value(PPV) are of less importance. Comparing the results the direct approach especially the logistic regression using the patterns of the markers gives the best option.

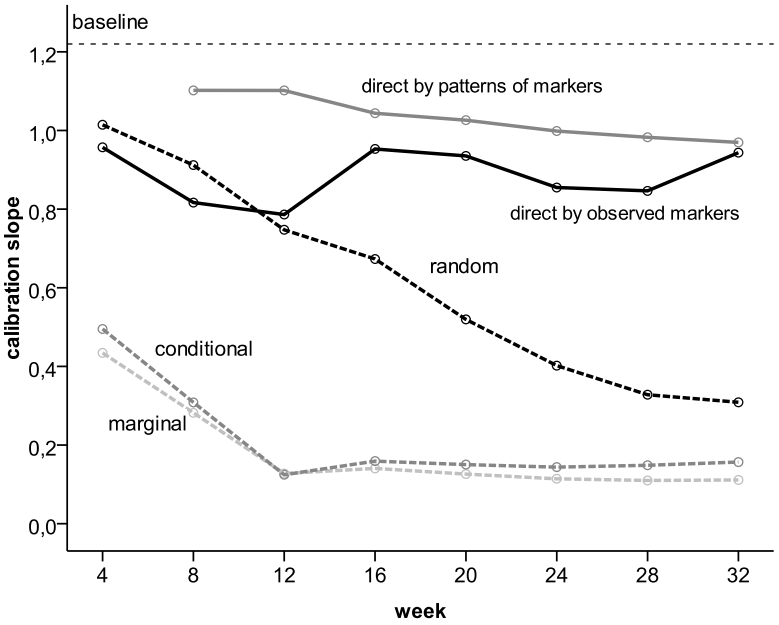


**Figure 6.** The estimated calibration slope for the different methods by visit week.



# DISCUSSION

Dynamic updates of prediction of treatment response are not done routinely when new information of longitudinal biomarkers becomes available. In this paper dynamic prediction methods for the case of a binary outcome are presented and compared. We introduce a direct approach extending the logistic regression model with the observed marker values along with time. We elaborate on the direct approach extending with a set of parameters describing the patterns of the biomarkers over time. In the indirect approach the distribution of the markers estimated by linear

**Table 1.** Results of different stopping rules with a selection of different classification cutoff's of the predicted response

| | Classification | Stop during Rx | | | | | Stop week 24 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method | Cutoff | % stop | PPV | NPV | SENS | SPEC | % stop | PPV | NPV | SENS | SPEC |
| direct method | 0.1 | 12.0 | 36.4 | 73.3 | 90.9 | 13.6 | 8.0 | 36.5 | 80.0 | 95.5 | 9.9 |
| by observed | 0.15 | 17.6 | 35.9 | 68.2 | 84.1 | 18.5 | 24.0 | 41.1 | 83.3 | 88.6 | 30.9 |
| markers | 0.2 | 26.4 | 37.0 | 69.7 | 77.3 | 28.4 | 31.2 | 45.3 | 87.2 | 88.6 | 42.0 |
| direct method | 0.1 | 13.6 | 38.0 | 82.4 | 93.2 | 17.3 | 11.2 | 37.8 | 85.7 | 95.5 | 14.8 |
| by patterns of | 0.15 | 16.8 | 39.4 | 85.7 | 93.2 | 22.2 | 24.8 | 42.6 | 87.1 | 90.9 | 33.3 |
| markers | 0.2 | 24.0 | 41.1 | 83.3 | 88.6 | 30.9 | 36.8 | 49.4 | 89.1 | 88.6 | 50.6 |
| indirect method | 0.001 | 44.0 | 50.0 | 83.6 | 79.5 | 56.8 | 22.4 | 39.2 | 78.6 | 86.4 | 27.2 |
| marginal | 0.01 | 59.2 | 54.9 | 78.4 | 63.6 | 71.6 | 52.8 | 52.5 | 80.3 | 70.5 | 65.4 |
| | 0.05 | 70.4 | 56.8 | 73.9 | 47.7 | 80.2 | 63.2 | 52.2 | 74.7 | 54.5 | 72.8 |
| indirect method | 0.001 | 48.8 | 51.6 | 82.0 | 75.0 | 61.7 | 23.2 | 39.6 | 79.3 | 86.4 | 28.4 |
| conditional | 0.01 | 64.8 | 59.1 | 77.8 | 59.1 | 77.8 | 57.6 | 52.8 | 77.8 | 63.6 | 69.1 |
| | 0.05 | 75.2 | 38.7 | 66.0 | 27.3 | 76.5 | 68.8 | 53.8 | 73.3 | 47.7 | 77.8 |
| indirect method | 0.1 | 18.4 | 38.2 | 78.3 | 88.6 | 22.2 | 0.0 | 35.2 | 0.0 | 100.0 | 0.0 |
| random | 0.15 | 20.8 | 39.4 | 80.8 | 88.6 | 25.9 | 1.6 | 34.1 | 0.0 | 95.5 | 0.0 |
| effect | 0.2 | 33.6 | 42.2 | 78.6 | 79.5 | 40.7 | 14.4 | 36.4 | 72.2 | 88.6 | 16.0 |
| | 0.3 | 44.0 | 47.1 | 80.0 | 75.0 | 54.3 | 38.4 | 45.5 | 81.3 | 79.5 | 48.1 |

mixed effect models are used to calculate posterior probabilities of treatment response. The methods result in a dynamic individualized update of the prediction of response. The approaches were applied to data on the peginterferon treatment of chronic hepatitis B and their predictive performance were compared.

The modelling approaches are very flexible in that they allow inclusion of more than one biomarker. The biomarkers may be described by complex mixed modelling if necessary, however one has to be aware not to overfit. For the indirect approach splines were used to describe the decline of viral load and ALT of a hepatitis B patient. Instead of linear mixed models, non-linear mixed models might be considered or ordinal distributed biomarkers might be modelled. Fieuws et al.[15] studied multivariate longitudinal profiles allowing a joint distribution of the random effects. They further[16] used non-linear mixed of longitudinal profiles designing a set of classification rules. Komárek et al.[17] relaxed the normal assumption of the random effects biomarkers studying a heteroscedastic multivariate normal mixture for the random effects. The direct approach with the observed biomarkers enables as the term say direct inclusion of any marker, ordinal or non-linear as well as inclusion of interaction over time.



The indirect method and the direct method, which uses the parameters describing the patterns of the biomarkers over time, have one small drawback being the computational programming, which is elaborate and time consuming and new estimates need to be established for a future subject before predictions can be calculated. In contrast, standard statistical procedures are available for the direct method of the observed markers and a prediction model can be expressed[7] which directly can be applied to a future subject. This model though, has limited memory of the patterns of the biomarkers (depending on inclusion of the markers at previous visits), which can be an advantage in the situation were the last observed biomarkers predicts the outcome well, and a disadvantage if the prediction of the outcome is better associated with the total pattern of the markers. In the situation of response of peginterferon treatment the last observed load predicts just as well as the estimated decline.

In our situation we studied a binary outcome. Brant and Morrell used the indirect method in several clinical studies.[9,10,18] Especially they focused on longitudinal measurements of the prostate specific antigen to predict prostate cancer. They observe more than two outcome categories (ex. no cancer, low risk, high risk) and constructs an elegant stopping rule depending on posterior probabilities over time. Our clinical situation is simpler, but therefore also allows us to easily study different stopping rules. In case of more than two outcome categories a generalized logit

approach to apply the direct approach would be necessary.
Maruyama[8] used the direct approach with an application to data on smoking cessation and the relation with longitudinal measurements of carving. They first estimated the decline of carving over time and then included the estimated individual slope in the logistic regression of smoking cessation. Their situation is very similar to ours. We adapted their method but reformulated it to a dynamic updating process.
In summary several dynamic prediction methods to update the prognosis for the individual patient are available and a significant improvement of the baseline prediction can be obtained. The direct approach had the best predictive performance in our application, while the indirect approaches behaved more like classifiers. Furthermore, the direct method is easy for practical application while standard statistical software is not yet available for the other approaches. Finally the methods are flexible and offer a new generation of prediction models.

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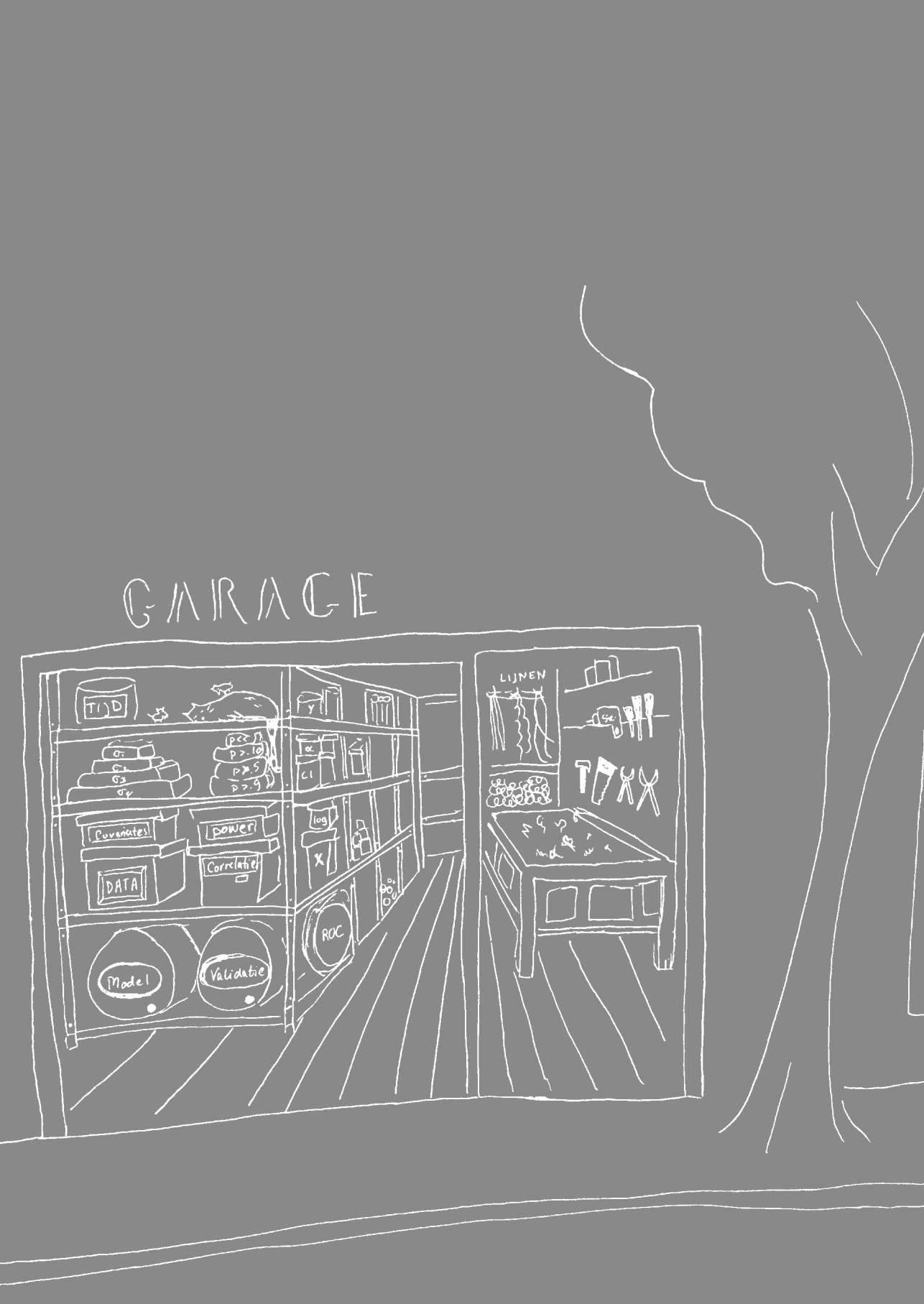
GARAGE
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# CHAPTER 2.5

# Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B patients using HBsAg and HBV DNA levels

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## ABSTRACT

Peginterferon alfa-2a results in a sustained response (SR) in a minority of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients. This study investigated the role of early on-treatment serum hepatitis B surface antigen (HBsAg) levels in the prediction of SR in HBeAg-negative patients receiving peginterferon alfa-2a. HBsAg (Abbott ARCHITECT) was quantified at baseline, during treatment (weeks 4, 8, 12, 24, 36 and 48) and follow-up (weeks 60 and 72) in the sera from 107 patients who participated in an international multicenter trial (peginterferon alfa-2a, n=53 versus peginterferon alfa-2a and ribavirin, n=54). Overall, 24 (22%) patients achieved SR (serum hepatitis B virus (HBV DNA) <10,000 copies/mL and normal alanine aminotransferase level at week 72). Baseline characteristics were comparable between sustained responders and non-responders. From week 8 onwards, serum HBsAg levels markedly decreased in sustained responders, whereas only a modest decline was observed in non-responders. However, HBsAg declines alone were of limited value in the prediction of SR (area under the receiver-operating characteristic curve (AUC) 0.59, 0.56 and 0.69 at weeks 4, 8 and 12, respectively). Combining HBsAg and HBV DNA declines allowed the best prediction of SR (AUC 0.74 at week 12). None of the 20 patients (20% of the study population) in whom a decrease in serum HBsAg level was absent and HBV DNA declined less than 2 log copies/mL exhibited a SR (NPV 100%).

Conclusion: At week 12 of peginterferon alfa-2a treatment for HBeAg-negative CHB a solid stopping rule was established using a combination of declines in serum HBV DNA and HBsAg level from baseline. Quantitative serum HBsAg in combination with HBV DNA enables on-treatment adjustment of peginterferon therapy in HBeAg-negative CHB.

# INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 to 400 million people worldwide and is responsible for 1 million deaths every year.[1] Hepatitis B e antigen (HBeAg-)negative chronic hepatitis B (CHB) represents a late phase in the course of the infection, which is recognized worldwide with an increasing prevalence.[2] Therapeutic intervention is often indicated for HBeAg-negative patients, because spontaneous remission rarely occurs and patients have more advanced liver disease in comparison with HBeAg-positive patients.[3]

In the last decade great strides have been made in the treatment of CHB, but the management of the HBeAg-negative type remains difficult. Nucleos(t)ide analogues are able to maintain suppression of viral replication in the majority of HBeAg-negative patients and are well tolerated,[4-5] but it is highly uncertain whether oral antiviral therapy can be discontinued.[6-8] In contrast to nucleos(t)ide analogues, one year of peginterferon therapy can result in an off-treatment sustained response (SR) in HBeAg-negative patients.[9-10] However, treatment with peginterferon is often complicated by the occurrence of side effects and a minority of patients with HBeAg-negative disease achieve SR. It is therefore a major challenge to identify patients who are likely to benefit from peginterferon therapy as early as possible during the treatment course.

HBV DNA quantification is widely used as a marker of viral replication to assess response to nucleos(t)ide analogues, but prediction of response to peginterferon by means of serum HBV DNA levels is difficult.[11-12] Advances in technology have enabled the development of a quantitative assay for hepatitis B surface antigen (HBsAg). The serum concentration of HBsAg appears to reflect the amount of covalently closed circular DNA (cccDNA) in the liver, which acts as a template for the transcription of viral genes.[13-14] Recently, several studies have suggested that serum HBsAg levels may be indicative of the likelihood of response to interferon-based therapy.[15-17] The aim of this study was to clarify the role of early on-treatment quantitative serum HBsAg in the prediction of SR in HBeAg-negative CHB patients treated with peginterferon alfa-2a.

# PATIENTS AND METHODS

## Patients

HBsAg levels were measured in sera from a total of 107 of 133 HBeAg-negative chronic hepatitis B patients who participated in an investigator-initiated, multicenter, randomized, double-blind, controlled trial.[9] Patients were randomly assigned in a one-to-one ratio to receive 180 μg peginterferon alfa-2a weekly and ribavirin 1000 mg (body weight <75 kg) or 1200 mg daily (body weight  75 kg) or peginterferon alfa-2a 180 μg weekly

and placebo daily. Duration of therapy was 48 weeks, followed by a 24-week observation period. Patients attended the outpatient clinic every 4 weeks. Results at the end of treatment (week 48) and at the end of follow-up (week 72) have been reported previously.[9] Patients who were treated according to the protocol and completed the follow-up phase were selected for the present study.

Eligible patients for the original study had been positive for HBsAg for more than 6 months; were HBeAg negative and anti-HBe positive on 2 occasions within 2 months before randomization; had had 2 episodes of elevated serum alanine aminotransferase (ALT) levels (>1.5 but ≤10 times the upper limit of normal (ULN) of the normal range) within 2 months prior to randomization and had a serum HBV DNA level >100,000 copies/mL (17,143 IU/mL). Exclusion criteria were: antiviral or immunosuppressive therapy within the previous 6 months; co-infection with hepatitis C, hepatitis D or human immunodeficiency virus (HIV); other acquired or inherited causes of liver disease; pre-existing cytopenia or decompensated liver disease. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave written, informed consent.



## Laboratory measurements

Serum HBsAg was quantified in samples taken at baseline, during the treatment period (weeks 4, 8, 12, 24, 36, 48) and during follow-up (weeks 60 and 72) using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL).[18] Serum HBV DNA was measured at the same time points using the Taqman polymerase chain reaction assay (Taqman HBV assay, Roche Diagnostics, lower limit of quantification: 35 copies/mL (6 IU/mL)). Transaminases were measured locally at the time of sampling in accordance with standard procedures. HBV genotype was assessed using the INNO-LiPA assay (Innogenetics).

## Liver histology

A liver biopsy was performed in all patients within one year before randomization. Necroinflammation grade (range 0-18) and fibrosis stage (range 0-6) were assessed using the Ishak scoring system.[19]

## Statistical analysis

Sustained response (SR), the predefined primary endpoint in the original study, was defined according to the EASL guidelines as the combined presence of serum HBV DNA level below 10,000 copies/mL (1,714 IU/mL) and normalization of ALT at the end of follow-up (week 72).[20] The association between baseline factors and SR was assessed by univariate logistic regression analyses. Predictive values of early on-treatment serum HBsAg, as well as HBV DNA and ALT levels (weeks 4, 8, and 12) were explored applying logistic regression analysis techniques. Discrimination, which is the ability to distinguish patients who will develop SR from those who will not, was quantified by the area under the receiver-operating characteristic curve (AUC). The best model-fit was assessed comparing the AUC and the Akaike's Information Criteria (AIC). Hereafter the optimal cut-off values for serum HBsAg and HBV DNA levels during treatment were established with the use of explanatory plots and the maximum chi-square approach to find a clinically useful rule for (dis)continuation of therapy.[21] SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA) were used to perform statistical analyses. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

# RESULTS

## Sustained response rate

Twenty-four (22%) of 107 patients developed SR. The number of sustained responders was comparable between the peginterferon alfa-2a monotherapy and the peginterferon alfa-2a and ribavirin combination therapy group (14 (26%) of 53 versus 10 (19%) of 54 patients, respectively, p=0.33). The two treatment groups were therefore pooled for further analysis. Among the 24 sustained responders, one patient cleared HBsAg from serum and developed anti-HBs.

## Baseline characteristics

Baseline characteristics of the 107 patients are shown in table 1. The mean pretreatment serum HBsAg level was 3.8 log IU/mL (range 1.1-5.0 log IU/mL) and the mean serum HBV DNA level was 6.8 log copies/mL (range 4.3-9.5 log copies/mL), both were stable during the screening period. There was no significant correlation between serum HBsAg and other factors at baseline including serum HBV DNA and ALT, HBV genotype, age, gender, body mass index (BMI) or liver histology. Baseline characteristics were comparable

for patients with and without SR, including age, gender, HBV genotype, serum ALT, HBV DNA and HBsAg levels and liver necroinflammatory and fibrosis scores (Table 1).

**Table 1.** Baseline characteristics according to SR

| Characteristics | All patients (n=107) | SR + (n=24) | SR – (n=83) | *P* value |
|---|---|---|---|---|
| Mean (SD) age, years | 42 (10) | 41 (11) | 42 (10) | 0.59 |
| Male (%) | 77 (72.0) | 16 (66.7) | 61 (73.5) | 0.51 |
| Ethnicity (%) | | | | 0.73 |
| Caucasian | 102 (95.3) | 23 (95.8) | 79 (95.2) | |
| Other | 5 (4.7) | 1 (4.2) | 4 (4.8) | |
| HBV genotype (%) | | | | 0.13 |
| A | 15 (14.0) | 0 | 15 (18.1) | |
| D | 85 (79.4) | 23 (95.8) | 62 (74.7) | |
| Other/mixed | 7 (6.5) | 1 (4.2) | 6 (7.2) | |
| Median (IQR) ALT* | 2.3 (1.6-4.1) | 2.0 (1.7-3.9) | 2.3 (1.6-4.1) | 0.82 |
| Mean (SD) HBV DNA, log copies/mL | 6.8 (1.2) | 6.9 (1.2) | 6.7 (1.2) | 0.52 |
| Mean (SD) HBsAg, log IU/mL | 3.8 (0.5) | 3.8 (0.4) | 3.8 (0.6) | 0.80 |
| Median (IQR) liver necroinflammation | 5 (4-7) | 5 (4-6) | 5 (4-7) | 0.52 |
| Median (IQR) liver fibrosis | 3 (1-3) | 2 (1-3) | 3 (1-3) | 0.57 |
| Cirrhosis† (%) | 3 (2.8) | 0 | 3 (3.6) | 1.0 |

*Multiples of upper limit of the normal range
†Ishak fibrosis score 5-6



## Serum HBsAg and HBV DNA levels during treatment and follow-up

Overall, the mean serum HBsAg concentration decreased significantly after 48 weeks of therapy (mean change compared to baseline -0.47 log IU/mL, p<0.001). HBsAg remained at end-of-treatment levels during post-treatment follow-up (mean change at week 72 compared to baseline -0.52 log IU/mL, p<0.001). Serum HBV DNA declined significantly during the treatment period as well (mean change at week 48 compared to baseline -3.29 log copies/mL p<0.001). In contrast to HBsAg levels, HBV DNA levels relapsed after treatment discontinuation (mean change at week 72 compared to baseline -1.55 log copies/mL, p=0.004).

A weak positive correlation was present between serum HBsAg and HBV DNA levels when all available samples were considered (R=0.35, p<0.001). From baseline until week 12, serum HBsAg and HBV DNA were not correlated (R<0.15, p>0.11). However, the correlation became stronger at the end of the treatment phase (week 48; R=0.36, p<0.001) and further increased at the end of follow-up (week 72; R=0.53, p<0.001).

## Serum HBsAg and HBV DNA levels according to response

Mean HBsAg declines from baseline for sustained responders and non-responders are shown in figure 1A. During the first 8 weeks of therapy mean serum HBsAg levels remained stable in both patient groups (Fig. 1A). From week 8 onwards however, HBsAg levels markedly decreased among the 24 patients who developed SR, whereas only a modest decrease in HBsAg level was observed in patients who failed to achieve SR ($p<0.05$ for comparison of HBsAg declines between patients with and without SR at all time points from week 8 with correction for multiple testing).

**Figure 1A**

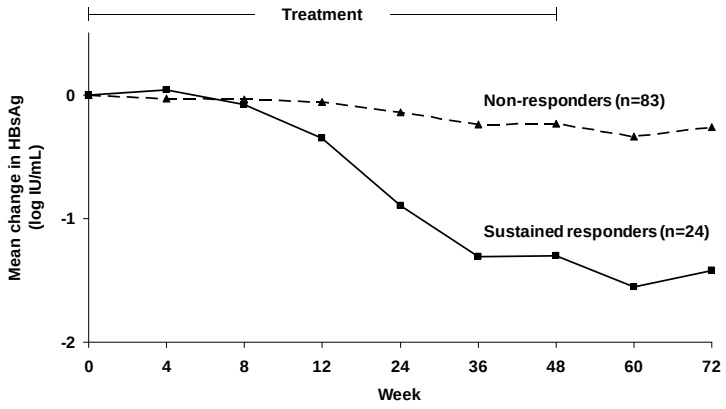


**Figure 1B**

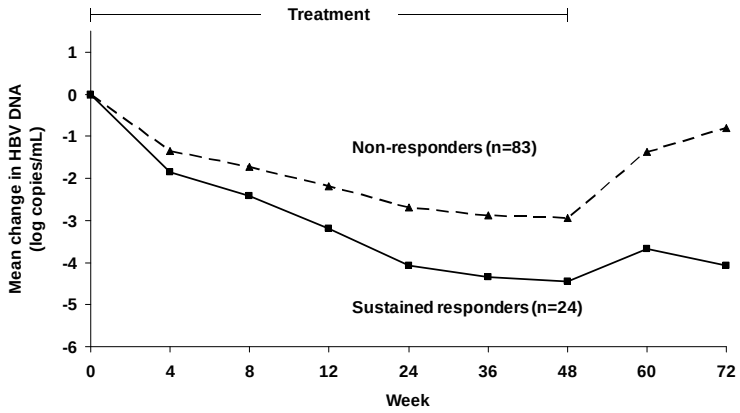


**Figure 1.** Mean change compared to baseline for HBsAg (A) and HBV DNA (B) levels in patients who achieved SR and those who did not.

Mean HBV DNA declines from baseline for patients with and without SR are displayed in figure 1B. A significant reduction in serum HBV DNA level was observed at week 4, in contrast to the later on-treatment decline in serum HBsAg level. Although the magnitude of on-treatment HBV DNA decline was larger in patients who eventually developed SR ($p<0.01$ for comparison of HBV DNA declines between patients with and without SR at all time points with correction for multiple testing), HBV DNA also decreased substantially in patients who did not achieve SR (Fig. 1B).
Serum ALT levels behaved similarly in sustained responders and non-responders during the treatment period and were not predictive of SR.

## Prediction of sustained response

The relationship between serum HBsAg and HBV DNA levels and subsequent achievement of SR was assessed at weeks 4, 8 and 12 of therapy. The performance of HBsAg and HBV DNA declines from baseline on SR was superior to absolute values. The AUC for declines in HBsAg and HBV DNA level is shown in figure 2. The reductions in HBsAg level at weeks 4 and 8 were not associated with SR using logistic regression analysis. HBsAg decline at week 12 was significantly associated with SR, but the overall discrimination remained unsatisfactory (AUC 0.59, 0.56 and 0.69 at weeks 4, 8 and 12, respectively).
In contrast to HBsAg declines, HBV DNA declines were associated with SR as early as week 4 of treatment. HBV DNA declines performed better with regard to the prediction of SR than HBsAg declines at weeks 4, 8 and 12 (Fig. 2). The best model-fit however, based on the AUC and AIC, was achieved through a combination of HBsAg and HBV DNA declines (AUC 0.74 at week 12). The performance of the model at week 24 did not



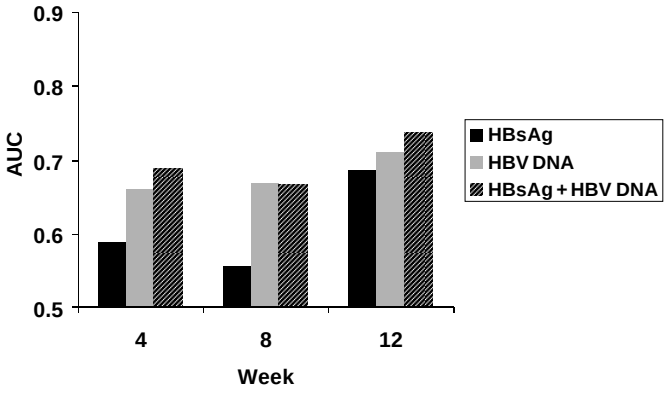


**Figure 2.** AUC for HBsAg decline from baseline, HBV DNA decline from baseline and a combination of these 2 markers for the prediction of SR.

improve significantly compared to week 12 (p=0.37). Treatment regimen was not associated with SR when added to the logistic regression models (p≥0.35 for all time points).

## Treatment algorithm

To find a clinically useful guiding rule, optimal cut-off values for a combination of HBsAg and HBV DNA decline at week 12 were established. We aimed to identify a stopping rule which enables discontinuation of therapy in patients who have a very low chance of SR, while maintaining more than 95% of sustained responders on treatment. Serum samples to measure HBsAg and HBV DNA decline at week 12 were available for 102 patients. Figure 3 illustrates the chance of SR within 4 patient groups defined according to the presence of HBsAg decline and/or HBV DNA decline ≥2 log copies/mL at week 12. None of the patients in whom a decline in serum HBsAg level was absent and HBV DNA decreased less than 2 log copies/mL (20% of the study population) exhibited a SR (NPV 100%). In contrast, patients in whom both these virological declines were achieved had the highest probability of SR (39%), which is almost double the overall response rate of 22%. Rates of SR were intermediate in patients with either a ≥2 log copies/mL decline in HBV DNA (24%) or a decline in HBsAg concentration only (25%). Separate analyses for the two treatment regimens (peginterferon alfa-2a with or without ribavirin) resulted in identical cut-off values for HBsAg and HBV DNA decline at week 12.

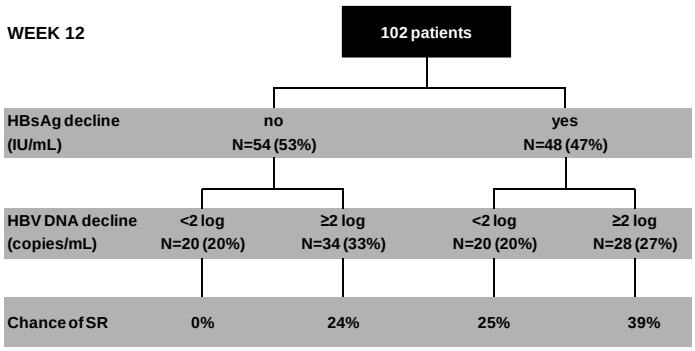


**Figure 3.** Algorithm showing chances of SR based on (1) HBsAg decline and (2) HBV DNA decline ≥2 log copies/mL at week 12 compared to baseline.

# DISCUSSION

HBeAg-negative CHB represents a difficult-to-treat population at high risk for liver-related complications.[3] All of the major practice guidelines recommend both

peginterferon and nucleos(t)ide analogues as initial treatment options[20, 22-23], but the optimal choice for individual patients remains controversial. Due to the higher chance of disease relapse after treatment discontinuation peginterferon is relatively less often prescribed to HBeAg-negative as compared to HBeAg-positive patients. A treatment course with peginterferon should however be considered for HBeAg-negative patients with a high likelihood of response, because a finite treatment course can lead to an off-treatment SR. Otherwise prolonged or indefinite treatment with a nucleos(t)ide analogue is likely. Unfortunately, baseline predictors of response to peginterferon are poorly defined in comparison with HBeAg-positive disease.[24-25] One study reported that baseline serum HBV DNA and ALT levels, patient age and gender, and infecting HBV genotype were significantly associated with response to peginterferon alfa-2a with or without lamivudine therapy,[26] but this was not confirmed in our patient population. Recent studies on peginterferon in HBeAg-negative patients have focussed on the identification of markers allowing on-treatment prediction of response.[15-17]

We found that accurate prediction of SR to peginterferon for HBeAg-negative disease in an early treatment phase is not possible based on serum HBsAg levels alone. However, combining on-treatment declines in serum HBsAg and HBV DNA concentration resulted in a solid stopping rule. At week 12, the absence of a decline in HBsAg level combined with less than 2 log copies/mL decrease in HBV DNA level identified a substantial proportion of the total study population (20%) in which therapy could be discontinued without losing sustained responders. In contrast, patients in whom both declines were present had the highest probability of SR (39%). This patient group should be encouraged to complete the 48-week treatment phase because they are the most likely group to benefit from therapy. Table 2 provides recommendations for (dis)continuation of therapy for patient groups based on the chance of developing SR. Obviously, the final decision to (dis)continue therapy is at the discretion of the treating physician, taking into account other factors like drug tolerability as well. Another important finding is that a guiding rule before 12 weeks of therapy could not be established because discrimination of



**Table 2.** Recommendations for continuation of peginterferon alfa-2a therapy for HBeAg-negative CHB at week 12.

| *Week 12 versus baseline* | | | |
|---|---|---|---|
| **HBsAg decline** | **HBV DNA decline ≥2 log copies/mL** | **Chance of SR** | **Recommendation to continue** |
| no | no | Absent | stop |
| no | yes | Intermediate | continue |
| yes | no | Intermediate | continue |
| yes | yes | High | strong recommendation for continuation |

serum HBsAg and HBV DNA levels during the first 8 weeks of treatment did not prove sufficient. Also, the decision to discontinue therapy should not be postponed, because the prediction of SR did not improve significantly at week 24 compared to week 12.

The kinetics of serum HBsAg and HBV DNA levels clearly differed during the treatment phase. HBV DNA decreased throughout the entire treatment period, while a later decline was observed in serum HBsAg levels. HBsAg and HBV DNA levels were not correlated at baseline and early during the treatment phase, further underlining the additional value of HBsAg levels in the prediction of SR. The added information that is provided by quantitative assessment of serum HBsAg may be explained by the dual antiviral and immunomodulatory mode of action of peginterferon. The on-treatment reduction in serum HBV DNA primarily reflects the direct antiviral effect of peginterferon. In contrast, the decline in serum HBsAg may be a marker of its immunomodulatory effects resulting in gradual clearance of infected hepatocytes from the liver through the induction of cytotoxic T-cell activity.[27] In line with these findings, it has been demonstrated that reductions in serum HBsAg mirror the decline in intrahepatic cccDNA.[13-14]

Recently high predictive values for on-treatment HBsAg declines at weeks 12 and 24 on sustained virological response (HBV DNA <70 copies/mL) were reported in a cohort of 48 patients treated with peginterferon alfa-2a for 48 weeks.[17] This finding was not confirmed in our larger study population, which was derived from a randomized controlled trial. This discrepancy may be generated by the substantial difference in response rates between the two studies. In the study by Moucari et al., 25 percent of patients developed a sustained virological response.[17] This response rate is substantially higher than in any peginterferon study for HBeAg-negative patients, suggesting that a selection bias may have affected the results of this retrospective study.

In our study SR had previously been defined as the combined presence of a serum HBV DNA level <10,000 copies/mL and a normal ALT level at 6 months after treatment discontinuation. One could argue that the HBV DNA threshold should have been set at a lower level. Indeed, off-treatment undetectability of serum HBV DNA by a sensitive PCR assay is a major virological endpoint and strongly associated with HBsAg clearance from serum in the years afterwards.[28] However, these preferred treatment endpoints occur infrequently in HBeAg-negative patients treated with peginterferon. In fact, another important goal of therapy for HBeAg-negative CHB is the induction of the HBsAg inactive carrier phase. Our endpoint of a serum HBV DNA level <10,000 copies/mL combined with a normal ALT level appears to differentiate reliably between inactive carriers and HBeAg-negative chronic hepatitis B patients.[29] In addition, large population studies have shown that HBsAg-positive patients with an HBV DNA concentration below this level of viral replication have a reduced risk of progression to cirrhosis and

hepatocellular carcinoma.[30-32] Furthermore, this HBV DNA threshold and the duration of follow-up correspond with the definition of response to peginterferon therapy according to the recent European guidelines and the pivotal studies on peginterferon in chronic hepatitis B, respectively.[10, 20, 33]

The large majority of our patients were of Caucasian origin and infected with HBV genotypes A and D. Responsiveness to interferon-based therapy appears to be lower in genotype D compared to other genotypes, which may explain the limited efficacy of peginterferon in our study population.[9-10, 26, 34] A recent retrospective analysis of 264 HBeAg-negative patients treated with peginterferon alfa-2a alone or in combination with lamivudine reported that pretreatment HBsAg levels varied according to genotype. The highest concentrations were found in patients infected with genotypes A and D. Although serum HBsAg levels decreased during the treatment phase in all genotypes, HBsAg decline was least pronounced in genotype D.[35] Therefore, our data on HBsAg decline need to be confirmed in genotypes B and C.

In summary, the current study shows that a combination of early quantitative serum HBsAg and HBV DNA levels allows the best selection of patients with HBeAg-negative CHB who will not respond to a 48-week course of peginterferon alfa-2a therapy. Discontinuation of peginterferon therapy and a switch to alternative treatment appears to be indicated in patients without a decline in HBsAg level combined with less than 2 log copies/mL decline in HBV DNA level at week 12.

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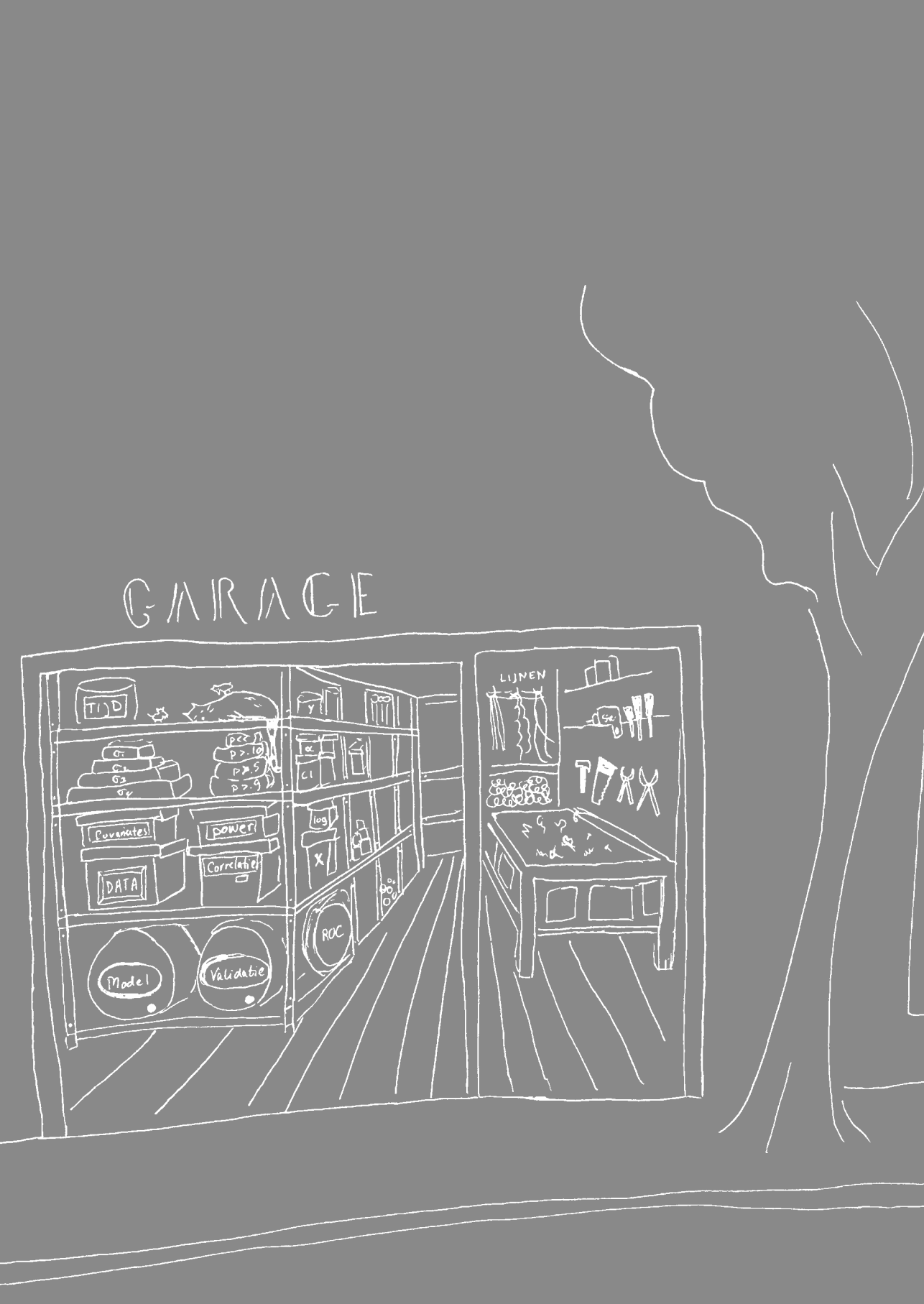
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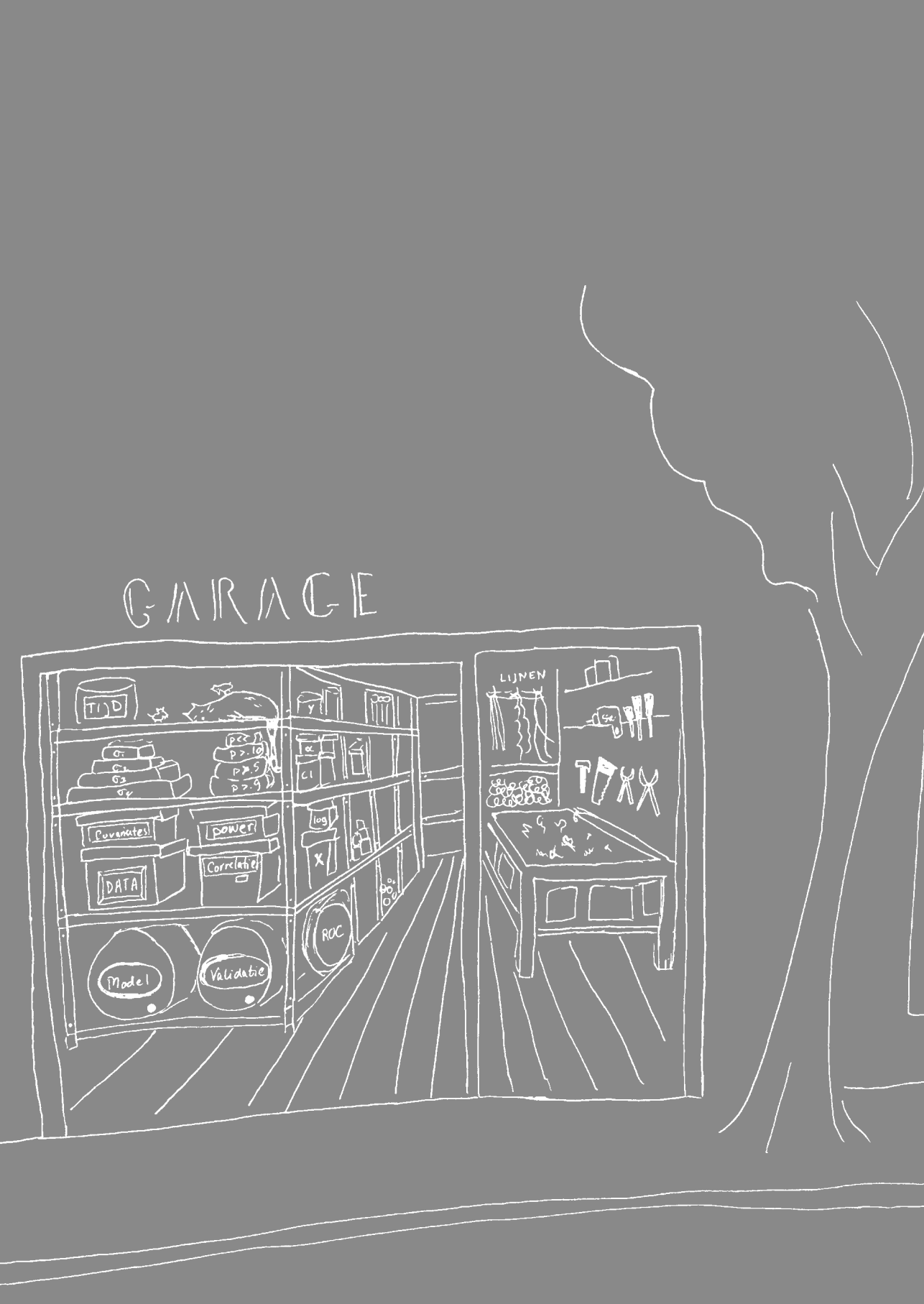
# CHAPTER 3

Statistical models of long-term treatment effects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# CHAPTER 3.1

## Long-term clinical outcome and effect of glycyrrhizin in 1093 chronic hepatitis C patients with non-response to interferon

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## ABSTRACT

Background: In chronic hepatitis C patients not responding to interferon, glycyrrhizin may be used for reducing disease activity.

Aim: To evaluate the effect of glycyrrhizin on the incidence of hepatocellular carcinoma (HCC) during long-term follow-up after interferon non-response.

Methods: We analyzed individual patient data of all consecutive patients treated with interferon in 12 major Japanese hospitals between 1990 and 1995 who showed no sustained response.

Results: We included 1093 patients. During a mean follow-up of 6.1±1.8 years, 107 patients developed HCC. Cox regression with time dependent variables showed that older age, male sex, higher alanine aminotransferase (ALT) and higher fibrosis stage were significantly associated with a higher risk for developing HCC. Response to glycyrrhizin, defined as ALT<1.5 x upper limit of normal, was significantly associated with a decreased incidence of HCC: hazard ratio 0.39 (95%CI 0.21-0.72; p<0.01).

G-estimation, used to correct for ALT as confounder, showed no significant benefit of glycyrrhizin in the overall study population.

Conclusion: This study provides some evidence that interferon non-responder patients with chronic hepatitis C and fibrosis stage 3 or 4 may have a reduced incidence of HCC if glycyrrhizin therapy leads to normalization of ALT levels.

# INTRODUCTION

Chronic hepatitis C is a major cause of liver disease worldwide. Infection with the hepatitis C virus may lead to a chronic inflammation of the liver, which is manifested in elevated liver enzymes such as alanine aminotransferase (ALT). This chronic inflammation may lead to fibrosis and subsequent cirrhosis. It has been estimated that the delay for developing cirrhosis is about thirty years, but the individual prognosis may vary substantially depending on factors such as age at infection, gender, alcohol abuse and co-infection with hepatitis B or the human immunodeficiency virus (HIV).[1]

Over the past fifteen years treatment regimens based on the administration of interferon have proven to be increasingly effective against hepatitis C. Combination treatment with pegylated interferon and ribavirin will lead to disappearance of the virus from the blood in 50% to 80% of the patients.[2,3] If the virus remains undetectable in the blood at 6 months after the end of treatment, we speak of a sustained virological response. Sustained virological response is almost always associated with normalization of serum ALT and a survival similar to the overall population.[4] There still remains a considerable proportion of patients who do not achieve a sustained virological response. These patients are in need of other therapeutic approaches. Various long-term interferon-based regimens are under investigation.[5,6]

In Japan, glycyrrhizin has been propagated as an anti-inflammatory drug, capable of minimizing disease activity in the chronically infected liver. Placebo controlled trials have proven that the administration of glycyrrhizin leads to a significant reduction of ALT levels in chronic hepatitis C patients.[7] The question remains whether this reduction of ALT levels leads to a reduced risk of liver-related morbidity and mortality. Ideally, one should design a randomised controlled trial with a prolonged follow-up of at least several years in order to investigate the effect of glycyrrhizin on these clinical endpoints. However, even when such a study would be restricted to cirrhotics, based on the incidence of HCC, decompensation and mortality,[8] it would take at least 5 years before we had an answer whether glycyrrhizin is a beneficial drug or not. Therefore we performed a large retrospective multicenter study, analyzing independently data collected in Japan, the only country so far where hepatologists have extensively used this compound. We were especially careful to minimize the various biases associated with retrospective studies and to apply the most sophisticated statistics designed for such studies.

# METHODS

## Study design

Japanese academic hospitals and major general hospitals were invited to participate in the study. Additional entry criteria were availability of data on previous treatment with interferon and on clinical outcomes.

All consecutive chronic hepatitis C patients who received interferon alpha treatment between January 1, 1990 and December 31, 1995 and who did not show a sustained virological response were included. Sustained virological response was defined as normal alanine aminotransferase (ALT) and negative HCV-RNA at the end of treatment and six months thereafter.

The ethics committees of all participating centers approved the protocol. In order to ensure privacy of the patients, the treating physician replaced patient names by a code before entry in the database.

## Patient selection

Data of all consecutive patients with chronic hepatitis C with non-response to previous interferon treatment were collected. Data were collected on separate case record forms, one per patient, by the local investigator. The case record forms were sent to the co-ordination center in Rotterdam, where the data were entered in a central database. Before the data were entered, they were checked and in case of doubt, contact was made with the local investigator.



## Data recorded

Information was obtained on demographics (age, gender) and on details of the interferon treatment (starting date, duration, and total dose) as well as the glycyrrhizin treatment (starting date, duration, total dose). Virological data (genotype, viremia), hematological (platelet count) and biochemical data (aminotransferase levels, bilirubin, and gamma glutamyltransferase) were measured in the certified laboratories of the participating hospitals and added to the case record form by the local investigator. Centrally, the results were corrected for local normal values.

Follow-up data were recorded every four weeks if available and included ALT-levels, start of glycyrrhizin treatment and the occurrence of HCC. Patients were considered to have a HCC if biopsy proved so or if ultrasound or computed tomography showed a focal lesion in the presence of a serum alpha-fetoprotein of > 400.

## Statistics

A data analysis plan was developed before closure of the database. The Kaplan Meier method was used to assess the occurrence of HCC over time in the overall population. Risk factors for development of hepatocellular carcinoma over time were assessed by time-dependent Cox-regression analysis. Baseline factors and medication during follow-up after interferon treatment were included in this analysis. The latter factor necessitated the time dependent form of Cox regression analysis. In order to avoid bias, cases were censured at the time of a second interferon-based treatment.

According to the data analysis plan, a second analysis was done to assess the effect of glycyrrhizin according to response. Response to glycyrrhizin was defined as ALT levels < 1.5 x upper limit of normal at the first measurement 3 months after initiation of treatment. Statistical analyses were performed using SPSS Windows version 11 (SPSS Inc, Chicago, IL, USA). Findings showed a strong influence of fibrosis and ALT elevation on development of HCC. Therefore, an additional analysis was done in a more homogeneous group of patients with advanced fibrosis.

Simply adjusting for ALT as a time-dependent covariate in a Cox model may lead to a biased estimate of the treatment effect, since higher ALT levels were associated with a higher probability of developing HCC and also of starting glycyrrhizin therapy (figure 1). In order to estimate the causal effect of time-dependent glycyrrhizin treatment in the presence of a time-dependent covariate ALT, we used the G-estimation described by Robins.[9] This method is designed to get an unbiased estimate of a treatment effect in

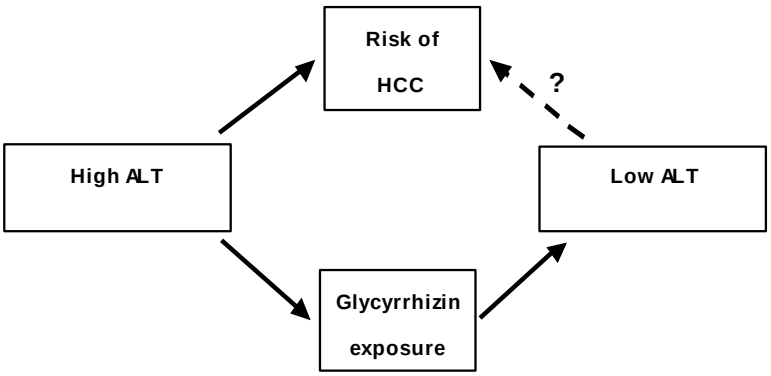


**Figure 1.** Elevated ALT-levels during follow-up were associated with a higher probability of receiving glycyrrhizin, but also lead to a higher probability of developing HCC.
As ALT-levels are lowered by glycyrrhizin treatment, ALT is regarded as a time-dependent covariate which is both a confounder and an intermediate. In order to investigate whether glycyrrhizin reduces the risk of developing HCC by lowering ALT levels (dotted arrow), sophisticated statistical analyses were required and a G-estimation was performed

the presence of a confounding variable, which is also intermediate. The G-estimation estimates the factor ψ. We use the exponent of -ψ, further referred to as *E*, as the factor by which the time towards development of HCC would be expanded (or contracted in case *E* is smaller than 1.0) if the treatment with glycyrrhizin would not have been given (Appendix 1). This G-estimation was carried out with a macro written in SAS (SAS Institute Inc, Cary, NC, USA).

# RESULTS

## Descriptives

A total of 1093 chronic hepatitis C patients with non-response to previous interferon therapy were included in the study. Follow-up started at six months after the end-of-treatment. During a mean follow-up of 6.1 years (SD 1.8) 26,450 visits were recorded. The mean duration of follow-up was 6.3 years (SD 1.8) for patients who were treated with glycyrrhizin and 6.0 years (SD 1.8) for patients who were not. Fifty-eight percent of the patients were males and the median age at time of inclusion was 52 years (range 17-81). Forty percent of the patients had acquired hepatitis C by blood transfusion. Further patient characteristics are shown in table 1.



**Table 1.** Descriptives.

| | Overall | Glycyrrhizin | no glycyrrhizin | p-value* |
|---|---|---|---|---|
| Number | 1093 | 465 | 628 | |
| M/F (%) | 628/455 (58/42) | 262/198 (56/43) | 366/257 (58/41) | 0.67 |
| Age, mean (range) | 52.2 (17-81) | 53.9 (29-80) | 50.9 (17-81) | <0.01 |
| Genotype | | | | <0.01 |
| 1 (%) | 750 (69) | 338 (73) | 334 (53) | |
| 2 (%) | 214 (20) | 90 (19) | 191 (30) | |
| 3 (%) | 9 (1) | 6 (1) | 68 (11) | |
| 4 (%) | 4 (0.4) | 0 (0) | 25 (4) | |
| Fibrosis stage | | | | <0.01 |
| 1 (%) | 451 (41) | 117 (25) | 334 (53) | |
| 2 (%) | 372 (34) | 181 (39) | 191 (30) | |
| 3 (%) | 203 (19) | 135 (29) | 68 (11) | |
| 4 (%) | 54 (5) | 29 (6) | 25 (4) | |
| ALT at t=0 | | | | <0.01 |
| < 1 x ULN (%) | 319 (29) | 81 (17) | 238 (38) | |
| 1 – 1.5 x ULN (%) | 225 (21) | 68 (15) | 157 (25) | |
| 1.5 – 2 x ULN (%) | 161 (15) | 65 (14) | 96 (15) | |
| 2 – 3 x ULN (%) | 159 (15) | 82 (18) | 77 (12) | |
| >3x ULN(%) | 222 (20) | 167 (36) | 55 (9) | |

* p-value of the difference between patients treated or not treated with glycyrrhizin. (Chi-square / Mann Whitney)

Four hundred and sixty-five patients received intravenous glycyrrhizin therapy, given as Stronger Neo Minophagen C (SNMC), which was started at various follow-up times. One hundred and sixty-four of these patients had advanced fibrosis. The mean treatment duration with glycyrrhizin was 4.1 years (SD 2.6), 79% of the patients received treatment for 3 years or longer. The patients received a mean dose of 506 mg glycyrrhizin (191 ml SNMC) per week (range 106-1855 mg). Six patients stopped treatment because of side effects. Other treatments given to the interferon non-responders were interferon plus ribavirin (n=23), ursodeoxycholic acid (n=657) and herbal medicines (n=93). The patients receiving interferon plus ribavirin were censored at the start of this treatment.

## Events

One hundred and seven patients developed HCC. We performed a Kaplan Meier analysis in order to investigate the influence of raised ALT levels on the risk of developing HCC (figure 2). In patients with normal ALT levels during the first year of follow-up, the 5-year incidence of HCC was 3.1% (95% CI 0.8-5.5). The incidence of HCC increased to 4.9 (95%CI 2.0-7.8) for ALT levels between 1 and 1.5 xULN, 8.3% (95%CI 4.1-12.5) for ALT levels between 1.5 and 2 xULN and 8.3% (95%CI 4.2-12.3) for ALT levels between 2 and 3 xULN. The highest occurrence of HCC was seen in patiënts with ALT levels above thrice the ULN during the first year of follow-up: 16.6% (95% CI 9.3-24.0).

Time dependent Cox regression analysis showed that older age, male sex, higher fibrosis stage and non-response to glycyrrhizin were significantly associated with a higher risk for developing HCC (table 2).

Subgroup analysis of patients with fibrosis stage 3 and 4 showed a trend towards less development of HCC among patients with a response to glycyrrhizin (hazard ratio=0.50 (95% CI 0.22-1.12, p=0.09).

Seventy-four percent (343/465) of the patients treated with glycyrrhizin had ALT levels above 1.5 xULN at the start of therapy and 66% (228/343) of these responded by decreased ALT-levels. In comparison, the rate of spontaneous ALT normalisation in patients with elevated ALT levels at start of follow-up who were not treated with glycyrrhizin was 33% (114/344) at 3 months after inclusion into the study. In an analysis of all 465 treated patients, patients with an ALT-response had a significant lower chance of developing HCC than non-responders; hazard ratio 0.39 (95% CI 0.21-0.72, p<0.01) (table 3). Cox regression analysis of untreated patients (patients censored at start of glycyrrhizin therapy) showed that spontaneous normalisation of ALT-levels at 4 months after start of follow-up, though twice less common than normalisation after initiation of glycyrrhizin, also tended to be associated with a lower chance of developing HCC (hazard ratio 0.44 (95% CI 0.19-1.02, p=0.06).

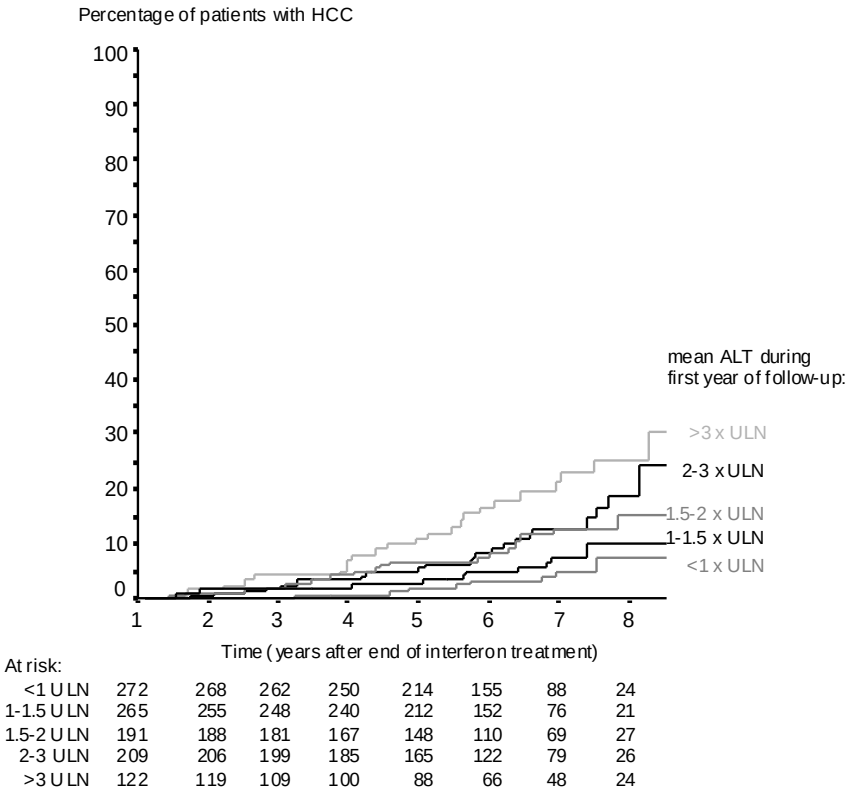




**Figure 2.** Kaplan Meier curve showing the development of hepatocellular carcinoma over time, according to mean ALT during the first year after interferon therapy. As the mean ALT was calculated over the first year, the time-scale starts at one year of follow-up.
Patients who did not fulfill one year of follow-up (n=7) and patients who developed HCC within the first year of follow-up (n=27) were excluded from this analysis.

## G-estimation

The G-estimation performed for the overall study-population, showed that the time towards development of hepatocellular carcinoma was not significantly influenced by glycyrrhizin treatment (*E*= 0.96 (95% CI 0.76-2.10).

There was a trend towards a prolonged time to development of HCC among patients with fibrosis stage 3 or 4 if they received glycyrrhizin; *E*= 1.17 (95% CI 0.65-2.29). Among patients with fibrosis stage 1 or 2, no beneficial effect of glycyrrhizin was seen during the observation period, but the number of events was too small to make a reliable estimate in this subgroup.

**Table 2.** Time dependent Cox regression analysis assessing risk factors for HCC (n=1093).

| | | Hazard Ratio | 95% CI | p-value |
|---|---|---|---|---|
| Sex | Male | 1 | | |
| | Female | 0.31 | 0.19-0.51 | <0.01 |
| Age | | 1.08 | 1.05-1.11 | <0.01 |
| ALT-levels at t=0 | < 1.5 x ULN | 1 | | |
| | > 1.5 x ULN | 1.58 | 0.92-2.70 | 0.10 |
| Alcohol | < 50 g / day | 1 | | |
| | > 50 g /day | 1.15 | 0.64-2.04 | 0.65 |
| Fibrosis stage | Fibrosis stage 1 | 1 | | |
| | Fibrosis stage 2 | 4.04 | 1.66-9.83 | <0.01 |
| | Fibrosis stage 3 | 8.75 | 3.56-21.5 | <0.01 |
| | Fibrosis stage 4 | 15.2 | 5.82-39.7 | <0.01 |
| Glycyrrhizin | No glycyrrhizin | 1 | | |
| | Glycyrrhizin, no ALT response | 2.03 | 1.21-3.42 | 0.01 |
| | Glycyrrhizin, ALT response | 0.81 | 0.41-1.60 | 0.54 |

The hazard ratios with their 95% confidence intervals and p-values associated with these factors are given. Hazard ratio <1.0 indicates a decreased risk for HCC. Older age, male sex, higher fibrosis stage and non-response to glycyrrhizin treatment were significantly associated with a higher risk of developing HCC. Sex, ALT, alcohol intake, fibrosis stage and glycyrrhizin treatment were entered as categorical values, age was entered as a continuous value.

**Table 3.** Time dependent Cox regression analysis assessing risk factors for HCC in patients who received glycyrrhizin treatment (n=465).

| | | Hazard Ratio | 95% CI | p-value |
|---|---|---|---|---|
| Sex | Male | 1 | | |
| | Female | 0.23 | 0.12-0.42 | <0.01 |
| Age | | 1.09 | 1.05-1.13 | <0.01 |
| ALT-levels at start of treatment | < 1.5 x ULN | 1 | | |
| | > 1.5 x ULN | 0.44 | 0.17-1.14 | 0.09 |
| Fibrosis stage | 1 | 1 | | |
| | 2 | 2.41 | 0.89-6.50 | 0.08 |
| | 3 | 3.35 | 1.26-8.92 | 0.02 |
| | 4 | 7.95 | 2.71-23.3 | <0.01 |
| Response to glycyrrhizin | No | 1 | | |
| | Yes | 0.39 | 0.21-0.72 | <0.01 |

The hazard ratios with their 95% confidence intervals and p-values associated with these factors are given. Hazard ratio <1.0 indicates a decreased risk for HCC. Older age, male sex and advanced fibrosis were significantly associated with a higher risk of developing HCC. Patients with an ALT response to glycyrrhizin had a significantly decreased chance of development of HCC, compare to non-responders. Sex, ALT, fibrosis stage and glycyrrhizin treatment were entered as categorial values, age was entered as a continuous value.

## DISCUSSION

The aim of this study was to determine the long-term clinical outcome of chronic hepatitis C patients who did not respond to interferon monotherapy and to evaluate the effect of glycyrrhizin treatment on the incidence of HCC in this group of patients.

During follow-up 107 patients developed HCC. This is well in accordance with data published by Yoshida et al., who presented the rates of development of HCC by age, sex and fibrosis stage in their population of non-sustained responders. Applying these rates to our dataset would lead to an expected number of 117 HCCs (95% CI 99-139) during 6.1 years of follow-up.[10] In our cohort the overall yearly incidence of HCC was 1.6%. Previous large cohort studies found a yearly incidence of 0.3 to 2.7% per year in Japanese non sustained responders to interferon treatment.[10-12] In the literature, lower rates of HCC development are described in patients who relapsed after an initial response and in patients with persistently low ALT levels.[13] Similarly, in our cohort, patients with lower baseline ALT levels had a smaller probability of developing HCC.

As chronic hepatitis C only progresses slowly, it is hard to evaluate the efficacy of treatment on clinical outcomes like mortality and development of HCC in randomized controlled trials. Therefore, "best" information should be derived from cohort studies. However, cohort studies are only reliable if the drop-out rate is low compared to the events. In retrospective cohort studies the risk of introducing bias is even larger. Incomplete capture of early clinical events, confounding bias and compliance bias have been described as possible confounders in retrospective studies.[14] In large randomized trials this problem is usually avoided, as unmeasured confounders are likely to be equally divided over the groups by randomization.

We executed this retrospective cohort analysis with great care to avoid these biases. Incomplete capture of clinical events could not play a role in our analysis as the development of HCC was monitored during the whole follow-up period. Secondly, confounding bias may have played a role as raised ALT-levels increased both the chance of receiving glycyrrhizin treatment as the risk of developing HCC. Sophisticated statistical analyses were used to correct for this confounder.[9,15,16]

Finally, compliance bias may have played a role in this study, as patients who are willing to attend the hospital several times a week for intravenous injections of glycyrrhizin are possibly also more likely to adhere to other protective types of behavior. However, the fact that the follow-up of patients who did not receive glycyrrhizin was similar to those who did, suggests that they were equally compliant in their hospital visits.

A previous study on the effect of glycyrrhizin on clinical outcome did show a significant protective effect on development of HCC.[17] In our study we refined the methodology by using an intention-to-treat approach. All patients who received glycyrrhizin were

included, even those who were treated for a short time. In this way we tried to avoid excluding patients who stopped their glycyrrhizin early because they died of HCC.
In the present study, we first used multiple regression analysis to assess the effect of glycyrrhizin. Overall, there was no significant effect, but in patients with fibrosis stage 3 and 4 there was a trend to a protective effect on development of HCC. An intention to treat analysis of all patients treated with glycyrrhizin showed that patients responding by decreased ALT levels had a significantly lower probability of developing HCC. A G-estimation was performed to address the problem of confounding by ALT levels. The latter analysis failed to show an overall beneficial effect of glycyrrhizin, but in patients with fibrosis stage 3 or 4 at the start of follow-up there was a trend towards a protective effect.
In conclusion, this study provides some evidence that interferon non-responder patients with chronic hepatitis C and fibrosis stage 3 or 4 may have a reduced incidence of HCC if glycyrrhizin therapy leads to normalization of ALT levels.

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# APPENDIX

The method of G-estimation by J.M.Robins offers a solution to estimate the causal effect of the time dependent glycyrrhizin-treatment on the development of HCC, in the presence of a time-dependent covariate ALT that is both a confounder and an intermediate variable. G-estimation of the parameter of a nested structural model estimates the expansion or contraction parameter $\psi$ of the time to event (HCC) due to the exposure to glycyrrhizin treatment. If, for instance the exponent of $-\psi$ (referred to as *E* in the text) = 1.20 the time to HCC is expanded by 20%, corresponding with a beneficial effect.

Fundamental for this approach is the assumption of no unmeasured confounders. This means that all covariates influencing both the decision to use glycyrrhizin and the HCC-free survival time should be measured. That means that given the covariates, the decision to start treatment is independent of the patient's (possibly counterfactual) HCC-free survival time under any treatment regime.

A pooled logistic regression analysis over all visits was applied, with glycyrrhizin therapy at visit k as outcome. This means that each subject contributed with multiple observations, one for each visit, until development of HCC or censoring. Covariates considered for inclusion in the model are baseline factors (age, sex, fibrosis stage, ALT and gamma glutamyltransferase at the start of the study) and the covariate history before visit k (ALT, glycyrrhizin treatment and concomitant medication at the two visits prior to visit k). Furthermore the number of weeks since the prior visit and the number of weeks since the start of the study were included in the model.

The parameter $\psi$ is g-estimated by extending the logistic model with sets of imaginary (counterfactual) HCC-free survival times, had glycyrrhizin-treatment never been given. Weights have been calculated to adjust for patients who are lost to follow-up or who are censored at a second interferon-based treatment.

Data description and annotation:

$T_i$ = Observed failure time for subject *i*.

$U_i$ = Time to failure (HCC) for subject *i* if never exposed to glycyrrhizin (=counterfactual failure time)
$Glycyrrhizin_i(t)$ = The treatment status of subject *i* at timepoint t.

The model that relates the observed data $T_i$ and $Glycyrrhizin_i(Ti)$ to the counterfactual failure time $U_i$ is assumed to be:

$$U_i(\psi) = \int_0^{T_i} \exp(\psi\, Glycyrrhizin_i(t))\, dt$$

The model of U as a function of $\psi$ describes the relation between the counterfactual failure time, the observed failure time and the use of glycyrrhizin over time.

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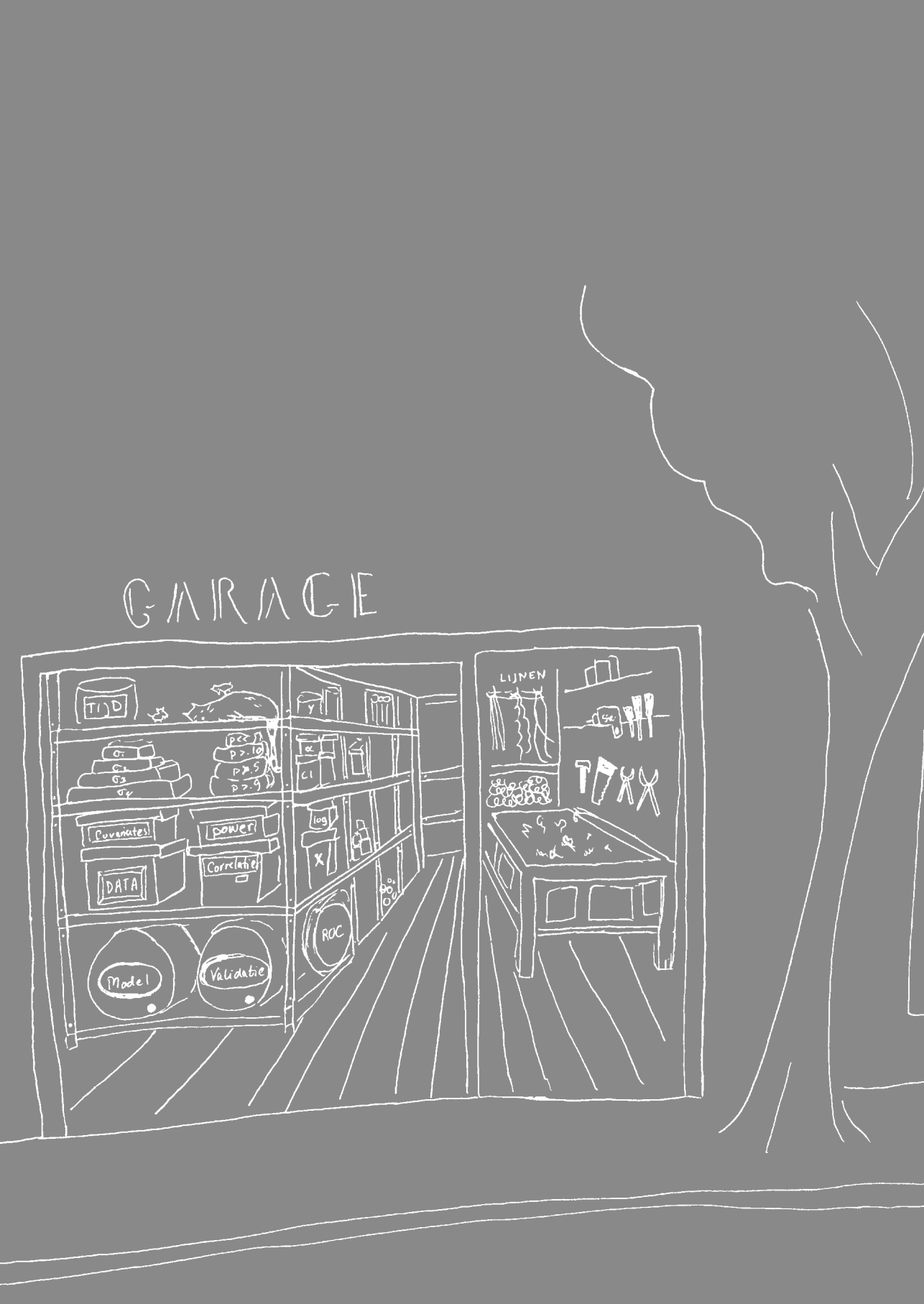
GARAGE
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ROC
Model
Validatie

# CHAPTER 3.2

## Discriminant analysis using a multivariate linear mixed model with a normal mixture in the random effects distribution

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## Abstract

We have developed a method to longitudinally classify subjects into two or more prognostic groups using longitudinally observed values of markers related to the prognosis. We assume the availability of a training data set where the subjects' allocation into the prognostic group is known. The proposed method proceeds in two steps as described earlier in the literature. First, multivariate linear mixed models are fitted in each prognostic group from the training data set to model the dependence of markers on time and on possibly other covariates. Secondly, fitted mixed models are used to develop a discrimination rule for future subjects. Our method improves upon existing approaches by relaxing the normality assumption of random effects in the underlying mixed models. Namely, we assume a heteroscedastic multivariate normal mixture for random effects. Inference is performed in the Bayesian framework using the Markov chain Monte Carlo methodology. Software has been written for the proposed method and it is freely available. The methodology is applied to data from the Dutch Primary Biliary Cirrhosis Study.

# Introduction

The Dutch Multicenter Primary Biliary Cirrhosis (PBC) Study is a prospective cohort study of patients with PBC with participation of 7 university hospitals and 39 general hospitals. Recruitment of patients started in January 1990 and follow-up data until April 2007 were available for analysis. Follow-up data were collected at approximately 3-monthly intervals in the first year and yearly intervals thereafter. In total, 375 patients were recruited with a median follow-up of 9.7 years. See Kuiper et al.[1] for details of the study. It is of clinical interest to predict the future patients' status. In this paper, we are interested in predicting whether the patient will suffer from a serious disease progression (liver related death or liver transplantation) in the first $T = 10$ years after the start of treatment by ursodeoxycholic acid (UDCA, 13–15 mg/day/kg of weight). Many factors are known to be related to progression of PBC and hence could be used to establish the prognosis. Here we consider the following three markers whose longitudinal profiles are available: bilirubin, albumin, alkaline phosphatase (AP). A prognostic group (status at $T = 10$ years) is also known for many patients. Our aim is to use these patients as a training data set, model the dependence of these markers on time and possibly other covariates (e.g., age) and subsequently use the developed models to predict longitudinally the prognostic group of future patients.

We now introduce the following notation. Let $\boldsymbol{Y}_{i,r} = (Y_{i,r,1}, \ldots, Y_{i,r,n_{i,r}})'$ be a random vector of the $r$-th marker ($r = 1, \ldots, R$) observed for the $i$-th patient ($i = 1, \ldots, N$) at time points $0 \leq t_{i,r,1} < \cdots < t_{i,r,n_{i,r}}$. Further, let $\boldsymbol{Y}_i = (\boldsymbol{Y}'_{i,1}, \ldots, \boldsymbol{Y}'_{i,R})'$ be the longitudinal profiles of all markers for the $i$-th patient and let $n_i = \sum_{r=1}^{R} n_{i,r}$ be the total number of marker measurements for the $i$-th patient. Finally, let $\boldsymbol{Y}_i(\tau)$ be a subvector of $\boldsymbol{Y}_i$ containing only the observations performed at times $t \leq \tau$. Let $\mathcal{P}_{g,i}(\tau)$ be the probability, known at time $\tau < T$ that the patient belongs to a prognostic group $g$ ($g = 0, \ldots, G-1$) at time $T$. In our application, $G = 2$, $g = 0$ refers to patients without a serious disease progression and $g = 1$ to patients who died because of a liver related cause or required liver transplantation in the first $T = 10$ years. The aim of this paper is to estimate $\mathcal{P}_{g,i}(\tau)$ using $\boldsymbol{Y}_i(\tau)$, the history of markers at time $\tau$.

In recent years, a number of approaches were suggested either to estimate $\mathcal{P}_{g,i}(\tau)$ (discriminant analysis) or to cluster patients (cluster analysis) into prognostic groups using linear mixed models for the observed markers $\boldsymbol{Y}_i(\tau)$. Lyles and Xu[2], Tomasko, Helms and Snapinn[3], Marshall and Barón[4], Brant et al.[5], Wernecke et al.[6] describe the discriminant or cluster analysis approaches using a single marker based on a classical linear mixed model (LMM). Discriminant analysis using a longitudinal history of

multiple markers was considered by Morrell et al.[7] and Marshall et al.[8] who based their discrimination rule on a multivariate (non)linear mixed model (M(N)LMM). Finally, Fieuws et al.[9] describe a discrimination procedure based on a multivariate generalized linear mixed model (MGLMM) which allowed them to include discrete markers as well.

All of the above approaches assume a normal distribution for the random effects in the underlying mixed model. Nevertheless, it is known that it is difficult to check this assumption which cannot be evaluated using commonly used empirical Bayes estimates of individual random effects due to their shrinkage (Verbeke and Lesaffre[10]). Consequently, Verbeke and Molenberghs[11], Chapter 7 conclude that non-normality of the random effects can only be detected by comparing the results obtained under the normality assumption with results obtained from fitting a mixed model with relaxed distributional assumptions for the random effects. Moreover, according to Komárek et al.[12], the most promising approach for discrimination based on longitudinal profiles is based on predictors of individual random effects and the distribution of these random effects. It is therefore natural that the correct specification of the random effects distribution plays an important role.



For these reasons, we are targeting a method in which the normality assumption of random effects is relaxed. A suitable semi-parametric model for an unknown distribution is a normal mixture. Verbeke and Lesaffre[10] used the homoscedastic version (variances of the mixture components are equal) as a model for random effects in LMM for a single marker. This approach relaxes the strong parametric assumption of the normal random effects distribution and also allows to cluster the longitudinal profiles in the absence of a training data set. The first objective of this paper is to generalize the model of Verbeke and Lesaffre[10] to (a) allow for multiple longitudinal markers in a computationally tractable manner; (b) consider more general heteroscedastic normal mixtures (variances of the mixture components are unequal) in the random effect distribution. The second objective of this paper is to apply the developed model to the training (Dutch PBC) data set and to discriminate future patients using their multivariate longitudinal profiles.

The paper proceeds as follows. The first Section describes the multivariate linear mixed model with a normal mixture in the random effects distribution. This approach will be used to model in each prognostic group the longitudinal evolution of the markers and their dependence on possible covariates. The estimation procedure for the proposed model is based on the Markov chain Monte Carlo methodology and is given in Section 'Estimation'. Section 'Discrimination procedure' explains how the fitted mixed models can be used to discriminate future patients into prognostic

groups. The methodology is illustrated on the Dutch PBC Study data in Section 'Application to PBC data'. We have also extended the R (R Development Core Team[13]) package `mixAK` (Komárek[14]) to apply the proposed methods in practice. The use of the package is briefly explained in the Appendix.

## A multivariate linear mixed model with normal mixture in the random effects distribution

In the multivariate linear mixed model (MLMM, Morrell et al.[7]), a standard linear mixed model is first specified for the $r$-th marker. That is,

$$\boldsymbol{Y}_{i,r} = \mathbb{X}_{i,r}\boldsymbol{\alpha}_r + \mathbb{Z}_{i,r}\boldsymbol{b}_{i,r} + \boldsymbol{\varepsilon}_{i,r} \qquad (i = 1, \ldots, N,\ r = 1, \ldots, R), \tag{1}$$

where $\mathbb{X}_{i,r}$ is a $n_{i,r} \times p_r$ covariate matrix for fixed effects and $\mathbb{Z}_{i,r}$ is a $n_{i,r} \times q_r$ covariate matrix for random effects in a model for marker $r$. Further, $\boldsymbol{\alpha}_r = (\alpha_{r,1}, \ldots, \alpha_{r,p_r})'$ is a vector of fixed effects for marker $r$, and $\boldsymbol{b}_{i,r} = (b_{i,r,1}, \ldots, b_{i,r,q_r})'$ is a vector of random effects for marker $r$ specific for the $i$-th subject. For computational convenience of the approach outlined in Section 'Estimation', a hierarchically centered parametrization of the LMM will be used here, i.e. $\mathrm{E}(\boldsymbol{b}_{i,r}) = \boldsymbol{\beta}_r = (\beta_{r,1}, \ldots, \beta_{r,q_r})'$ with matrices $(\mathbb{X}_{i,r}, \mathbb{Z}_{i,r})$ of full column rank. That is, the vector $(\boldsymbol{\alpha}_r', \boldsymbol{\beta}_r')'$ is a vector of fixed effects in a classical sense. In the remainder of the paper we let $\boldsymbol{\alpha} = (\boldsymbol{\alpha}_1', \ldots, \boldsymbol{\alpha}_R')'$ and $\boldsymbol{\beta} = (\boldsymbol{\beta}_1', \ldots, \boldsymbol{\beta}_R')'$ be the vectors of fixed effects and means of random effects for all considered markers, respectively. Further, let $p = \sum_{r=1}^{R} p_r$ be the length of the vector $\boldsymbol{\alpha}$ and $q = \sum_{r=1}^{R} q_r$ be the length of the vector $\boldsymbol{\beta}$ also equal to the total dimension of random effects. Finally, $\boldsymbol{\varepsilon}_{i,r} = (\varepsilon_{i,r,1}, \ldots, \varepsilon_{i,r,n_{i,r}})'$ is the vector of random errors for the measurements of the $r$-th marker on the $i$-th subject. The errors are assumed to be mutually independent and normally distributed. However, we allow the residual variances corresponding to different markers to differ. Hence, for $\boldsymbol{\varepsilon}_i = (\boldsymbol{\varepsilon}_{i,1}', \ldots, \boldsymbol{\varepsilon}_{i,R}')'$ we assume $\boldsymbol{\varepsilon}_i \sim \mathcal{N}(0, \Sigma_i)$, where $\Sigma_i$ is a block-diagonal matrix with each diagonal block being equal to $\sigma_r^2 I_{n_{i,r}}$, where $\sigma_r^2$ is the residual variance of the $r$-th marker. Note that the MLMM (1) can now be written as a standard LMM as

$$\boldsymbol{Y}_i = \mathbb{X}_i\boldsymbol{\alpha} + \mathbb{Z}_i\boldsymbol{b}_i + \boldsymbol{\varepsilon}_i \qquad (i = 1, \ldots, N), \tag{2}$$

where $\mathbb{X}_i$ is a $n_i \times p$ block-diagonal matrix with matrices $\mathbb{X}_{i,1}, \ldots, \mathbb{X}_{i,R}$ on the diagonal and similarly $\mathbb{Z}_i$ is a $n_i \times q$ block-diagonal matrix with matrices $\mathbb{Z}_{i,1}, \ldots, \mathbb{Z}_{i,R}$ on the diagonal.

The correlation between single observations made on the same subject (between two measurements of either the same marker or two different markers) is introduced by specifying a joint distribution for $\boldsymbol{b}_i = (\boldsymbol{b}'_{i,1}, \ldots, \boldsymbol{b}'_{i,R})'$, the vector of $i$-th subject specific random effects pertaining to all $R$ markers. Particularly, the covariance matrix $\text{var}(\boldsymbol{b}_i)$ is assumed to be a general (unstructured) positive definite matrix $\mathbb{D}$. The model is finalized by specifying the distribution of random effects. Morrell et al.[7] or Fieuws et al.[9] assume a normal distribution, i.e., $\boldsymbol{b}_i \overset{\text{i.i.d.}}{\sim} \mathcal{N}(\boldsymbol{\beta}, \mathbb{D})$ which together with the representation (2) allows them to fit model (1) using standard software like R (R Development Core Team[13]) package `lme4` (Bates and Maechler[15]) or SAS `PROC MIXED/NLMIXED`.

For reasons mentioned in the introduction we replace the normality assumption here by a heteroscedastic normal mixture. More precisely, we assume that

$$\boldsymbol{b}_i \;=\; \boldsymbol{s} + \mathbb{S}\,\boldsymbol{b}_i^*, \qquad \boldsymbol{b}_i^* \overset{\text{i.i.d.}}{\sim} \sum_{k=1}^{K} w_k\, \mathcal{N}(\boldsymbol{\mu}_k, \mathbb{D}_k), \tag{3}$$

where $\boldsymbol{s}$ is a fixed shift vector, $\mathbb{S}$ a fixed diagonal scale matrix and $\boldsymbol{b}_i^*$ are shifted and scaled random effects. Further, $\boldsymbol{w} = (w_1, \ldots, w_K)'$ is a vector of non-negative mixture weights satisfying $\sum_{k=1}^{K} w_k = 1$, $\boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K$ are mixture means and $\mathbb{D}_1, \ldots, \mathbb{D}_K$ are mixture covariance matrices. The number of mixture components $K$ is assumed to be pre-specified. Note that the shift $\boldsymbol{s}$ and the scale $\mathbb{S}$ are included in the specification of the model (3) only to improve numerical stability of the estimation procedure described in Section 'Estimation'. However, it is possible to set $\boldsymbol{s} = 0$ and $\mathbb{S} = I_q$.

The model parameters which need to be estimated are

$$\boldsymbol{\theta} = \big(\boldsymbol{\alpha}',\, \boldsymbol{w}',\, \boldsymbol{\mu}'_1, \ldots, \boldsymbol{\mu}'_K,\, \text{vec}(\mathbb{D}_1), \ldots, \text{vec}(\mathbb{D}_K),\, \sigma_1^2, \ldots, \sigma_R^2\big)', \tag{4}$$

where $\text{vec}(\mathbb{D}_k)$ is a vector with the elements of the lower triangle of the matrix $\mathbb{D}_k$. Note that the means of random effects ($\boldsymbol{\beta}$) and the overall covariance matrix of random effects $\mathbb{D}$ are determined by the parameters of the mixture as

$$\mathbb{D} = \text{var}(\boldsymbol{b}_i) \;=\; \mathbb{S}\left[\sum_{k=1}^{K} w_k \left\{\mathbb{D}_k + \Big(\boldsymbol{\mu}_k - \sum_{j=1}^{K} w_j\,\boldsymbol{\mu}_j\Big)\Big(\boldsymbol{\mu}_k - \sum_{j=1}^{K} w_j\,\boldsymbol{\mu}_j\Big)'\right\}\right]\mathbb{S}'. \tag{5}$$

In the model of Verbeke and Lesaffre[10], maximum-likelihood estimation was used. Applied to the above heteroscedastic mixture (3) results however in an unbounded likelihood (McLachlan and Basford[16]). Here, we will use a Bayesian approach where, with some care, the problem of unbounded likelihood is tackled by using suitable prior distributions for mixture covariance matrices, see Section 'Prior distributions'.

# Estimation

The MLMM (1) can be regarded as a standard LMM (2) with $\mathbb{X}$ and $\mathbb{Z}$ matrices supplemented by zeros. However, maximum-likelihood based estimation routines that ignore the specific sparse structure of $\mathbb{X}$ and $\mathbb{Z}$ matrices are inefficient and encounter numerical problems even with normally distributed random effects ($K = 1$ in expression (3)). For example, Fieuws et al.[9] used a pairwise fitting approach of Fieuws and Verbeke[17] to avoid numerical problems. Another route would be to use methods for sparse matrices, see, e.g., R package `Matrix` (Bates and Maechler[18]). Here we adopt the Bayesian approach with Markov chain Monte Carlo (MCMC) estimation. The MCMC approach proved to be a useful machinery for problems involving hierarchically specified models (like linear mixed models) and models involving mixture distributions. Our prior distributions for the model parameters are weakly informative such that the posterior summary statistics correspond closely to maximum-likelihood estimates.

## Prior distributions

To specify the model from a Bayesian point of view, prior distributions have to be assigned to model parameters. The vector $\boldsymbol{\theta}$ of model parameters is supplemented by latent quantities (values of random effects $\boldsymbol{b} = (\boldsymbol{b}_1', \ldots, \boldsymbol{b}_N')'$), variance hyperparameters $\boldsymbol{\gamma}_b = (\gamma_{b,1}, \ldots, \gamma_{b,q})'$, $\boldsymbol{\gamma}_\varepsilon = (\gamma_{\varepsilon,1}, \ldots, \gamma_{\varepsilon,R})'$ (see below), and further, by other parameters pertaining to the hierarchical structure of the model which simplify the subsequent computations (component allocations $\boldsymbol{u} = (u_1, \ldots, u_N)'$, see below) in the spirit of the Bayesian data augmentation approach of Tanner and Wong[19]. This leads to the vector of parameters

$$\boldsymbol{\psi} = (\boldsymbol{\theta}', \boldsymbol{b}', \boldsymbol{\gamma}_b', \boldsymbol{\gamma}_\varepsilon', \boldsymbol{u}')', \tag{6}$$

for which the joint prior distribution will be specified hierarchically.

It is well known (see, e.g., Diebolt and Robert[20]) that, due to the problem of an unbounded likelihood mentioned above, mixture models do not allow for improper priors. Nevertheless, several proper, however weakly informative prior distributions for mixture problems have been suggested in the literature leading to the proper posterior distribution (see Diebolt and Robert[20], Roeder and Wasserman[21], Richardson and Green[22]). In this paper, we exploit the approach of Richardson and Green[22] adapted to our needs and to hierarchical linear models (see, e.g., Gelman et al.[23], Chapter 15). Let $p$ be a generic symbol for a distribution. The joint prior distribution

for our MLMM is factorized as

$$
\begin{aligned}
p(\boldsymbol{\psi}) = \; & p(\boldsymbol{w}, \boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K, \mathbb{D}_1, \ldots, \mathbb{D}_K, \boldsymbol{\gamma}_b, \boldsymbol{\alpha}_1, \ldots, \boldsymbol{\alpha}_R, \sigma_1^2, \ldots, \sigma_R^2, \boldsymbol{\gamma}_\varepsilon, \boldsymbol{b}, \boldsymbol{u}) \\
= \; & p(\boldsymbol{w}) \times \Big\{ \prod_{k=1}^{K} p(\boldsymbol{\mu}_k, \mathbb{D}_k^{-1} \,|\, \boldsymbol{\gamma}_b) \Big\} \times p(\boldsymbol{\gamma}_b) \qquad (7) \\
& \Big\{ \prod_{r=1}^{R} p(\boldsymbol{\alpha}_r) \times p(\sigma_r^{-2} \,|\, \gamma_{\varepsilon,r}) \times p(\gamma_{\varepsilon,r}) \Big\} \times \\
& \Big\{ \prod_{i=1}^{N} p(\boldsymbol{b}_i \,|\, u_i, \boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K, \mathbb{D}_1, \ldots, \mathbb{D}_K) \times p(u_i \,|\, \boldsymbol{w}) \Big\}.
\end{aligned}
$$

Particular parts of expression (7) are:

$$
p(\boldsymbol{w}) \sim \mathcal{D}(\delta, \ldots, \delta), \qquad (8)
$$

$$
p(\boldsymbol{\mu}_k, \mathbb{D}_k^{-1} \,|\, \boldsymbol{\gamma}_b) = p(\boldsymbol{\mu}_k) \times p(\mathbb{D}_k^{-1} \,|\, \boldsymbol{\gamma}_b) \sim \mathcal{N}(\boldsymbol{\xi}_{b,k}, \mathbb{C}_{b,k}) \times \mathcal{W}(\zeta_b, \Theta_b),
$$

$$
\Theta_b = \mathrm{diag}(\gamma_{b,1}, \ldots, \gamma_{b,q}), \qquad k = 1, \ldots, K, \qquad (9)
$$

$$
p(\boldsymbol{\gamma}_b) = \prod_{l=1}^{q} p(\gamma_{b,l}) \sim \prod_{l=1}^{q} \mathcal{G}(g_{b,l}, h_{b,l}), \qquad (10)
$$

$$
p(\boldsymbol{\alpha}_r) = \prod_{l=1}^{p_r} p(\alpha_{r,l}) \sim \prod_{l=1}^{p_r} \mathcal{N}(\xi_{\alpha_r,l}, c_{\alpha_r,l}^2), \qquad r = 1, \ldots, R, \qquad (11)
$$

$$
p(\sigma_r^{-2} \,|\, \gamma_{\varepsilon,r}) \sim \mathcal{G}(\zeta_{\varepsilon,r}/2, \gamma_{\varepsilon,r}^{-1}/2), \qquad r = 1, \ldots, R, \qquad (12)
$$

$$
p(\gamma_{\varepsilon,r}) \sim \mathcal{G}(g_{\varepsilon,r}, h_{\varepsilon,r}), \qquad (13)
$$

where $\mathcal{D}(\delta, \ldots, \delta)$ denotes a Dirichlet distribution with parameters $\delta, \ldots, \delta$, $\mathcal{W}(\zeta, \Theta)$ denotes a Wishart distribution with $\zeta$ degrees of freedom and a scale matrix $\Theta$, and $\mathcal{G}(g, h)$ is a gamma distribution with parameters $g$ and $h$. The last two parts of expression (7) correspond to mixture model (3) where additionally, component allocations $\boldsymbol{u} = (u_1, \ldots, u_N)'$, $u_i \in \{1, \ldots, K\}$ $(i = 1, \ldots, N)$ are introduced. If we use the following prior distributions,

$$
p(\boldsymbol{b}_i \,|\, u_i, \boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K, \mathbb{D}_1, \ldots, \mathbb{D}_K) \sim \boldsymbol{s} + \mathbb{S}\mathcal{N}(\boldsymbol{\mu}_{u_i}, \mathbb{D}_{u_i}), \qquad i = 1, \ldots, N, \quad (14)
$$

$$
p(u_i \,|\, \boldsymbol{w}) \sim \mathrm{P}(u_i = k) = w_k, \qquad k = 1, \ldots, K, \;\; i = 1, \ldots, N, \qquad (15)
$$

prior (7) whereby the vector $\boldsymbol{u}$ is integrated out, is the same as when the terms $p(\boldsymbol{b}_i \,|\, u_i, \boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K, \mathbb{D}_1, \ldots, \mathbb{D}_K) \times p(u_i \,|\, \boldsymbol{w})$ are replaced by $p(\boldsymbol{b}_i \,|\, \boldsymbol{w}, \boldsymbol{\mu}_1, \ldots, \boldsymbol{\mu}_K, \mathbb{D}_1, \ldots, \mathbb{D}_K) \sim \boldsymbol{s} + \mathbb{S} \sum_{k=1}^{K} w_k \mathcal{N}(\boldsymbol{\mu}_k, \mathbb{D}_k)$, i.e., by normal mixture (3).
For particular choices of the fixed hyperparameters related to the normal mixture, $\delta$, $\boldsymbol{\xi}_{b,k}$, $\mathbb{C}_{b,k}$ $(k = 1, \ldots, K)$, $g_{b,l}$, $h_{b,l}$ $(l = 1, \ldots, q)$, and leading to a weakly

informative prior distribution, we refer to Komárek[14], where in his notation the terms $y_{i,j}$ are replaced by reasonable initial values of the random effects. These can be, for example, equal to their empirical Bayes estimates from separate (for $r = 1, \ldots, R$) maximum-likelihood fits of models derived from (1). A weakly informative prior for the fixed effects $\boldsymbol{\alpha}$ is obtained by setting $\xi_{\alpha_r,l}$ to zero, and $c^2_{\alpha_r,l}$ ($r = 1, \ldots, R, l = 1, \ldots, p_r$) to a large positive number, e.g., 10 000 (it is necessary to check that the variance of the posterior distribution is considerably smaller). Finally, adapting recommendations of Richardson and Green[22], the following values of the fixed hyperparameters $\zeta_{\varepsilon,r}$, $g_{\varepsilon,r}$, $h_{\varepsilon,r}$ ($r = 1, \ldots, R$) related to the prior distribution of the error terms lead to a weakly informative prior: a small positive number for $\zeta_{\varepsilon,r}$ and $g_{\varepsilon,r}$, $h_{\varepsilon,r} = 10/R^2_{\varepsilon,r}$, where $R_{\varepsilon,r}$ is a range of residuals from separate (for $r = 1, \ldots, R$) initial maximum-likelihood fits of models derived from (1).

## Posterior distribution and Markov chain Monte Carlo

Given the parameters $\boldsymbol{\psi}$, i.e. parameters for which the prior distribution has been specified in (7), the likelihood corresponding to model (1) takes a relatively simple form, i.e.,

$$\begin{aligned} L(\boldsymbol{\psi}) &= \prod_{i=1}^{N} p(\boldsymbol{y}_i \mid \boldsymbol{\psi}) = \prod_{i=1}^{N}\prod_{r=1}^{R} p(\boldsymbol{y}_{i,r} \mid \boldsymbol{\alpha}_r,\, \boldsymbol{b}_{i,r},\, \sigma_r^2) \\ &\sim \prod_{i=1}^{N}\prod_{r=1}^{R} \mathcal{N}(\mathbb{X}_{i,r}\boldsymbol{\alpha}_r + \mathbb{Z}_{i,r}\boldsymbol{b}_{i,r},\, \sigma_r^2\, I_{n_{i,r}}). \end{aligned} \tag{16}$$

Let $\boldsymbol{y}$ be the observed values of all longitudinal markers from the whole data. Bayesian inference is based on a sample from the posterior distribution $p(\boldsymbol{\psi} \mid \boldsymbol{y}) \propto L(\boldsymbol{\psi})\, p(\boldsymbol{\psi})$ obtained using the Markov chain Monte Carlo method with a block Gibbs sampler.

To improve the numerical properties of the MCMC algorithm, it is useful to choose the shift vector $\boldsymbol{s}$ and the scale matrix $\mathbb{S}$ (see expression (3)) such that the shifted and scaled random effects $\boldsymbol{b}_i^*$ have approximately zero mean and unit variances. For this reason we recommend to set $\boldsymbol{s}$ to the estimated means and diagonal elements of the $\mathbb{S}$ matrix to the estimated standard deviations from separate (for $r = 1, \ldots, R$) maximum-likelihood fits of models derived from (1). Further, note that a sample from the posterior distribution $p(\boldsymbol{\theta} \mid \boldsymbol{y})$ is directly available, simply by ignoring the $\boldsymbol{\gamma}_b$, $\boldsymbol{\gamma}_\varepsilon$, $\boldsymbol{b}$, $\boldsymbol{u}$ parts of sampled values of the vector $\boldsymbol{\psi}$.

The R package `mixAK` (Komárek[14]) has been extended to handle the MLMM (1). Whenever possible, the R implementation exploits the block-diagonal structure of

$\mathbb{X}$ and $\mathbb{Z}$ matrices from the "stacked" specification (2) of the MLMM to enhance the computational speed. The package is freely available from CRAN at `http://CRAN.R-project.org/package=mixAK`.

## Estimates of individual values of random effects

We now explore the conditional distributions $p(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i,\, \boldsymbol{\theta})$ and $p(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, \boldsymbol{\theta})$ resulting from the Bayesian specification of the model. This will be useful for the developments in next section. The distribution $p(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta})$ $(i = 1, \ldots, N,\ k = 1, \ldots, K)$ is in fact the full conditional distribution for $\boldsymbol{b}_i$ and it is used in the MCMC algorithm to update the values of random effects. It can be shown that it is a (multivariate) normal distribution with mean and covariance matrix

$$\mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta}) = \boldsymbol{s} + \mathbb{S}\,\mathbb{Q}_{i,k}^{-1}\boldsymbol{\eta}_{i,k}, \tag{17}$$

$$\mathrm{var}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta}) = \mathbb{S}\,\mathbb{Q}_{i,k}^{-1}\,\mathbb{S}', \tag{18}$$

where $\mathbb{Q}_{i,k}^{-1}$ is the covariance matrix and $\boldsymbol{\eta}_{i,k}$ is the canonical mean of the conditional distribution $p(\boldsymbol{b}_i^* \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta})$ of shifted and scaled random effects given by

$$\mathbb{Q}_{i,k} = \mathbb{Q}_{i,k}(\boldsymbol{y}_i,\, \boldsymbol{\theta}) = \mathbb{S}'\mathbb{Z}_i'\Sigma_i^{-1}\mathbb{Z}_i\mathbb{S} + \mathbb{D}_k^{-1}, \tag{19}$$

$$\boldsymbol{\eta}_{i,k} = \boldsymbol{\eta}_{i,k}(\boldsymbol{y}_i,\, \boldsymbol{\theta}) = \mathbb{S}'\mathbb{Z}_i'\Sigma_i^{-1}\left(\boldsymbol{y}_i - \mathbb{X}_i\boldsymbol{\alpha} - \mathbb{Z}_i\boldsymbol{s}\right) + \mathbb{D}_k^{-1}\,\boldsymbol{\mu}_k. \tag{20}$$

Note that expression (17) can alternatively be written as

$$\begin{aligned}&\mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta})\\ &= \boldsymbol{s} + \mathbb{S}\left[\mathbb{D}_k\mathbb{S}'\mathbb{Z}_i'(\mathbb{Z}_i\mathbb{S}\mathbb{D}_k\mathbb{S}'\mathbb{Z}_i' + \Sigma_i)^{-1}\left\{\boldsymbol{y}_i - \mathbb{X}_i\boldsymbol{\alpha} - \mathbb{Z}_i\left(\boldsymbol{s} + \mathbb{S}\,\boldsymbol{\mu}_k\right)\right\} + \boldsymbol{\mu}_k\right],\end{aligned} \tag{21}$$

which resembles (taking into account the changes due to inclusion of the shift $\boldsymbol{s}$, scale $\mathbb{S}$ and non-zero mean $\boldsymbol{\mu}_k$) a classical expression for empirical Bayes estimation of a random effect in a standard linear mixed model with normally distributed random effects (see Verbeke and Molenberghs[11], Section 7.2).

On the other hand, the conditional distribution $p(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, \boldsymbol{\theta})$ can serve as a basis for inference on random effects which closely corresponds to classical approaches since all latent parameters, especially the component allocation $u_i$, are integrated out and we only condition on observed data and parameters in a classical sense. The mean of this distribution is given by

$$\widetilde{\boldsymbol{b}}_i = \mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, \boldsymbol{\theta}) = \sum_{k=1}^{K} w_{i,k}(\boldsymbol{y}_i,\, \boldsymbol{\theta})\ \mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\, u_i = k,\, \boldsymbol{\theta}), \tag{22}$$

where

$$w_{i,k}(\boldsymbol{y}_i,\,\boldsymbol{\theta}) = \frac{w_k\,|\mathbb{D}_k|^{-1/2}\,|\mathbb{Q}_{i,k}|^{-1/2}\,\exp\left\{-\frac{1}{2}\left(\boldsymbol{\mu}_k{}'\mathbb{D}_k^{-1}\boldsymbol{\mu}_k\;-\;\boldsymbol{\eta}_{i,k}{}'\mathbb{Q}_{i,k}^{-1}\boldsymbol{\eta}_{i,k}\right)\right\}}{\sum_{j=1}^{K} w_j\,|\mathbb{D}_j|^{-1/2}\,|\mathbb{Q}_{i,j}|^{-1/2}\,\exp\left\{-\frac{1}{2}\left(\boldsymbol{\mu}_j{}'\mathbb{D}_j^{-1}\boldsymbol{\mu}_j\;-\;\boldsymbol{\eta}_{i,j}{}'\mathbb{Q}_{i,j}^{-1}\boldsymbol{\eta}_{i,j}\right)\right\}}$$

$(k = 1, \ldots, K)$. (23)

The Bayesian estimate of $\boldsymbol{b}_i$ integrating out the uncertainty with which the parameters $\boldsymbol{\theta}$ are estimated is the posterior mean of $\widetilde{\boldsymbol{b}}_i$. That is, $\mathrm{E}\big\{\mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\,\boldsymbol{\theta}) \,\big|\, \boldsymbol{y}\big\}$, where the second expectation is done over the different possible $\boldsymbol{\theta}$ values. For $\boldsymbol{\theta}^{(1)}, \ldots, \boldsymbol{\theta}^{(M)}$, the (MCMC) sample from the posterior distribution $p(\boldsymbol{\theta} \,|\, \boldsymbol{y})$, $\widetilde{\boldsymbol{b}}_i^{(m)} = \mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\,\boldsymbol{\theta}^{(m)})$ $(m = 1, \ldots, M)$ and $\mathrm{E}\big\{\mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\,\boldsymbol{\theta}) \,\big|\, \boldsymbol{y}\big\}$ is estimated as

$$\widehat{\boldsymbol{b}}_i = \widehat{\mathrm{E}}\big\{\mathrm{E}(\boldsymbol{b}_i \,|\, \boldsymbol{y}_i,\,\boldsymbol{\theta}) \,\big|\, \boldsymbol{y}\big\} = \frac{1}{M}\sum_{m=1}^{M} \mathrm{E}\big(\boldsymbol{b}_i \,\big|\, \boldsymbol{y}_i,\,\boldsymbol{\theta}^{(m)}\big) = \frac{1}{M}\sum_{m=1}^{M} \widetilde{\boldsymbol{b}}_i^{(m)}, \qquad (24)$$

leading to the Bayesian estimate of the $i$-th individual value of random effects.

## Discrimination procedure

To develop a discrimination procedure, we assume that a training data set is available for which we know the allocation of the involved subjects (patients, longitudinal profiles) to prognostic groups ($g = 0, \ldots, G-1$). Let $\boldsymbol{y}^g = \{\boldsymbol{y}_i : i \in$ prognostic group $g\}$ be the observed values of the longitudinal markers for subjects in the training data set belonging to prognostic group $g$. Each prognostic group is characterized by MLMM written as (1) or written in a condensed way as (2), with the random effects distribution specified by the mixture (3), i.e.,

$$\left.\begin{aligned}
&\boldsymbol{Y}_i \;=\; \mathbb{X}_i^g\boldsymbol{\alpha}^g \;+\; \mathbb{Z}_i^g\boldsymbol{b}_i \;+\; \boldsymbol{\varepsilon}_i,\\
&\boldsymbol{b}_i \;=\; \boldsymbol{s}^g \;+\; \mathbb{S}^g\,\boldsymbol{b}_i^*,\\
&\boldsymbol{b}_i^* \overset{\text{i.i.d.}}{\sim} \textstyle\sum_{k=1}^{K} w_k^g\,\mathcal{N}(\boldsymbol{\mu}_k^g,\,\mathbb{D}_k^g),\\
&\boldsymbol{\varepsilon}_i \sim \mathcal{N}(0,\,\mathrm{diag}(\sigma_1^{g\,2}, \ldots, \sigma_1^{g\,2}, \ldots, \sigma_R^{g\,2}, \ldots, \sigma_R^{g\,2}))\\
&(i \in \text{ prognostic group } g).
\end{aligned}\right\} \qquad (25)$$

Let $\boldsymbol{\theta}^g = \big(\boldsymbol{\alpha}^{g\prime},\; \boldsymbol{w}^{g\prime}, \boldsymbol{\mu}_1^{g\prime}, \ldots, \boldsymbol{\mu}_K^{g\prime}, \mathrm{vec}(\mathbb{D}_1^g), \ldots, \mathrm{vec}(\mathbb{D}_K^g),\; \sigma_1^{g\,2}, \ldots, \sigma_R^{g\,2}\big)'$ be the model parameters for group $g$. Note that not only the values of parameters $\boldsymbol{\theta}^g$ but also the structure of the MLMM (25), for example the structure of matrices $\mathbb{X}_i^g$ and $\mathbb{Z}_i^g$, may differ between prognostic groups.

For each $g = 0, \ldots, G-1$, model (25) is estimated using subjects from the respective prognostic group in the training dataset. That is, samples $\boldsymbol{\theta}^{g,(1)}, \ldots, \boldsymbol{\theta}^{g,(M)}$ are obtained from the posterior distribution $p\big(\boldsymbol{\theta}^g \,\big|\, \boldsymbol{y}^g\big)$. Furthermore, each prognostic group is assigned prevalences $\pi_0, \ldots, \pi_{G-1}$, $\sum_{g=0}^{G-1} \pi_g = 1$ which play the role of prior pertinence probabilities for the discrimination procedure.

Let $\boldsymbol{Y}_{new} = (\boldsymbol{Y}'_{new,1}, \ldots, \boldsymbol{Y}'_{new,R})'$ be the history of relevant markers for a new subject. Without loss of generality, we assume that $\boldsymbol{Y}_{new}$ contains only the history up to time $\tau < T$ at which we want to estimate $\mathcal{P}_{g,new} = \mathcal{P}_{g,new}(\tau)$ $(g = 0, \ldots, G-1)$, probabilities at time $\tau$ that the new subject belongs to either of prognostic groups. Let $n_{new,1}, \ldots, n_{new,R}$ be the lengths of vectors $\boldsymbol{Y}_{new,1}, \ldots, \boldsymbol{Y}_{new,R}$. Further, let $\mathbb{X}^g_{new}$, $\mathbb{Z}^g_{new}$ $(g = 0, \ldots, G-1)$ be the corresponding covariate matrices for a new subject under the $G$ models (25). Finally, let $\Sigma^g_{new} = \text{diag}(\sigma_1^{g2}, \ldots, \sigma_1^{g2}, \ldots, \sigma_R^{g2}, \ldots, \sigma_R^{g2})$, with $\sigma_r^{g2}$ repeated $n_{new,r}$ times $(r = 1, \ldots, R)$.

A general framework for discriminant analysis based on mixed models fitted in a frequentist approach is reviewed by Morrell, Brant and Sheng[24]. They show how classification can be based on a fitted (a) marginal distribution of observed markers (*marginal prediction*); (b) conditional distribution of observed markers given suitable predictors of random effects (*conditional prediction*); (c) distribution of random effects (*random effects prediction*). We will follow their taxonomy and explain how these approaches are applied with the MLMM with a normal mixture in the random effects distribution fitted using a Bayesian method.



Given the model parameters, the strength of the allocation of the new subject to the $g$-th diagnostic group is characterized by a predictive density $f_{g,new} = f(\boldsymbol{y}_{new}; \boldsymbol{\theta}^g)$ whose particular expression is discussed below. In a ML approach, $f_{g,new}$ is estimated as $\widehat{f}_{g,new} = f(\boldsymbol{y}_{new}; \widehat{\boldsymbol{\theta}}^g)$, where $\widehat{\boldsymbol{\theta}}^g$ is the MLE of the parameters for the model in group $g$. In a Bayesian approach, $\widehat{f}_{g,new}$ equals the posterior predictive density, estimated from the posterior sample $\boldsymbol{\theta}^{g,(1)}, \ldots, \boldsymbol{\theta}^{g,(M)}$ as

$$\widehat{f}_{g,new} = \frac{1}{M} \sum_{m=1}^{M} f\big(\boldsymbol{y}_{new}; \boldsymbol{\theta}^{g,(m)}\big). \tag{26}$$

The estimated allocation of the new subject to group $g$ is based on a combination of the prior probabilities $\pi_0, \ldots, \pi_{G-1}$ and the estimated values of the predictive densities $\widehat{f}_{1,new}, \ldots, \widehat{f}_{G,new}$ using Bayes' rule leading to

$$\widehat{\mathcal{P}}_{g,new} = \frac{\pi_g \, \widehat{f}_{g,new}}{\sum_{h=0}^{G-1} \pi_h \, \widehat{f}_{h,new}} \qquad (g = 0, \ldots, G-1). \tag{27}$$

As reviewed by Morrell, Brant and Sheng[24], there are three natural ways of specifying the predictive density $f_{g,new}$ for the purpose of discrimination based on longitudinal

profiles and fitted mixed model leading to *marginal*, *conditional* and *random effects* prediction.

## Marginal prediction

For marginal prediction, the predictive density $f_{g,new}$ is equal to the marginal density of $\boldsymbol{Y}_{new}$ where the term marginal reflects the fact that the random effects are integrated out. That is, for our model

$$f^{marg}_{g,new} = f^{marg}(\boldsymbol{y}_{new};\, \boldsymbol{\theta}^g) \equiv p\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{\theta}^g\big) = \sum_{k=1}^{K} w^g_k \, p_k\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{\theta}^g\big), \qquad (28)$$

where $p_k\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{\theta}^g\big)$ is the density of $\mathcal{N}\Big(\mathbb{X}^g_{new}\boldsymbol{\alpha}^g + \mathbb{Z}^g_{new}(\boldsymbol{s}^g + \mathbb{S}^g\boldsymbol{\mu}_k),\ \mathbb{V}^g_{new,k}\Big)$ with $\mathbb{V}^g_{new,k} = \mathbb{Z}^g_{new}\mathbb{S}^g\mathbb{D}^g_k\mathbb{S}^{g\prime}\mathbb{Z}^{g\,\prime}_{new} + \Sigma^g_{new}$.

## Conditional prediction

For conditional prediction, the predictive density $f_{g,new}$ is equal to the conditional density of $\boldsymbol{Y}_{new}$ given the estimated values of individual random effects. That is, for our model

$$f^{cond}_{g,new} = f^{cond}(\boldsymbol{y}_{new};\, \boldsymbol{\theta}^g) \equiv p\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{b}_{new} = \widetilde{\boldsymbol{b}}^g_{new},\, \boldsymbol{\theta}^g\big) \qquad (29)$$

which is a density of $\mathcal{N}\Big(\mathbb{X}^g_{new}\boldsymbol{\alpha}^g + \mathbb{Z}^g_{new}\widetilde{\boldsymbol{b}}^g_{new},\ \Sigma^g_{new}\Big)$. As explained in Section 'Estimates of individual values of random effects', a suitable estimate of the individual random effects, denoted by $\widetilde{\boldsymbol{b}}^g_{new}$, is the mean of the conditional distribution $p(\boldsymbol{b}_{new} \,|\, \boldsymbol{y}_{new},\, \boldsymbol{\theta}^g)$ which is computed using an expression analogous to (22), with $\boldsymbol{y}_i$, $\boldsymbol{b}_i$, $\boldsymbol{\theta}$ replaced by $\boldsymbol{y}_{new}$, $\boldsymbol{b}_{new}$, $\boldsymbol{\theta}^g$, respectively.

## Random effects prediction

Random effects prediction is based on the distribution of the individual random effects. The predictive density $f_{g,new}$ is then equal to the density of $\boldsymbol{b}_{new}$ evaluated at the estimated value of the random effect, i.e., at $\widetilde{\boldsymbol{b}}^g_{new}$. Hence, in our case,

$$f^{rand}_{g,new} = f^{rand}(\boldsymbol{y}_{new};\, \boldsymbol{\theta}^g) \equiv p\big(\widetilde{\boldsymbol{b}}^g_{new} \,\big|\, \boldsymbol{\theta}^g\big) = \sum_{k=1}^{K} w^g_k p_k\big(\widetilde{\boldsymbol{b}}^g_{new} \,\big|\, \boldsymbol{\theta}^g\big), \qquad (30)$$

where $p_k\big(\widetilde{\boldsymbol{b}}^g_{new} \,\big|\, \boldsymbol{\theta}^g\big)$ is the density of $\mathcal{N}\big(\boldsymbol{s}^g + \mathbb{S}^g\boldsymbol{\mu}^g_k,\ \mathbb{S}^g\mathbb{D}^g_k\mathbb{S}^{g\prime}\big)$.

## Focus of marginal, conditional and random effects prediction

Spiegelhalter et al.[25] define the 'focus' of the Bayesian model which is given by considered factorization of the marginal distribution $p(\boldsymbol{y}_{new})$ of new data. We shall show that the marginal, conditional and random effects predictions correspond to different model focuses in the spirit of Spiegelhalter et al.[25]. In our context, there are two obvious possibilities for factorization of $p(\boldsymbol{y}_{new})$. Let $\Theta^g$ be the parameter space for $\boldsymbol{\theta}^g$. With factorization

$$p(\boldsymbol{y}_{new}) = \int_{\Theta^g} p\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{\theta}^g\big) p(\boldsymbol{\theta}^g)\, d\boldsymbol{\theta}^g, \tag{31}$$

the model is focused on $\Theta^g$, i.e., on the mean evolution of the markers over time. Further, let $\boldsymbol{\theta}_1^g = \big(\boldsymbol{\alpha}^{g\prime}, \sigma_1^{g2}, \ldots, \sigma_R^{g\,2}\big)'$ and $\boldsymbol{\theta}_2^g = \big(\boldsymbol{w}^{g\prime}, \boldsymbol{\mu}_1^{g\prime}, \ldots, \boldsymbol{\mu}_K^{g\,\prime}, \mathrm{vec}(\mathbb{D}_1^g), \ldots, \mathrm{vec}(\mathbb{D}_K^g)\big)'$ be the parts of $\boldsymbol{\theta}^g$ corresponding to (1) the fixed effects and variances of random errors and (2) the distribution of the random effects. Let $\Theta_1^g$ and $\Theta_2^g$ be the corresponding parameter spaces. Finally, let $\Psi = \mathbb{R}^q$ be the parameter space for the vector $\boldsymbol{b}_{new}$ of random effects. The marginal distribution $p(\boldsymbol{y}_{new})$ can alternatively be factorized as

$$p(\boldsymbol{y}_{new}) = \int_{\Psi\times\Theta_1^g} p\big(\boldsymbol{y}_{new} \,\big|\, \boldsymbol{b}_{new},\, \boldsymbol{\theta}_1^g\big)\, p(\boldsymbol{b}_{new})\, p(\boldsymbol{\theta}_1^g)\, d\boldsymbol{b}_{new}\, d\boldsymbol{\theta}_1^g, \tag{32}$$

where

$$p(\boldsymbol{b}_{new}) = \int_{\Theta_2^g} p\big(\boldsymbol{b}_{new} \,\big|\, \boldsymbol{\theta}_2^g\big)\, p(\boldsymbol{\theta}_2^g)\, d\boldsymbol{\theta}_2^g, \tag{33}$$

and the model is focused on $\Psi\times\Theta_1^g$, i.e., on the patient specific evolution of markers over time.

That is, comparing expressions (28) and (31) we conclude that the marginal prediction is based on the likelihood of the Bayesian model focused on the mean evolution of the markers over time. Further, expressions (29) and (33) reveal that the conditional prediction is based on the likelihood of the Bayesian model focused on the patient specific evolution of the markers over time where, however, the nuisance parameter (the vector of random effects) is replaced by a plug-in estimate. Hence, the conditional prediction ignores variability in the estimation of the individual random effects. It is seen from expressions (30) and (33) that the random effects prediction in fact uses the likelihood of the Bayesian model for random effects focused on the parameters of the distribution of random effects, i.e., focused on the patient specific evolution of the markers over time. Finally, note that the marginal and conditional predictions work on the level of the observables and have to take into account also the error (within subjects) variability. On the other hand, the random effects pre-

diction uses only the latent characteristics of the subjects with the between subjects variability.

## Application to PBC data

As an illustration, we have applied our methodology to the Dutch PBC study data described in the introduction. Of the 375 patients, 178 patients are known to be alive at $T = 10$ years without needing a liver transplantation. They will be further referred to as prognostic group 0. A total of 41 patients died from a liver related cause or needed a liver transplantation during the first 10 years and will be further referred as prognostic group 1. The remaining 156 patients can be divided in three categories for whom the prognostic group is unknown because loss of follow-up (14 patients), or is unknown because the follow-up time was less than 10 years (112 patients). The third category consists of 30 patients who died in the first $T = 10$ years from another than a liver related cause. These 156 patients could be considered as the third prognostic group in our methodology. However, the results of our discrimination procedure can be better exemplified with $G = 2$ groups by means of sensitivity, specificity and receiver operating curves (ROC). Since the main objective of this section is to illustrate and explore the performance of our methodology, we will therefore consider only the two prognostic groups mentioned above. Hence our training data consist of 178 + 41 patients. The markers used to predict the prognostic group include values of: bilirubin, albumin, alkaline phosphatase (AP). Hence, $R = 3$ in model (1). To better satisfy mixed model (1), the natural logarithm of AP was used as the marker instead of the original AP value. Figure 1 shows the observed longitudinal profiles of the markers, separately for Group 0 and Group 1. We can observe that the bilirubin levels in Group 0 are rather low and stable over time whereas they start to increase dramatically from a specific time point for many patients in Group 1. Albumin levels are in general higher in Group 0 than in Group 1 while the reverse is true for AP. For practically all patients in Group 1, log(AP) is almost never negative whereas in Group 0 negative values of log(AP) are quite frequent.

Although not required with our approach, the mixed models (1) for the PBC data will have the same structure in both prognostic groups. Namely, for each of three markers ($r = 1, 2, 3$) and both groups ($g = 0, 1$), the $\mathbb{X}_{i,r}^{g}$ matrix contains two columns: age of the patient at start of treatment (median 54.7) and a dose of ursodeoxycholic acid (UDCA) in mg/day (median 750). Consequently, the vector $\boldsymbol{\alpha}^{g}$ of fixed effects has a length of 6 for $g = 0, 1$. Matrices $\mathbb{Z}$ correspond to

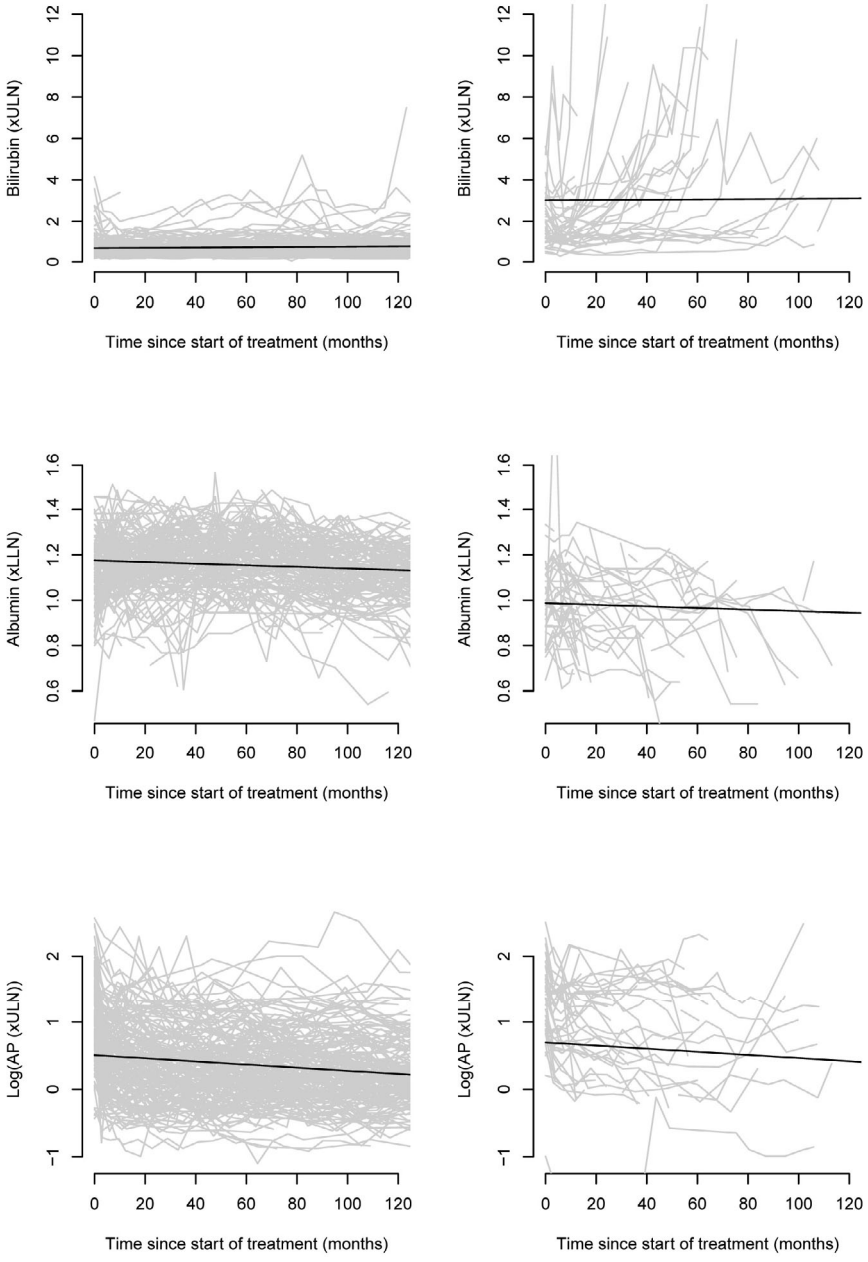


**Figure 1.** Dutch PBC Study. Observed evolution (in grey) of serum bilirubin, albumin and alkaline phosphatase levels for Group 0, patients who were alive without liver transplantation at time T = 10 years (left panel) and for Group 1, patients who encountered either liver related death or liver transplantation by time T = 10 years (right panel). Black solid lines show fitted mean profiles of a patient with median values of included covariates.

a random intercept and time effects (time in months). That is, each matrix $\mathbb{Z}_{i,r}^{g}$ contains an intercept column and the column of the times at which the markers have been measured. Hence, in each prognostic group, there are 6-dimensional random effects. Weakly informative priors as described in Section 'Prior distributions' have been used for the model parameters. Models with $K = 1$ and $K = 2$ mixture components have been fitted for comparative purposes. The reported results are based on 10 000 iterations of 1:10 thinned MCMC after a burn-in period of 5 000 iterations. MCMC sampling took about 120s for Group 0 and about 30s for Group 1 on an Intel Core 2 Duo 3 GHz CPU with 3.25 GB RAM. Convergence of the MCMC was evaluated using the R package `coda` (Plummer et al.[26]).

Tables 1 and 2 show posterior summary statistics for the fixed effects, means and standard deviations of the random effects and standard deviations of the error terms in models with $K = 2$ mixture components. The results for models with $K = 1$ are similar. The mean trajectories for a patient with median values of the covariates and based on the posterior medians of the model parameters from Table 1 are shown as black lines in Figure 1. The fitted mean profiles confirm differences between both prognostic groups and our earlier conclusions taken from the observed longitudinal profiles. The fact that the observed profiles differ from the fitted mean profiles more considerably in Group 1 than in Group 0 is seen also from the posterior medians of

**Table 1.** Dutch PBC Study. Posterior summary statistics for fixed effects (α) and means of random effects (β) in models with K = 2.

| | Group 0 | | Group 1 | |
|---|---|---|---|---|
| | **Posterior Median** | **95% HPD Interval** | **Posterior Median** | **95% HPD Interval** |
| **Bilirubin** | | | | |
| intercept | 1.13 | (1.07, 1.20) | -2.48 | (-3.02, -1.98) |
| time (months) | $0.77 \cdot 10^{-3}$ | $(-0.02, 1.61) \cdot 10^{-3}$ | 0.11 | (0.06, 0.17) |
| age (years) | $-0.78 \cdot 10^{-2}$ | $(-0.87, -0.69) \cdot 10^{-2}$ | 0.056 | (0.049, 0.062) |
| UDCA dosis (mg/day) | $-0.59 \cdot 10^{-4}$ | $(-1.00, 0.12) \cdot 10^{-4}$ | $0.85 \cdot 10^{-3}$ | $(0.34, 1.34) \cdot 10^{-3}$ |
| **Albumin** | | | | |
| intercept | 1.26 | (1.21, 1.31) | 1.27 | (1.13, 1.40) |
| time (months) | $-0.30 \cdot 10^{-3}$ | $(-0.50, -0.10) \cdot 10^{-3}$ | $-0.33 \cdot 10^{-2}$ | $(-0.54, -0.12) \cdot 10^{-2}$ |
| age (years) | $-0.11 \cdot 10^{-2}$ | $(-0.27, 0.05) \cdot 10^{-2}$ | $-0.47 \cdot 10^{-2}$ | $(-0.93, 0.00) \cdot 10^{-2}$ |
| UDCA dosis (mg/day) | $-0.35 \cdot 10^{-4}$ | $(-0.96, 0.24) \cdot 10^{-3}$ | $-0.07 \cdot 10^{-5}$ | $(-19.41, 18.63) \cdot 10^{-5}$ |
| **Alkal. phosph.** | | | | |
| intercept | 3.66 | (3.48, 3.84) | 7.38 | (6.74, 8.00) |
| time (months) | $-0.50 \cdot 10^{-2}$ | $(-0.61, -0.38) \cdot 10^{-2}$ | -0.021 | (-0.042, -0.003) |
| age (years) | $-0.30 \cdot 10^{-1}$ | $(-0.32, -0.29) \cdot 10^{-1}$ | -0.053 | (-0.058, -0.046) |
| UDCA dosis (mg/day) | $0.30 \cdot 10^{-4}$ | $(-0.52, 1.12) \cdot 10^{-4}$ | $-0.15 \cdot 10^{-2}$ | $(-0.19, -0.11) \cdot 10^{-2}$ |

**Table 2.** Dutch PBC Study. Posterior summary statistics for standard deviations of random effects (square roots of diagonal elements of the matrix **D**) and error terms ($\sigma_1$, $\sigma_2$, $\sigma_3$) in models with K = 2.

| | Group 0 | | Group 1 | |
|---|---|---|---|---|
| | **Posterior Median** | **95% HPD Interval** | **Posterior Median** | **95% HPD Interval** |
| **Bilirubin** | | | | |
| intercept | 0.39 | (0.30, 0.51) | 1.24 | (0.66, 2.12) |
| time (months) | 0.0050 | (0.0034, 0.0071) | 0.17 | (0.09, 0.27) |
| Error | 0.24 | (0.24, 0.25) | 1.34 | (1.24, 1.44) |
| **Albumin** | | | | |
| intercept | 0.12 | (0.10, 0.14) | 0.18 | (0.12, 0.28) |
| time (months) | 0.0010 | (0.0009, 0.0012) | 0.0045 | (0.0026, 0.0071) |
| Error | 0.069 | (0.067, 0.071) | 0.10 | (0.09, 0.10) |
| **Alkal. phosph.** | | | | |
| intercept | 1.18 | (1.03, 1.36) | 1.82 | (1.32, 2.40) |
| time (months) | 0.0070 | (0.0059, 0.0084) | 0.046 | (0.026, 0.077) |
| Error | 0.62 | (0.61, 0.64) | 0.97 | (0.89, 1.04) |



the standard deviations of the random effects given in Table 2 which are higher in Group 1 than in Group 0. Especially for bilirubin, the random effects are clearly necessary to model the between-patients variation of the longitudinal evolution.

Further, we computed the posterior predictive density of the random effects in all considered models and explored in more detail the univariate and pairwise bivariate marginal densities. We conclude that in models with $K = 2$, neither of these densities is clearly bimodal. Nevertheless, in many cases, the two components mixture helped to capture skewness in the distribution of the random effects. As an illustration, Figure 2 shows the estimated pairwise marginal densities for two selected pairs in models with $K = 1$ and $K = 2$ in Group 1.

Let $0 \leq t_{i,1} < \cdots < t_{i,n_i^*}$ be the visit times of the $i$-th patient when either of three considered markers was measured. In the Dutch PBC study, all three markers were intended to be measured at each visit which would imply $n_i^* = n_{i,1} = n_{i,2} = n_{i,3}$ and $t_{i,j} = t_{i,1,j} = t_{i,2,j} = t_{i,3,j}$ for all $i = 1, \ldots, N$ and $j = 1, \ldots, n_i^*$. However, due to practical circumstances, not always all markers could have been obtained at each visit. This is, though, not a difficulty for our estimation method. In that case, times $t_{i,1}, \ldots, t_{i,n_i^*}$ denote simply the set of distinct visit times out of $t_{i,1,1}, \ldots, t_{i,1,n_{i,1}}, t_{i,2,1}, \ldots, t_{i,2,n_{i,2}}, t_{i,3,1}, \ldots, t_{i,3,n_{i,3}}$. To evaluate the discrimination procedure, we have used cross-validation and computed the values of $\mathcal{P}_{0,i}(\tau)$ and $\mathcal{P}_{1,i}(\tau)$ for values of $\tau \in \{t_{i,1}, \ldots, t_{i,n_i^*}\}$ based on MCMC samples obtained from the data without the $i$-th patient. As prior group probabilities (prevalences), we

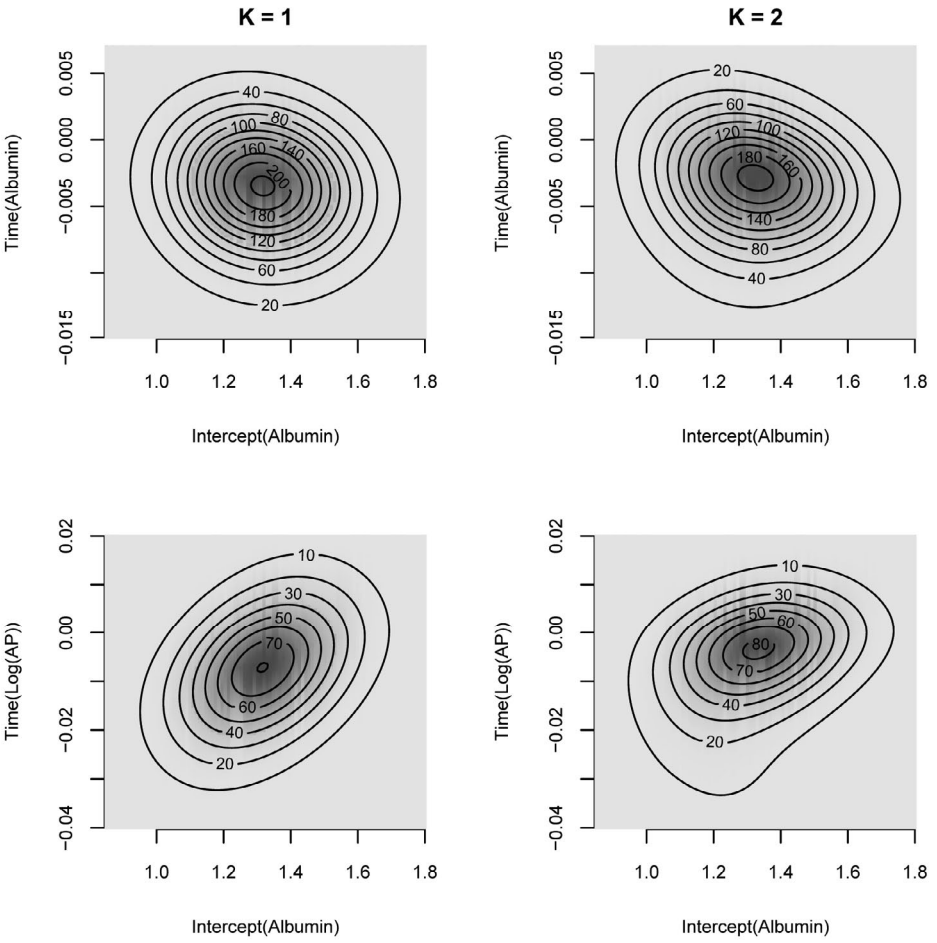


**Figure 2.** Dutch PBC Study. Estimates of selected pairwise densities from the joint distribution of the random effects in the model in Group1 for K=1 (left panel) abd K=2 (right panel). The upper panel shows the estimated distribution of ($b_{i,3}$, $b_{i,4}$)'(random intercept and time effect in the model for albumin), the lower panel shows the estimated distribution of ($b_{i,3}$, $b_{i,6}$)'(random intercept in the model for albumin and the random time effect in the model for log(alkaline phosphatase)).

used $\pi_0 = 0.8$, $\pi_1 = 0.2$ which approximately correspond to the relative sizes of the prognostic groups in the training data set. All three prediction approaches described in Section 'Discrimination procedure' have been used for models with $K = 1$ and $K = 2$ mixture components. The random effects prediction with $K = 2$ showed the best results, as will be illustrated.

The evolution of the cross-validated values of $\mathcal{P}_{1,i}(\tau)$, i.e. probabilities at time $\tau$ that the subject $i$ encounters serious disease progression by $T = 10$ years obtained from the random effects prediction is shown in Figure 3. Note that for a perfect

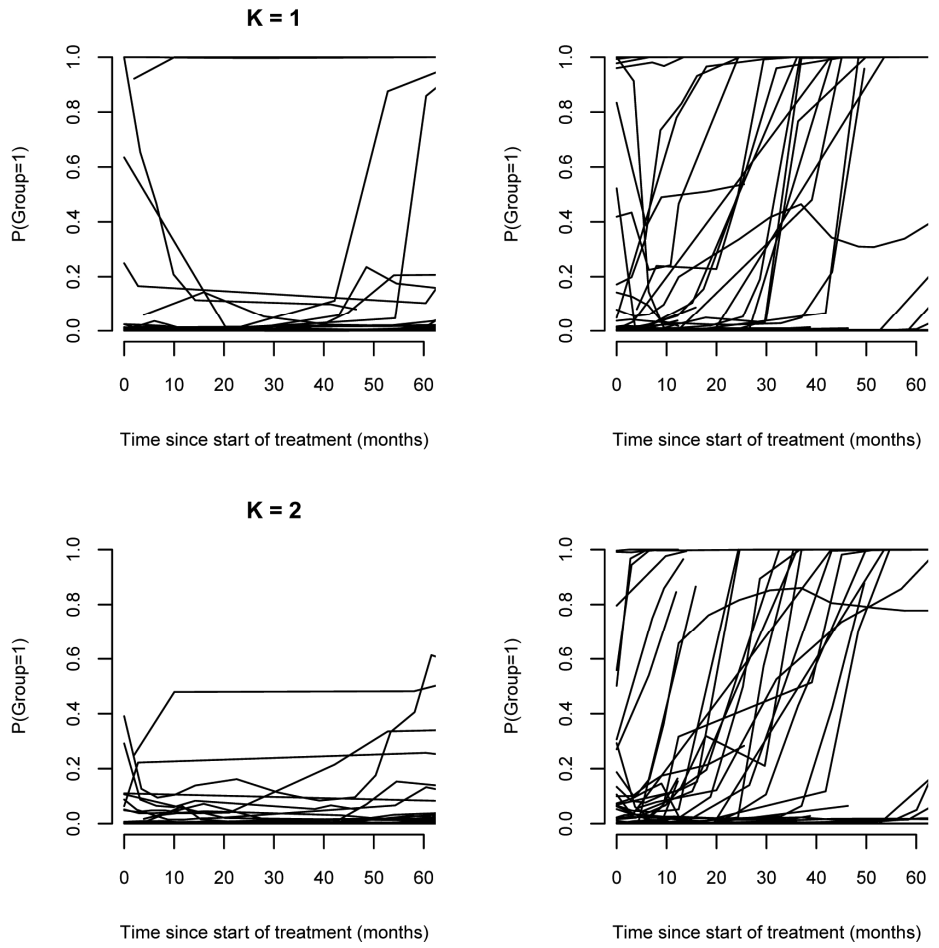


**Figure 3.** Dutch PBC Study. Evolution of cross-validated $P_{1,i}(\tau)$ (probability of being classified in Group 1) from random effect prediction based on mixed models with K = 1 (upper panel) and K = 2 (lower panel) for Group 0,
patients who were alive without liver transplantation at time T = 10 years (left panel) and for Group 1, patients who encountered either liver related death or liver transplantation by time T = 10 years (right panel).

discrimination procedure the values of $\mathcal{P}_{1,i}(\tau)$ would be zero for all $\tau$ for subjects from Group 0 (left panel of Figure 3) while conversely the values of $\mathcal{P}_{1,i}(\tau)$ would be one for all $\tau$ for subjects from Group 1 (right panel of Figure 3). It is seen that with $K = 2$ the values of $\mathcal{P}_{1,i}(\tau)$ in Group 0 remain close to zero for most subjects which is not always the case when the model with $K = 1$ was used. In Group 1, the values of $\mathcal{P}_{1,i}(\tau)$ go to one for most subjects, however, the difference between $K = 1$ and $K = 2$ is practically negligible.

Further, we explored the properties of the discrimination procedures based on $\mathcal{P}_{g,i}(\tau)$ from different models ($K = 1, 2$) and different discrimination approaches (marginal, conditional, random effects) in the following way. For each subject, each model and discrimination approach, the evolution $\{\mathcal{P}_{1,i}(\tau) : \tau \leq t_{i,n_i}\}$ was approximated from computed values of $\mathcal{P}_{1,i}(t_{i,1}), \ldots, \mathcal{P}_{1,i}(t_{i,n_i^*})$ as piecewise linear (as shown in Figure 3). The values of $\mathcal{P}_{1,i}(t)$, for $t = 0, 1, \ldots, 60$ months, from patients whose last visit happened at time $t$ or later were subsequently used to draw receiver operating curves (ROC) and to compute related areas under the ROC (AUC). Figure 4 clearly shows the superiority of the random effects prediction in this case and also a visible improvement when a two component normal mixture is used for the random effects distribution compared to a single component normal distribution. More insight is given in Figure 5 which shows ROCs for $t = 0, 6, 12, 18, 24, 36$ months and in Table 3 which provides the sensitivity values for specificity values equal to 0.99, 0.95 and 0.90. They show that with the random effects prediction, it is possible to predict already at $t = 18$ months the patient's status at $T = 120$ months with a rather high specificity and sensitivity (e.g. with $K = 2$, specificity of 0.90 and sensitivity of 0.733 is obtained at $t = 18$ months).

Furthermore, we examined how the multivariate mixed model (1) based on $R = 3$ markers improves the prediction of the patient's status at $T = 10$ years compared to separate mixed models for each marker. Hence, we separately fitted the three mixed models to each of the considered markers. In each model, $K = 2$ mixture components were used to model the distribution of the random effects. Figure 6

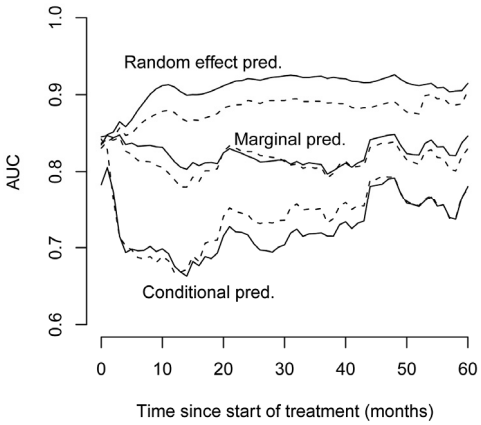


**Figure 4.** Dutch PBC Study. Evolution of the area under the ROC curve over time for different types of prediction methods based on mixed models with K = 1 and K = 2 mixture components. Solid line: K=2, dashed line: K=1.

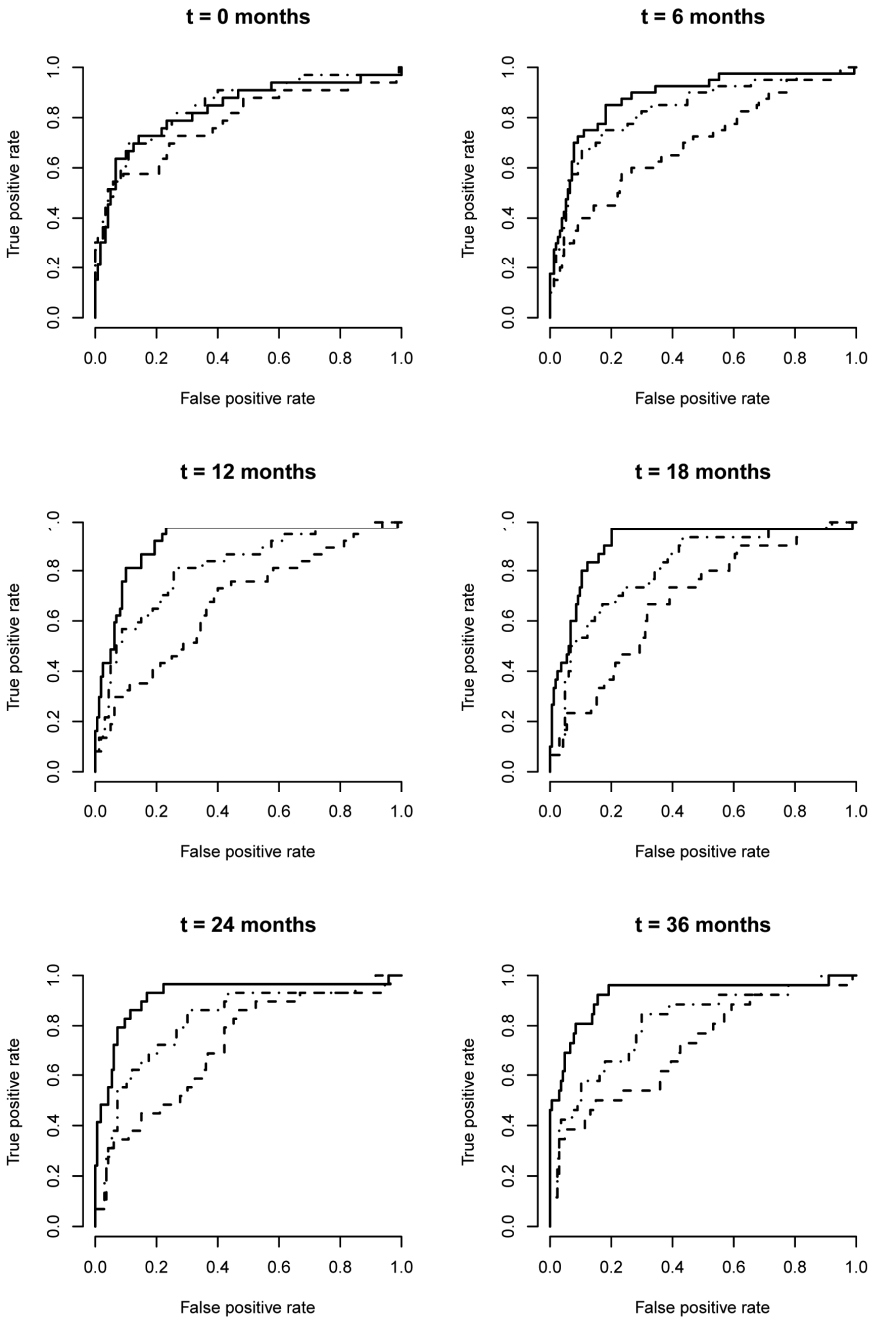


**Figure 5.** Dutch PBC Study. Receiver operating curves of the prediction at t = 0, 6, 12, 18, 24, 36 months based on cross-validated values of Pg,i( ) from a mixed model with K = 2 mixture components. Solid line: random effect prediction, dashed line: conditional prediction, dotted-dashed line: marginal prediction.

**Table 3.** Dutch PBC Study, random effect prediction with K=1 and K=2 mixture components. Sensitivity of the prediction at t=0, 6, 12, 18, 24, 36, 48, 60 months for specificity of 0.99,0.95, 0.90.

| | Specificity | | | | | |
|---|---|---|---|---|---|---|
| | 0.99 | | 0.95 | | 0.90 | |
| | K=1 | K=2 | K=1 | K=2 | K=1 | K=2 |
| **Time** | **Sensitivity** | | | | | |
| 0 months | 0.152 | 0.212 | 0.545 | 0.515 | 0.606 | 0.667 |
| 6 months | 0.175 | 0.175 | 0.450 | 0.425 | 0.675 | 0.725 |
| 12 months | 0.081 | 0.216 | 0.541 | 0.486 | 0.649 | 0.811 |
| 18 months | 0.033 | 0.267 | 0.467 | 0.433 | 0.600 | 0.733 |
| 24 months | 0.034 | 0.414 | 0.552 | 0.552 | 0.759 | 0.828 |
| 36 months | 0.115 | 0.500 | 0.577 | 0.692 | 0.731 | 0.808 |
| 48 months | 0.381 | 0.619 | 0.619 | 0.667 | 0.667 | 0.810 |
| 60 months | 0.429 | 0.500 | 0.643 | 0.714 | 0.714 | 0.857 |

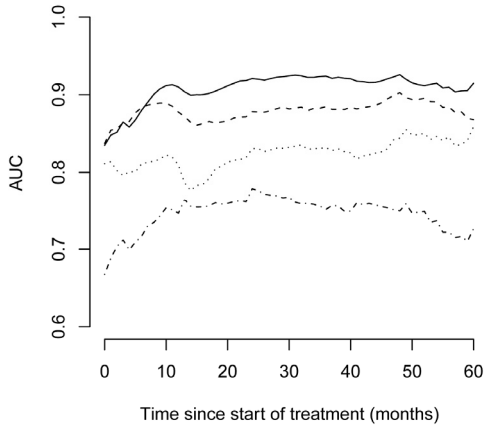


**Figure 6.** Dutch PBC Study. Evolution of the area under the ROC curve over time for random effect prediction for multivariate model (solid line) , and models with a single marker- bilirubin (dashed line), albumin (dotted line), log(alkaline phosphatase) (dotted line). In all models K=2 mixture components were used.

compares the AUC of the random effects prediction from the multivariate mixed model and the AUCs of the random effects' prediction from the single models for each marker. It is seen that prediction based on the multivariate model is better than with any of the single predictions. With this exercise, it is also seen that bilirubin provides the most information to predict the patient's status at $T = 10$ years.

Finally, we remark that in this illustration we concentrated on the evaluation of the discriminant procedure and have not paid much attention to the correct specification of the mean structure of the underlying mixed models. In fact, we let random

effects correct the mean structure to get the patient specific profile. Nevertheless, a further improvement of the mean structure may lead to a further improvement of the discriminant procedure. Further, we have also fitted models with $K = 3$ mixture components and compared the resulting discriminant rule to these described above. However, the solution with $K = 3$ performed worse than the above procedures based on $K = 1$ or $K = 2$ implying that the structure of the models with $K = 3$ is already overparametrized. Note that with $K = 3$, the dimension of the parameter space increases with $1 + 6 + 21 = 28$ in each prognostic group.

## Discussion

In this paper, we have generalized the discriminant analysis of multivariate longitudinal profiles by assuming a normal mixture in the random effects distribution in the mixed model. The application of our approach to the PBC Dutch Study data showed some improvements compared to the methodology based on mixed models with normal random effects. Due to the fact that the normal mixture serves as a semi-parametric model for the unknown random effects distribution, the first obvious question is how to choose $K$, the number of mixture components. In general, models with a different number of components can be fitted and then compared by means of a suitable measure of model complexity and fit like the deviance information criterion (DIC, Spiegelhalter et al.[25]), or the penalized expected deviance (PED, Plummer[27]). Alternatively, posterior distributions of deviances under different models can be compared (Aitkin, Liu and Chadwick[28]). Nevertheless, when discrimination is of primary interest and a training data set is available then it is preferable to choose the optimal model by evaluating the resulting discrimination rule by, e.g. means of cross-validation, as was done in Section 'Application to PBC data'.

In our specification of the MLMM, we assumed that the errors $\varepsilon_{i,r,j}$ ($i = 1, \ldots, N,\ r = 1, \ldots, R,\ j = 1, \ldots, n_{i,r}$) are independent and hence the markers $Y_{i,r,j}$ are conditionally independent given the random effects. Hence, one can further generalize the proposed model by using a more general covariance structure for the vectors of errors $\boldsymbol{\varepsilon}_{i,r}$ ($i = 1, \ldots, N,\ r = 1, \ldots, R$ as was done, e.g., by Shah, Laird and Schoenfeld[29] or Morrell et al.[7] With such generalization, the results of Section 'Application to PBC data' can even improve. Further, it is certainly possible to relax the normality assumption on random effects in several other directions than was done in this paper. For example, a multivariate t-distribution (see, e.g., Pinheiro, Liu and Wu[30]) or a mixture of multivariate t-distributions (Lin, Lee and Ni[31]) for

the distribution of random effects would make the mixed model more robust against possible outliers.

In Section 'Application to PBC data', we defined the prognostic groups by a status at a pre-specified time, i.e. $T = 10$ years. In other words, our prognostic groups are determined by the fact whether a particular event (serious disease progression) happened or not by time $T$. With our discriminant procedure, we have only attempted to predict the probability that the time to the event is below or above a pre-specified $T$. By using the methods for joint modeling of longitudinal and time-to-event data (see, e.g., Tsiatis and Davidian[32]), the mixed model for markers could be combined with a model for times-to-event and a more detailed picture could be obtained. Alternatively, one could classify the patients over time as being in one of pre-defined states (e.g., healthy/diseased in a stable state/serious disease progression) and then the model transition probabilities from one state to another as functions of markers and other covariates. This can be achieved by hidden Markov models (see, e.g., Jackson and Sharples[33]).

Finally, besides discriminant analysis, clustering of the longitudinal profiles in situations when a training data set is not available received considerable attention in the literature over the last decade, see, e.g., De la Cruz-Mesía, Quintana and Marshall[34]. Note that our approach allows directly for clustering as the procedure described in Verbeke and Lesaffre[10].

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# Appendix

We briefly show here how to run the MCMC sampling and perform subsequent discrimination with the PBC data in R using the extended version of the R package `mixAK` (Komárek[14]). It is assumed that the training data for both groups are stored in `data.frame`s called `Data0` and `Data1` whose structure is depicted in Table 4. Note that it is not necessary that at each visit, measurements of all three markers have been taken. For example, for patient `id`=1, the bilirubin value at his last visit at time 119.69 is unknown. Nevertheless, the values of albumin and alkaline phosphatase obtained at this visit are still used in the estimation of the mixed model. The MCMC algorithm to obtain a sample from the posterior distribution of model parameters in Group 0 in a model with $K = 2$ mixture components (specified within the argument `prior.b`) is run using the following code. The sampled values and additional information are stored in an object `mod0`.

```
> library("mixAK")
> mod0 <- GLMM_MCMC(y = Data0[, c("bili", "albu", "lap")],
     dist = c("gaussian", "gaussian", "gaussian"),
     id = Data0[, "id"],
     x = list(bili = Data0[, c("age", "dosis")],
              albu = Data0[, c("age", "dosis")],
              lap  = Data0[, c("age", "dosis")]),
     z = list(bili = Data0[, "time"],
              albu = Data0[, "time"],
              lap  = Data0[, "time"]),
     random.intercept = c(bili = TRUE, albu = TRUE, lap = TRUE),
     prior.b = list(Kmax = 2),
     nMCMC = c(burn = 5000, keep = 10000, thin = 10, info = 500))
```

Basic summary statistics of the posterior distribution can be seen (output not shown) with

```
> print(mod0)
```

Similarly, the sample from the posterior distribution of model parameters in Group 1 can be obtained and stored in an object `mod1`.

Further suppose that the values of the observed longitudinal markers and related covariates for new patients are stored in a `data.frame` `DataNew` which has the same structure as shown in Table 4. The values of $\mathcal{P}_{g,i}(\tau)$ for $i \in$ group of new patients

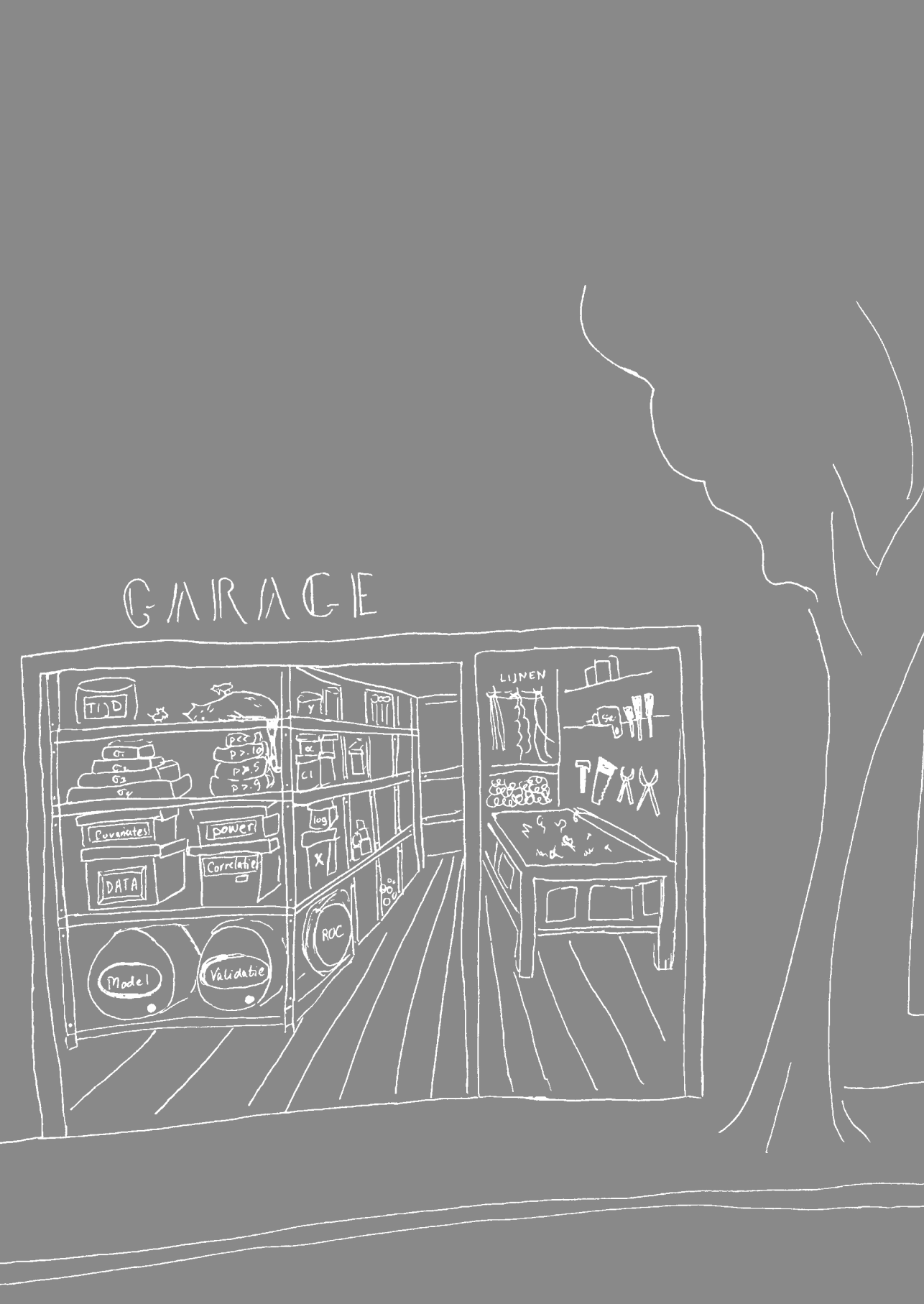
**Table 4.** Structure of the data.frame with the data to be used in R: id is identification of patient, time is time of the visit in months, age is age at baseline, dosis is dosis of UDCA medication, bili, albu, ap are obtained values of longitudinal markers.

| id | time | age | dosis | bili | albu | ap |
|---|---|---|---|---|---|---|
| 1 | 0.00 | 52.38 | 600 | 0.64 | 1.22 | 3.24 |
| 1 | 1.84 | 52.38 | 600 | 0.50 | 1.14 | 1.69 |
| . | . | . | . | . | . | . |
| . | . | . | . | . | . | . |
| . | . | . | . | . | . | . |
| 1 | 119.69 | 52.38 | 1200 | NA | 1.26 | 1.25 |
| 2 | 0.00 | 52.72 | 600 | 4.14 | 1.19 | 9.91 |
| . | . | . | . | . | . | . |
| . | . | . | . | . | . | . |
| . | . | . | . | . | . | . |

and $\tau$ corresponding to visit times of these new patients (given in column `time` of the `data.frame` `DataNew`) are computed using:

```
clust <- GLMM_longitDA(mod = list(mod0, mod1),
     w.prior = c(0.8, 0.2),
     y = DataNew[, c("bili", "albu", "lap")],
     id = DataNew[, "id"],
     time = DataNew[, "time"],
     xz.common = TRUE,
     x = list(bili = DataNew[, c("age", "dosis")],
              albu = DataNew[, c("age", "dosis")],
              lap  = DataNew[, c("age", "dosis")]),
     z = list(bili = DataNew[, "time"],
              albu = DataNew[, "time"],
              lap  = DataNew[, "time"]))
```

Note that for each patient and each time $\tau$ when $\mathcal{P}_{g,i}(\tau)$ is computed, the above mentioned code considers the whole history of a particular patient by time $\tau$ by checking the variables `id` and `time`. The resulting object `clust` is a `list` with components named `ident` (identification information), `marg`, `cond`, `ranef` (matrices with $G$ columns containing computed values of $\mathcal{P}_{g,i}$ for the three discrimination approaches described in this paper). For more details, see documentation of the package `mixAK`.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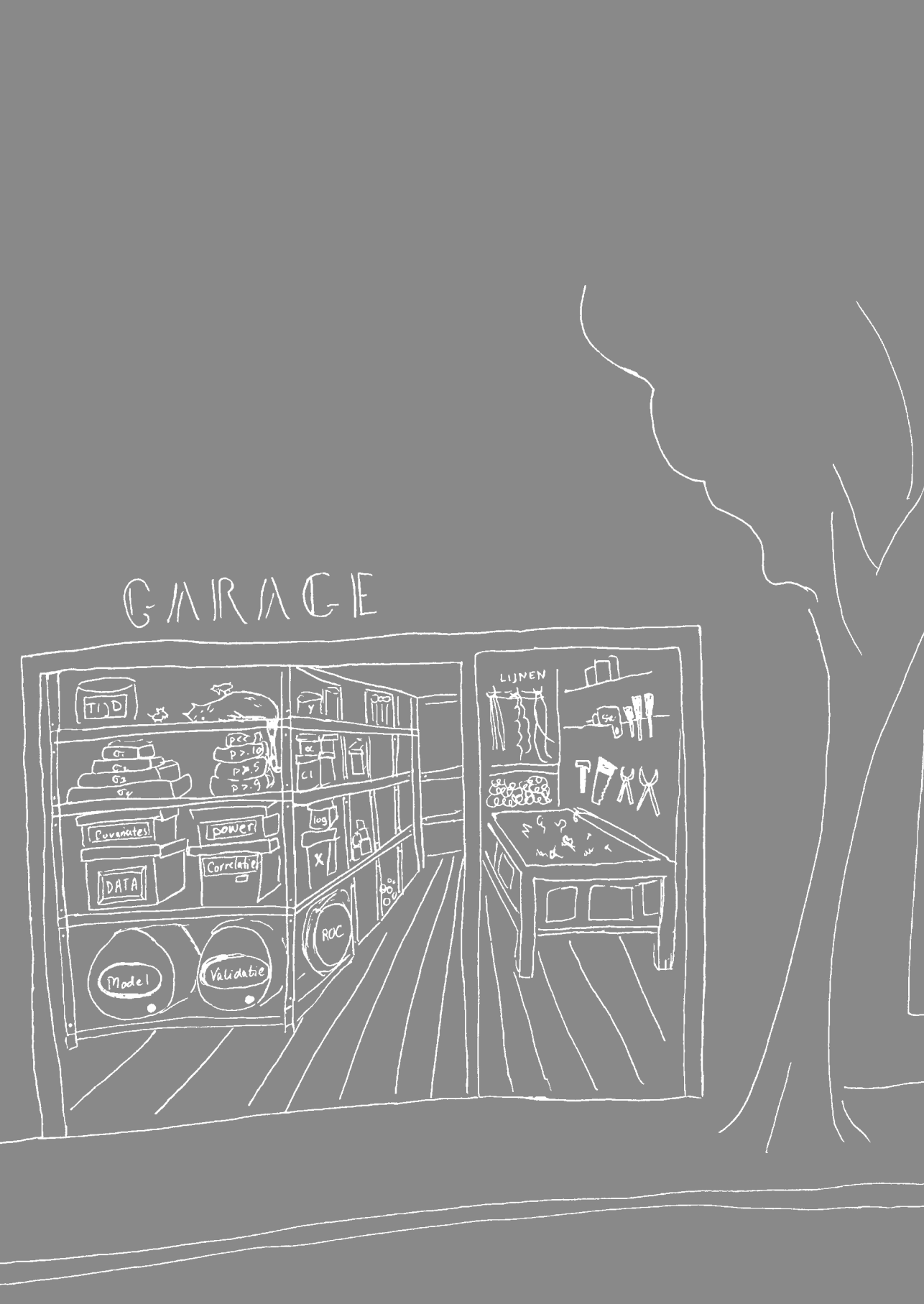
# CHAPTER 4

Statistical models of early treatment effects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# CHAPTER 4.1

## Viral dynamics during and after entecavir therapy in patients with chronic hepatitis B


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## ABSTRACT

Nucleoside analogues inhibit hepatitis B virus (HBV) replication. Entecavir, a new guanine nucleoside, has also been shown to reduce covalently closed circular DNA (cccDNA) to undetectable levels in woodchucks chronically infected with hepatitis virus. Mathematical description of changes in viral load during and after therapy may help to understand the several events that take place during nucleoside analogue treatment.

Ten chronic hepatitis B patients were evaluated with a mathematical model during and after withdrawal of four doses of entecavir. Blood was drawn for HBV DNA measurement at frequent intervals. Non-linear modelling was used to fit individual patient data.

The median effectiveness in blocking viral production is 96% (n = 10, range 87–98%). The median half-life of viral turn-over was 16 h (range 12–29 h). The median half-life of infected hepatocytes was 257 h ( = 10.7 days) (n = 9, range 112–762 h). Rebound of viral replication also followed a bi-phasic return to baseline levels.

In Conclusions decay and rebound of viral concentration during and after entecavir therapy, respectively, showed a biphasic pattern. Both can be described with a mathematical model. Data on levels of cccDNA in the liver in these patients could be helpful in supporting the parameters as calculated with the model.

# INTRODUCTION

In patients with chronic hepatitis B infection, annual clearance of HBsAg and HBeAg is estimated at 1 and 10%, respectively. HBeAg clearance, which is immune mediated, is improved by alpha interferon therapy resulting in HBeAg seroconversion in 30–40% of patients.[1–4] In particular, those patients with an immune-tolerant status, a large part of which is originating from Asian countries, do not show a favourable response to alpha interferon therapy.[5] Recently, lamivudine has been registered as a second option for the treatment of chronic hepatitis B patients. Whereas HBeAg seroconversion is a solid end-point for alpha interferon therapy, durability of HBeAg seroconversion after withdrawal of lamivudine therapy needs to be evaluated. Reports on this end-point are contradictory.[6–8] Recurrence of viral activity is attributed to the remnant covalently closed circular DNA (cccDNA) inside the nucleus of hepatocytes which is not affected by lamivudine.[9–10] Entecavir, a new guanine nucleoside analogue which is currently under investigation in phase II studies, is believed to be capable of interfering with cccDNA.[11–13] This consideration is based on observations in woodchucks chronically infected with the woodchuck hepatitis B virus (HBV). Short-term entecavir therapy markedly reduces cccDNA levels in the liver of woodchucks[14] and rebound of virus after withdrawal of therapy in woodchucks.[15] Moreover, maintenance therapy in woodchucks with once weekly dosing regimens is able to reduce cccDNA in the liver to undetectable levels.[16]

Mathematical modelling can be used to evaluate the mechanism of action of entecavir on both viral decline during and the return of virus after withdrawal of therapy. In previous modelling studies on the effect of nucleoside analogues in a chronic hepatitis B infection, it has been shown that viral decline can be divided into two phases: a first phase of turn-over of free virus and a second phase of death of infected hepatocytes.[17–18] Return of virus after withdrawal of therapy has never been evaluated in detail, but may be helpful in clarifying the mechanisms that take place during viral replication.

We therefore conducted a study to model viral decline during entecavir therapy and viral return after withdrawal of entecavir therapy.

# PATIENTS AND METHODS

## Study design

All patients who were treated in the Academic Hospital Rotterdam, The Netherlands, in a study on the safety and efficacy of entecavir were recruited for a study on viral dynamics. Patients were treated in a 1 month, double-blind, placebo-controlled dose escalating study on the safety and efficacy of entecavir (0.05, 0.1, 0.5, 1.0 mg) vs. placebo with a follow-up of 6 months. During the first month of therapy, HBV DNA was measured

at day 1 at t =0 and 8 h, at day 2 at t =24 and 32 h and at days 3, 4, 7, 10, 14, 21 and 28. Follow-up after withdrawal of therapy was documented with HBV DNA measurements at days 29, 30, 31, 32, 35, 38, 42, 49, 56 and months 3, 4, 5 and 6.

## Selection of patients

Patients were screened for eligibility on two occasions which had to be at least 2 weeks apart. Eligible patients included men and woman older than 18 years with a chronic hepatitis B infection as documented by HBsAg positivity in the serum for over 24 weeks before the start of therapy and HBV DNA of .20 Meq/ml measured with the Chiron hybridization bDNA assay. Patients had to have a compensated liver disease as documented by laboratory and clinical evaluation. Both HBeAg positive and HBeAg negative patients could be included. Previous antiviral therapy with alpha interferon, other nucleoside analogues or immunosuppressive therapy was permitted but these drugs had to be withdrawn 6 months before the start of therapy in this trial. Patients were excluded if they were co-infected with the hepatitis C virus, the hepatitis D virus or the human immunodeficiency virus (HIV), had another concomitant liver disease, had a history of pancreatitis, or had a history of any form of chronic headaches. Both male and female patients had to practice a reliable method of contraception.



## Assays

HBV DNA was quantified with a Digene Hybrid Capture tube liquid hybridization assay (calibrated on the EUROHEP standard[19]). If HBV DNA declined below 1.5 x $10^6$ geq/ml (the limit of detection of this liquid hybridization assay) during therapy, it was reassessed with the quantitative PCR (Roche, Amplicor Diagnostics, Almere, The Netherlands, calibrated on the EUROHEP standard; lower limit of detection of 1000 geq/ml). HBV polymerase mutant analysis was performed with the INNO-LiPA strip (Innogenetics, Ghent, Belgium).[20]

## Modelling of viral decline

A bi-phasic model previously applied for viral decline in chronic hepatitis C patients during alpha interferon therapy was used to describe viral decay, by means of viral dynamic parameters, during entecavir therapy.[21] In short, viral decline in this model is described by the following equation:

$V(t) = V_0 \{ A \exp(-\lambda_1 t) + (1-A) \exp(-\lambda_2 t)\}$

where $\lambda_1$ is the slope of the first phase of viral decline, $\lambda_2$ is the slope of the second phase of viral decline, $A = (\varepsilon c - \lambda_2) / (\lambda_1 - \lambda_2)$, $\lambda_{1/2} = ½ \{ (c + \delta) \pm [(c - \delta)^2 + 4 (1 - \varepsilon) (1-\eta) c\delta]^{½}\}$, V0 is the initial viral load, t is time, d is the death rate of productively infected cells, c is the clearance rate of the free virus, $\varepsilon$ is the effectiveness of entecavir in blocking virion production from infected cells, and $\eta$ is the effectiveness of entecavir in blocking de novo infection of susceptible cells.

The bi-phasic return of virus after withdrawal of therapy was described by application of a similar bi-phasic model as an inverse image of the biphasic decline in viral load during antiviral therapy:

$V(t) = V_0 / \{ A \exp(\lambda_1 t) + (1-A) \exp(-\lambda_2 t)\}$

## Statistics

Patients were fitted individually. Due to the small sample size, all patients on entecavir therapy were evaluated as one group. Non-linear modelling was used to fit both the bi-phasic model and the inverse bi-phasic model, executed in the PROC NLIN in SAS 6.12. The Mann–Whitney test was used to compare the difference between dose groups in rebound of viral replication after withdrawal of entecavir. The Kruskal–Wallis test was applied to calculate the difference in the dose of entecavir with regard to parameters of viral return. Significant difference was achieved if $p<0.05$.

# RESULTS

Eleven patients participated in the study: three patients received 0.05 mg, two 0.1 mg, two 0.5 mg, three 1.0 mg and one placebo. Ten patients on entecavir therapy were evaluated for viral dynamic parameters of viral decay. Nine patients were evaluated for re-appearance of viral replication after withdrawal from therapy; one patient was withdrawn from therapy after 1 week because of a serious adverse event and not included in the analysis for return of viral replication.

Baseline characteristics are shown in Table 1. Six patients were male and four were female. The median age of the entecavir-treated population was 35 years (range 18–63 years). Three patients were Asian and four patients were Caucasian. The majority of patients (70%) were treated with lamivudine previously. Patient 4 and patient 10 had detectable mutant virus against lamivudine at the start of entecavir therapy (YIDD and

**Table 1.** Baseline characteristics

| | Entecavir 0.05 mg (n = 3) | Entecavir 0.1 mg (n = 3) | Entecavir 0.5 mg (n = 2) | Entecavir 1.0 mg (n = 2) |
|---|---|---|---|---|
| Sex (M:F) | 1:2 | 2:1 | 1:1 | 2:0 |
| Age (years, range) | 23 (20–63) | 39 (29–51) | 38 (18–58) | 27 (19–35) |
| Race | | | | |
| Asian | 0 | 2 | 0 | 1 |
| Caucasian | 1 | 1 | 1 | 1 |
| Other | 2 | 0 | 1 | 0 |
| Previous lamivudine therapy | 3 | 3 | 0 | 1 |
| HBeAg positivity | 3 | 3 | 2 | 2 |
| HBV DNA (geq/ml) (median, range) | $3.11x10^8$–$5.35x10^9$ | $4.83x10^8$–$1.52x10^9$ | $1.68x10^9$–$1.5x10^{10}$ | $5.52x10^7$–$3.34x10^9$ |
| ALTxULN (median, range) | 1 (1–1.5) | 1.2 (1–3.4) | 1.6 (1–2.2) | 1.3 (1–1.5) |

YVDD, respectively). All patients were positive for HBeAg at the start of therapy. Median baseline HBV DNA was 1.68 x $10^9$ geq/ml (range 5.52 x $10^7$–1.50 x $10^{10}$ geq/ml), and median elevation of ALT at baseline was 1.1 x the upper limit of normal (ULN) (range 1–3.5). Viral decay was determined during 28 days of entecavir therapy (Figure 1). The median effectiveness of blocking viral replication in all ten patients on entecavir therapy was 96% (range 87–98%). Turn-over of free virus was 16 h (median; range 12–29 h, n = 10), and turn-over of infected hepatocytes was estimated to be 10.7 days (range 5.2–31.8 days, n = 9). For calculation of the viral decline during the second phase, patient 10 was excluded. This patient discontinued medication after 1 week due to a serious adverse event (Table 2). Entecavir was still capable of blocking viral replication in both patients with detectable lamivudine-induced mutant virus (effectiveness in blocking viral production of 87 and 98%, respectively).

Rebound of viral replication was followed until 6 months after withdrawal of therapy (n = 9, excluding patient 10) (Table 2, Figure 1). For mathematical description of return of viral concentration, the inverse of the bi-phasic model for viral decay describes the observed patient data accurately. The doubling time was 129 h (median; range 62–247 h) and the slope of the second phase of the rebound in viral concentration approaches zero in all patients (median 0.0016, range 20.051 to 10.03). The change from the first to the second phase of viral return was calculated to be at a median of 30 days (range 12–109 days). No relation between viral load at the moment of withdrawal of entecavir and the first and second phase of viral rebound with the dose of entecavir could be found. However, the three patients in the higher dose groups showed a more gradual return of viral replication to baseline levels than did the six patients in the lower dose groups (p = 0.024).

**Table 2.** Dynamic parameters during and after 1 month of entecavir therapy

| Patient (mg) | During entecavir therapy | | | | After withdrawal of entecavir therapy | | |
|---|---|---|---|---|---|---|---|
| | Effictiveness in blocking viral production (%) | Half-life first phase (h) | Half-life second phase (days) | Initial viral load (qeq/ml) | Doubling time of virus (h) | Duration of fast increase of viral concentration (first phase of viral return) (days) | Viral load at the end of follow-up (qeq/ml) |
| 1 (0.05) | 96 | 24 | 18.6 | $3.40x10^8$ | 95 | 23 | $2.58x10^9$ |
| 2 (0.05) | 97 | 18 | 10.7 | $2.25 x10^9$ | 129 | 40 | $1.83x10^9$ |
| 3 (0.05) | 96 | 22 | 15.2 | $6.63 x10^9$ | 120 | 30 | $4.21x10^9$ |
| 4 (0.1) | 87 | 16 | 31.8 | $1.92 x10^9$ | 140 | 16 | $1.05x10^9$ |
| 5 (0.1) | 96 | 13 | 5.2 | $5.18 x10^8$ | 62 | 14 | $2.32x10^7$ |
| 6 (0.1) | 89 | 29 | 25.0 | $7.03 x10^8$ | 102 | 12 | $3.06x10^8$ |
| 7 (0.5) | 97 | 12 | 4.7 | $1.44 x10^9$ | 247 | 101 | $2.24x10^9$ |
| 8 (0.5) | 95 | 13 | 5.6 | $8.16 x10^9$ | 244 | 89 | $5.35x10^9$ |
| 9 (1.0) | 92 | 16 | 10.7 | $3.61 x10^7$ | 230 | 109 | $8.33x10^8$ |
| 10 (1.0) | 98 | 17 | NA* | $3.04 x10^9$ | NA* | NA* | NA* |
| Median | 96 | 16 | 10.7 | $1.92 x10^9$ | 129 (~5 days) | 30 | $1.05x10^9$ |

* Not applicable; this patient was withdrawn from therapy after 1 week

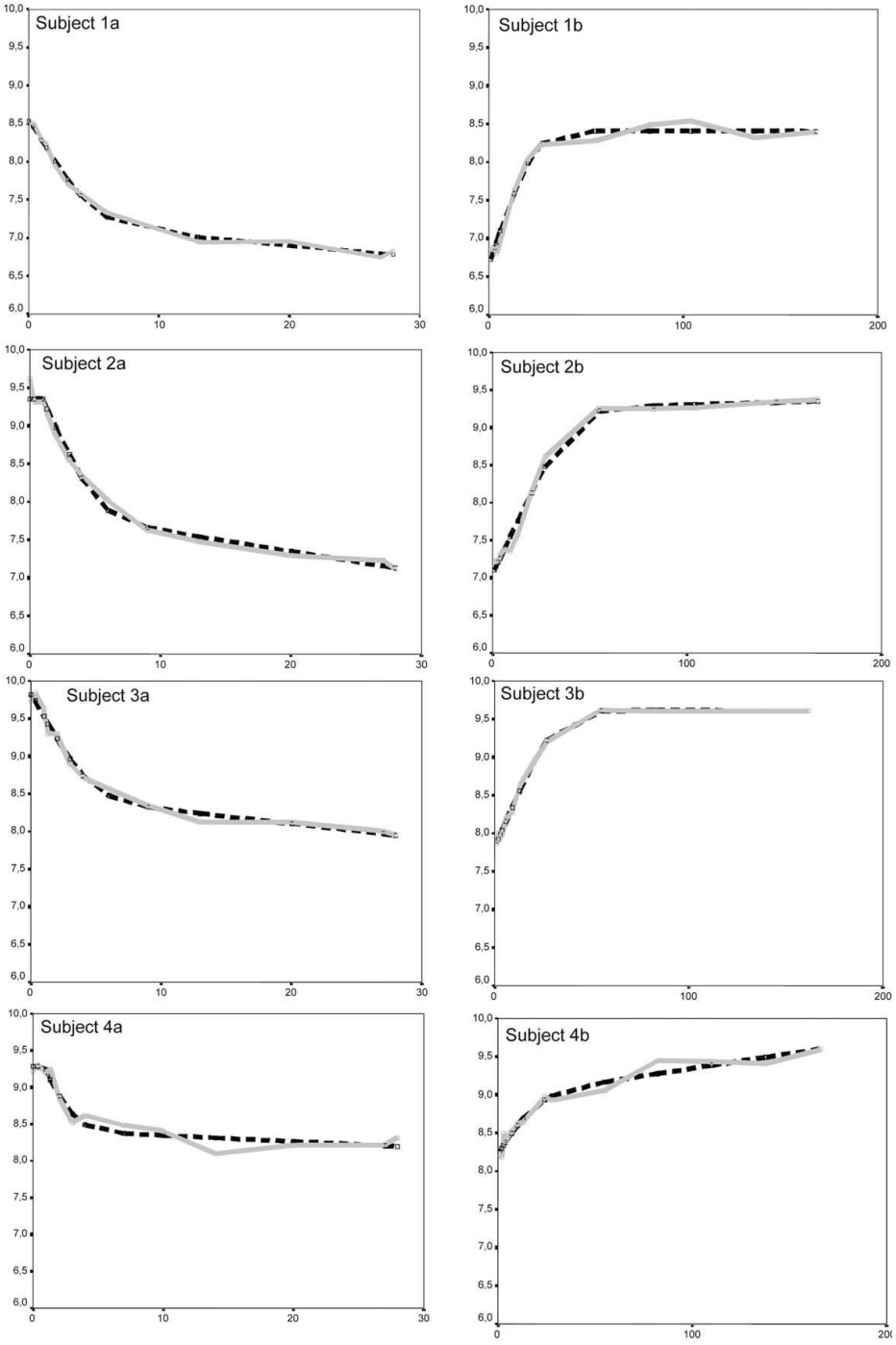
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**Figure 1.**

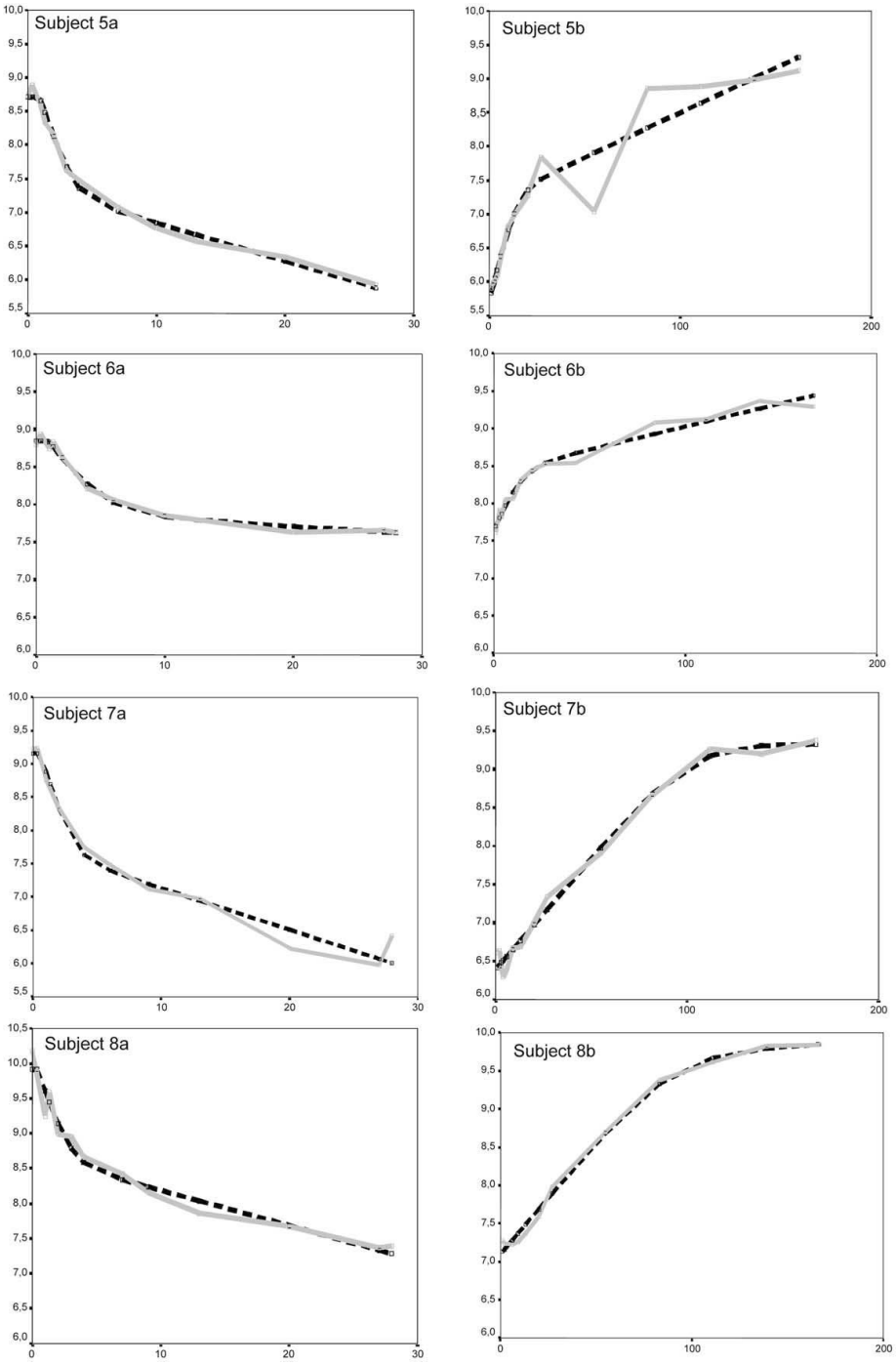
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**Figure 1.**

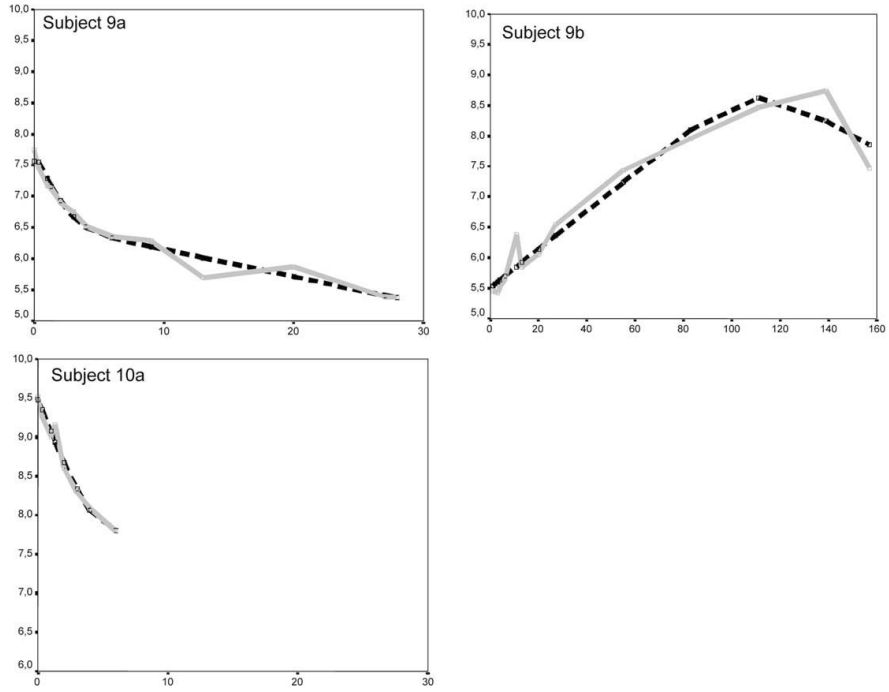


**Figure 1.** Viral decline and rebound during and after withdrawal of entecavir therapy. (A) Viral decline in ten patients. X-axis: 0–30 days; Y-axis: level of HBV DNA on a log scale. (B) Viral rebound in nine patients. X-axis: 0–200 days; Y-axis: level of HBV DNA on a log scale.



# DISCUSSION

The main action of nucleoside analogues is inhibition of viral replication through termination of the proviral chain. Lamivudine, which has been evaluated most extensively, has not been shown to have any effect on cccDNA in vitro systems.[9-10] As a result, lamivudine therapy should be continued for a long time in order to be able to eliminate the virus through cell division and death of infected cells. It has been calculated that therapy should be continued for many years to achieve complete eradication of the virus from the liver.[22] Unfortunately, indefinite prolongation of nucleoside analogue therapy will not result in complete eradication of the virus due to a cumulative incidence of viral resistance (40–60% after 2–3 years of lamivudine monotherapy).[23–25]

As a result, one should aim for a compound which does exhibit two features: interference with viral replication as well as a reduction of infected hepatocytes. Entecavir has proved to cause minimal side-effects during short-term application[26] and in vitro data imply the possible effect on cccDNA.[14] Therefore, therapy with this drug may reduce the amount of infected cells to a greater extent and in a shorter amount of time.

Although the results of this study are based on a small number of patients, the antiviral activity during short-term therapy with entecavir seems somewhat greater than during lamivudine therapy[27] and lower than during adefovir dipivoxil therapy,[17] although it should be realized that this is a head-to-head comparison and randomized studies are needed to identify the actual differences in parameters between these nucleoside analogues. Our analysis is based on four low doses of entecavir during the first study in chronic hepatitis B patients; some of these doses might have been insufficient for the optimal treatment of HBV. Moreover, the majority of our patients had previously failed lamivudine therapy which could also result in a less favour-able response to re-introduction of another antiviral agent. Entecavir did show continuing activity in patients with detectable lamivudine-induced mutant virus. Theoretically, the second phase of more gradual decline in viral concentration may be influenced by death of infected hepatocytes and turn-over of cccDNA harbouring cells. After the first 28 days of therapy, the decline of viral concentration can be either slower than, equal to, or faster than observed during the second phase. We do not observe a difference in the slope of the second phase between lamivudine-and entecavir-treated patients in a head-to-head comparison. We could therefore speculate that in both lamivudine and entecavir treated patients, this second phase is primarily influenced by death of infected hepatocytes. If entecavir exhibits a direct effect on cccDNA, this effect may surface only during a longer treatment period.

All nine patients who were evaluated after withdrawal of therapy showed a bi-phasic pattern with an initial fast increase of viral replication followed by a more or less steady state. This initial fast return of viral replication, which was calculated to last 30 days, could reflect the production capacity of the reservoir of hepatocytes that is still infected with HBV, as well as infection of non-infected hepatocytes leading to a larger productivity. The part of the cccDNA pool which is not affected by entecavir can be used as a template from which the virus can re-initiate replication once the inhibitor has been removed. This implies that a larger pool of still infected hepatocytes could result in a faster return of viral replication to baseline level. The second phase of this model represents a steady state of viral production, counteracted by turn-over of free virus and infected hepatocytes.

Patients who were treated with the two higher doses of entecavir (0.5 and 1.0 mg) showed a more gradual increase in HBV DNA to baseline levels than those patients who were treated with lower doses, even though viral load was suppressed to the same extent in all dose groups. Slower return of viral replication in the high dosed groups may therefore be due to a smaller remnant pool of infected hepatocytes and not to the extent of viral

suppression in serum. The latter explanation has previously been used as an explanation for the slower return of viral replication after withdrawal of lamivudine therapy.[28]
In conclusion, these data show that both decay of viral concentration as well as rebound of hepatitis B viral concentration can be fitted with a mathematical model. Entecavir is effective in patients infected with wildtype and variant HBV. In the future, data on the actual amount of cccDNA in the liver of these entecavir-treated patients could be helpful in supporting the outcome of the parameter estimates.

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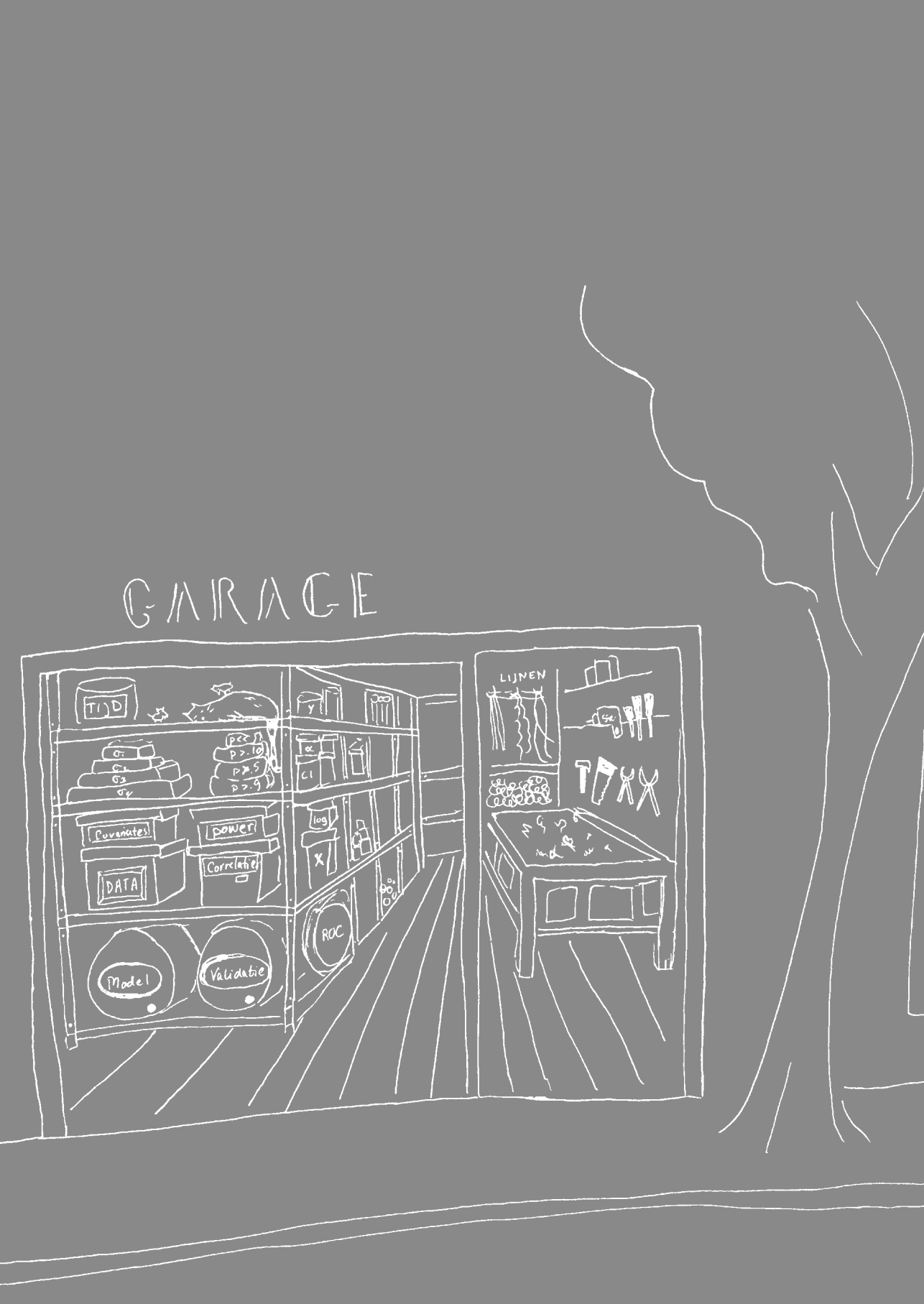
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# CHAPTER 4.2

## Viral dynamics during tenofovir therapy in patients infected with lamivudine-resistant hepatitis B virus mutants

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## ABSTRACT

Tenofovir, an antihuman immunodeficiency virus (HIV) drug, has activity against lamivudine-resistant hepatitis B virus (HBV) mutants. To describe the efficacy of tenofovir in patients with lamivudine-resistant hepatitis B we applied two investigative approaches based on mathematical models of viral dynamics: the individual nonlinear fitting and the mixed-effect group fitting approaches.

Eleven chronic HBV patients on lamivudine for a median of 176 weeks (range: 72–382) with YMDD mutation-related HBV-DNA breakthrough received 'add-on' tenofovir 300 mg once-daily, while maintaining their existing therapy. Sequential sera were taken at day 1 (t = 0 and t = 8 h), days 2, 4, 7, 10, 14, 21, 28 and every 4 weeks thereafter, and HBV-DNA levels were assessed using a validated quantitative polymerase chain reaction (PCR) assay.

Median baseline log HBV-DNA was 8.62 (range: 6.48  9.76 log HBV-DNA). Tenofovir treatment resulted in a mean (±SD) log HBV-DNA decline of 1.37 ± 0.51 in the first phase, 2.54 ± 0.91 after 4 weeks, and 4.95 ± 0.90 log HBV-DNA after 24 weeks. The median effectiveness of blocking viral replication in the individual fit model was 93% (range: 73–99) for $\eta$=0 and 93% (range: 59–99) for $\eta$=1. There was only a small difference between the efficacy parameter '$\varepsilon$' of the individual nonlinear fitting and mixed-effect group fitting on the biphasic exponential model.

These data show that tenofovir has good efficacy in blocking viral replication in HBV patients with lamivudine induced drug-resistant HBV mutants, but effectiveness varies greatly among individuals. Both models can be used to describe viral decay during tenofovir therapy.

# INTRODUCTION

Treatment of hepatitis B virus (HBV) infection with standard interferon-α produces a durable response in one-fifth to one-third of patients but has undesirable side-effects and must be administered subcutaneously three times per week.[1–3] Although lamivudine treatment also produces a modest response rate with few side-effects, prolonged treatment is often necessary to prevent relapse on cessation of therapy, and continuous treatment can lead to the development of lamivudine resistance.[4–5] Phenotypic lamivudine resistance, with the emergence of YMDD drug-resistant mutants in the polymerase gene of the HBV, leads to an increase in serum HBV-DNA levels. This suggests that there is a clinical need for new antiviral agents that adequately inhibit DNA-polymerase activity, both in wild type and in mutant virus populations.

The search for drug-resistant mutants is usually initiated after an increase in serum HBV-DNA load has been observed.[6] Studies have shown that the lamivudine mutations are localized in two major domains of the reverse transcriptase (RT) region of the polymerase gene.[7–8] Analyses of the YMDD region of the C-domain of the polymerase gene have shown that, in the case of resistance, methionine (rtM204) is replaced either by valine (rtM204V), isoleucine (rtM204I) or serine (rtM204S). The valine (rtM204V) variant is, in most cases, accompanied by another mutation (leucine to methionine; rtL180M) in the B-domain.[9] A mixture of YMDD variants can exist in one individual.

Tenofovir disoproxil fumarate, an acyclic nucleotide analogue RT inhibitor, appears to be effective against the YMDD drug-resistant mutant population. In vitro studies, tenofovir demonstrated a combination of low cytotoxicity and antiviral efficacy. It was equally effective at inhibiting wild-type HBV-DNA replication and at inhibiting DNA replication in the YMDD variant, rtM180V.[10] Clinical studies investigating the effect of tenofovir on HBV replication have shown that it has significant activity against lamivudineresistant mutants both in chronic HBV patients and in human immunodeficiency virus (HIV)/HBV co-infected patients.[11–17]

Mathematical modelling provides a tool for evaluating the effect of antiviral therapy. It can provide insight into the speed and variability in patterns of viral decay, which may be useful in the design of future treatment strategies. The decay curve of HBV during therapy with nucleoside analogues exhibits a biphasic decline during the first 4 weeks of treatment. Analysis of these viral kinetics can be used to calculate both the effectiveness of therapy in inhibiting viral production as well as the clearance of cells infected with HBV. We have used two previously published models to describe viral decline during treatment in chronic hepatitis B patients and investigate the viral dynamics of HBV replication after the addition of tenofovir to lamivudine therapy.[18–19]

# PATIENTS AND METHODS

## Patients

Eleven chronic hepatitis B patients [all liver-biopsy proven or serum hepatitis B surface antigen (HBsAg)-positive for at least 6 months] with breakthrough HBV-DNA on lamivudine therapy received tenofovir 300 mg once daily while maintaining their existing therapy, which included lamivudine. Five of these patients were co-infected with HIV. Sequential sera, taken at day 1 (at t = 0 and t = 8 h), days 2, 4, 7, 10, 14, 21, 28 and every 4 weeks thereafter, were quantitatively assessed for HBV-DNA. The presence of YMDD mutants was determined at t = 0 and t = 28 days.

## Virological measurements

The HBV-DNA was isolated using the MagnaPure LC isolation station (Roche Applied Science, Penzberg, Germany) with a modified protocol HBV-02 in which the proteinase K digestion occurred first.[20] HBV-DNA serum levels were quantitatively assessed using the HBV-DNA TaqMan assay and calibrated using EUROHEP HBV-DNA standards.[21] The TaqMan assay enabled accurate quantitative determination to levels of 1000 copies/mL.[20]

At days 1 and 28, HBV polymerase mutant analysis was performed on HBV-DNA using a Line Probe assay (INNO-LiPA HBV DR; Innogenetics N.V., Ghent, Belgium).[22] Where the INNO-LiPA assay was indeterminate, sequence analysis was used. A selected genome region of the polymerase gene was amplified and sequenced with particular primers described earlier.[23]

## Models for viral dynamics during the first 4 weeks of treatment

Mathematical modelling of viral decline was previously described by Neumann et al.[18] for hepatitis C and Nowak et al. and Tsiang et al.[24–25] for hepatitis B. We have used Neumann's biphasic-exponential model to describe the viral decay during the first 28 days of treatment in our patients:

$$V(t) = V_0 \{A \exp[-\lambda_1 t] + (1 - A) \exp[-\lambda_2 t]\}$$

where, $V_0$ = initial viral load; $\lambda_1$ = slope of the first phase of viral decline; $\lambda_2$ = slope of the second phase of viral decline; $A = (\varepsilon c - \lambda_2)/(\lambda_1 - \lambda_2)$; $\lambda_{1,2} = 1/2\{(c + \delta) \pm [(c - \delta)^2 + 4(1 - \varepsilon)(1 - \eta) c\delta]^{1/2}\}$; t = time; $\delta$ = death rate of productively infected cells; c = clearance rate of free virus; $\varepsilon$ = effectiveness of tenofovir in blocking virion production in infected cells;

$\eta$ = effectiveness of tenofovir in blocking the de novo infection of uninfected cells. The first-phase decline reflects the clearance rate of free virus from plasma; the second-phase decline reflects the death rate of productively infected cells.

We used two different approaches to describe viral decay: individual nonlinear fitting and mixed-effect group fitting. Mixed modelling implies a group-wise analysis while each patient retains his or her own subject-specific decline by introducing random effects on all parameters. All variables as well as all patient data are related; based on these data, group effects can be derived and compared. In the group fit approach, the random effect of $\lambda 1$ was set to zero, because of lack of variation between individuals. This indicates that $\lambda 1$ is stable and therefore justifies the choice of a fixed $\lambda 1$.

The nonlinear modelling approach, which was used to fit the biphasic model, was conducted in the NLINMIX macro in SAS 8.02.

Neumann et al.[18] assumed that $\eta = 0$ (there was no block of de novo infection of uninfected cells), while Tsiang et al.[24] and Nowak et al.[25] assumed that $\eta = 1$ (there was a complete block of infection of uninfected cells). If $\eta = 1$, then $\lambda_2 = \delta$, and A reflects antiviral efficacy. We have explored both assumptions in both models (individual vs group fit) as a possibility and we report the mathematical efficacy for all four situations.

## Models of viral dynamics during the first 24 weeks of treatment

Validated models of HBV viral kinetics are available only for the first 4 weeks of therapy and are unavailable for later viral kinetics. The viral kinetics patterns have been classified according to the definitions used by Neumann et al. for describing HBV-DNA early kinetics in chronic hepatitis B patients treated with adefovir dipivoxil.[26] We modified these existing definitions to describe the first 24 weeks of kinetics, taking into account the availability of frequent quantitative HBV-DNA measurements in the first 4 weeks of tenofovir treatment. First, we investigated viral decay in the first week (first-phase), then we examined decay in the following 23 weeks (second-phase), which we further divided into two periods (up to 4 weeks, days 8–28 and up to 24 weeks, days 29–168).

Definitions of viral kinetic patterns in the first-phase (days 1–7):

1 Rapid (R): decline of =1 log
2 Slow (S): decline between 0.5 and 1 log
3 Flat (F): decline of <0.5 log

Definitions of viral kinetic patterns in the second-phase (days 8–28 and 29–168):

1 Rapid: decline of >1 log/4 weeks

2 Slow: decline between 0.2 and 1 log/4 weeks over 23 weeks
3 Flat: decline of <0.2 log/4 weeks over 23 weeks
4 Beyond detection (BD):
HBV-DNA below the level of detection (<1000 copies/mL)
5 Rebound (Rebound): a transient (only one time-point) increase of >1 log

The Wilcoxon signed rank test was used to assess change in log HBV-DNA from baseline. Factors with a P-value <0.05 were considered significant.

# RESULTS

## Patient demographics

Eleven patients were evaluated for viral dynamics. In 10 patients, viral decay was evaluated at the time-points noted. In one patient, data were only available for the first 10 days and then the patient was lost to follow up. Patient characteristics at baseline are described in Table 1. Six patients were Asian and five were Caucasian.



**Table 1** Patient characteristics at baseline

| Patient | Sex | Age (years) | Type of infection | Duration of lamivudine (weeks) | HBV-DNA (gEq/mL) | HBeAg status | YMDD variant | ALT* (IU/L) |
|---|---|---|---|---|---|---|---|---|
| A | M | 53 | HIV/HBV | 382 | $4.1 \cdot 10^8$ | Positive | YVDD | 165 |
| B | M | 39 | HIV/HBV | 282 | $5.8 \cdot 10^9$ | Positive | YVDD | 98 |
| C | M | 36 | HIV/HBV | 166 | $3.0 \cdot 10^6$ | Positive | YVDD | 53 |
| D | M | 40 | HIV/HBV | 91 | $4.8 \cdot 10^9$ | Positive | YVDD | 46 |
| E | M | 36 | HIV/HBV | 313 | $6.1 \cdot 10^6$ | Positive | YVDD/YIDD | 46 |
| F | M | 28 | HBV | 162 | $1.5 \cdot 10^9$ | Positive | YVDD | 37 |
| G | M | 26 | HBV | 72 | $4.1 \cdot 10^8$ | Positive | YVDD | 44 |
| H | M | 32 | HBV | 164 | $5.9 \cdot 10^7$ | Negative | YVDD | 781 |
| I | M | 41 | HBV | 274 | $4.2 \cdot 10^8$ | Positive | YVDD | 121 |
| J | F | 26 | HBV | 178 | $1.6 \cdot 10^8$ | Positive | YSDD | 14 |
| K | F | 26 | HBV | 176 | $4.3 \cdot 10^7$ | Positive | YIDD | 55 |

*Upper limit of normal formales = 41; upper limit of normal for females = 31.
HIV, human immunodeficiency virus; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase.

## HBV-DNA levels

Mean (±SD) baseline log HBV-DNA was 8.31 ± 1.07 (median 8.62; range: 6.48 9.76). The use of tenofovir resulted in a mean log HBV-DNA decline of 1.37 ± 0.51 in the first-phase, 2.54 ± 0.91 (median 2.34; range: 1.33  4.02) after 4 weeks of tenofovir treatment and a mean decline of 4.95 ± 0.90 log HBV-DNA (median 5.05; range: 3.64 5.94) after 24 weeks

of treatment. The decline in HBV-DNA was significant at the time-points noted (P = 0.003 for the change from baseline to the transition between the first-and the second-phase, P = 0.005 for the change from baseline to 4 weeks and P = 0.005 for the change from baseline to 24 weeks). In five patients, treatment achieved HBV-DNA levels below the level of 1000 copies/mL. One patient had loss of hepatitis B e antigen (HBeAg), without seroconversion to anti-HBe.

## Lamivudine resistance and transaminase levels

The HBV polymerase mutant analyses at day 28 showed the presence of baseline mutations in nine patients; patient F showed a mixed population of YVDD and YMDD variants. In one patient, the level of serum alanine aminotransferase (ALT) was >1.1 Upper Limit Normal (ULN) after 24 weeks of treatment with tenofovir. In this patient, the ALT level after 24 weeks of treatment was higher than pretreatment ALT levels.

## Safety and tolerability

Tenofovir disoproxil fumarate was generally well-tolerated; none of the patients had abnormal renal function (data available for 10 patients) or phosphorous levels (n = 8).

## Models of viral dynamics

Estimates of the parameters of efficacy, based on the biphasic model with individual nonlinear fitting and mixed-effect group fitting, are shown in Table 2. The median effectiveness of blocking viral replication in the individual fit was 93% (range: 73–99) for $\eta = 0$ and 93% (range: 59–99) for $\eta = 1$. The half-life of free virus was 21.18 h (median; range: 16.23–47.34), the half-life of infected hepatocytes was 5.77 days (median; range: 3.06–33.24) when assessed by the individual fit. Similarly, with the group fit, the half-life of free virus was 21.54 h and the half-life of infected hepatocytes was 5.24 days.

On treatment with tenofovir, distinct patterns of response were observed. All patients showed a similar biphasic decline pattern in the first 4 weeks of treatment (Fig. 1a–k). The combined data for the group fit for the data set clearly demonstrates biphasic decline pattern (Fig. 2).

In nine patients, the first-phase response was rapid (Fig. 1a–k). Six of the nine patients followed this rapid first-phase by an initially rapid second-phase. However, in this study, the rate of viral decay in the first week of treatment did not appear to determine the rate in the following phase (the next 3 weeks of treatment). Some patients had rapid decay

**Table 2.** Parameter estimates based on the biphasic model with individual nonlinear fitting and mixed-effect group fitting.

| | Individual fit median (range) | Group fit median (range, subject-specific fit) |
|---|---|---|
| Ln (initial viral load) | 19.43 (15.25-22.50) | 19.17 (15.65-22.87) |
| Clearance rate of free virus | 0.79 (0.35-1.02) | 0.76 (0.76-0.77) |
| $\delta$ (if $\eta$= 0) | 0.12 (0.02-0.23) | 0.14 (0.073-0.22) |
| $\delta$ (if $\eta$ = 1) | 0.11 (0.02-0.20) | 0.13 (0.058-0.21) |
| $\varepsilon$ (if $\eta$ = 0) | 0.93 (0.73-0.99) | 0.94 (0.81-0.97) |
| $\varepsilon$ (if $\eta$ = 1) | 0.92 (0.59-0.99) | 0.91 (0.77-0.95) |
| Half-life (ln2/c) | 21.18 h (16.23-47.34) | 21.54 h (21.74-21.97) |
| Half-life (ln2/d) (if $\eta$ = 0)) | 5.77 days (3.06-33.24) | 5.24 days (3.16-9.52) |

$\delta$, death rate of productively infected cells; $\varepsilon$, effectiveness of tenofovir in blocking virion production in infected cells; $\eta$, effectiveness of tenofovir in blocking the *de novo* infection of uninfected cells.



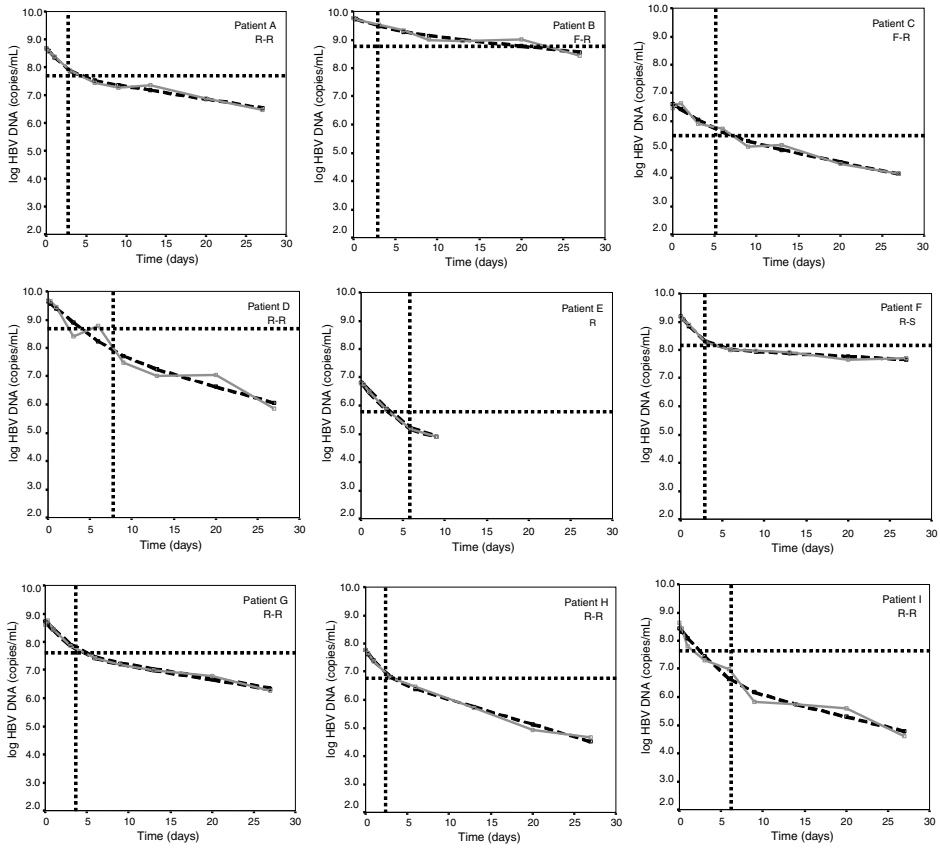


**Figure 1.**

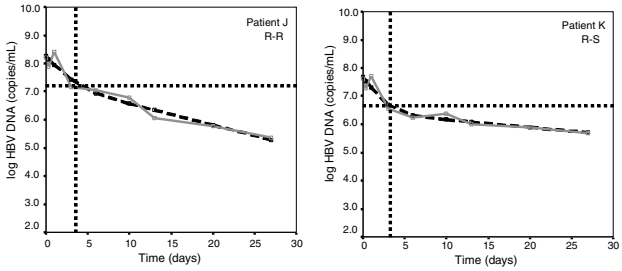


**Figure 1.** Viral decline during the first 4 weeks of tenofovir therapy in 11 lamivudine-resistant patients. Each individual patient could be fitted using the biphasic model. The vertical straight dotted line represents the time of transition from the first-to the second-phase for each individual patient. When describing the different patterns of viral decay, the first week represents the first-phase; the second-phase begins at day 8. In this study, the first-phase was categorized to one of the three patterns according to the rate of hepatitis B virus (HBV)-DNA decline in the first 7 days: rapid (R) with a decline of =1 log, slow (S) for a decline between 0.5 and 1 log, or flat (F) for a decline of <0.5 log. The horizontal straight dotted line is placed 1 log below the initial viral load of the patient. During the second-phase, the pattern of viral decay was also categorized according to the rate of decline. The following definitions were used: R for rapid declines of >1 log HBV-DNA over the 4-week period, S for slow declines of between 0.2 and 1 log HBV-DNA over 4 weeks, F for flat declines of <0.2 log HBV-DNA during the 4 weeks, and beyond detection (BD) when the HBV-DNA level fell below the level of detection. Straight grey line: observed HBV-DNA data. Black large dotted line: fitted HBV-DNA data.
log HBV DNA (copies/mL)

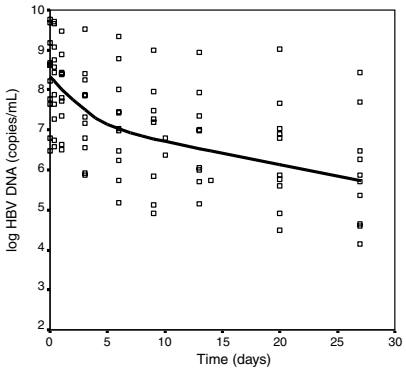


**Figure 2.** Overall viral decline during the first 4 weeks of tenofovir therapy in 11 lamivudine-resistant patients, by mixed-effect group fitting. Open squares: observed hepatitis B virus (HBV)-DNA data. Straight black line: group fit HBV-DNA data.

in the first-phase, followed by slow decay in the second-phase (patients F and K), others had 'flat' viral decay in the first-phase followed by rapid decay in the second-phase (patients B and C).

After the initial rapid decline in viral load of the first-phase, the response in the following weeks was highly variable between the individual patients (Fig. 3a–j). The variability of

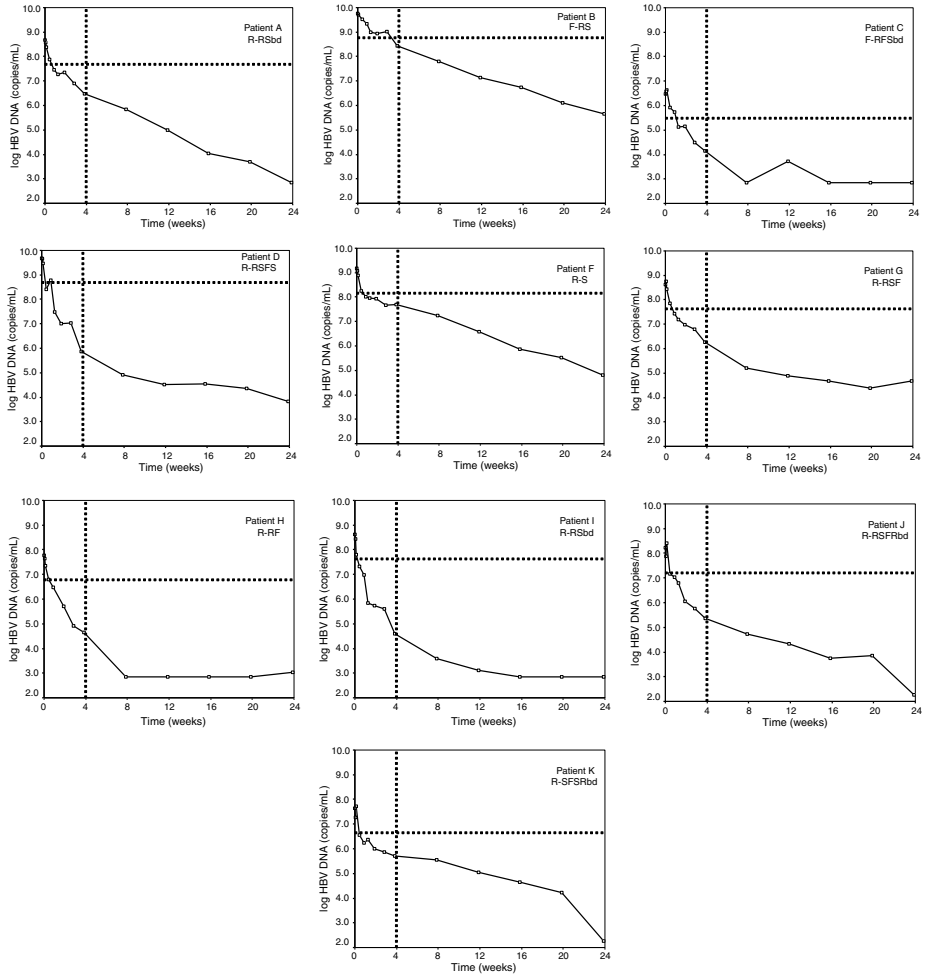


**Figure 3.** Viral decline during 24 weeks of tenofovir therapy in 11 lamivudine-resistant patients who continued lamivudine treatment. The first-phase was categorized to one of three patterns, according to the rate of hepatitis B virus (HBV)-DNA decline in the first 4 weeks of treatment: R for rapid declines of ¼1 log, S for slow declines between 0.5 and 1 log or F for flat declines of <0.5 log. In the second-phase, viral decay patterns were categorized according to the rate of decay over 4-weekly segments of the following 23-week period: R for rapid declines of >1 log/4 weeks over the 23-week period, S for slow declines of between 0.2 and 1 log/4 weeks over 23 weeks, F for declines of <0.2 log/4 weeks over 23 weeks, beyond detection (BD) for patients where the level of HBV-DNA fell below the level of detection, and Rebound for where patients experienced a transient (only one time-point) increase of >1 log. Because of lack of data, patient E is not described. The horizontal straight dotted line is placed 1 log below the initial viral load of the patient; the vertical straight dotted line is placed at the 4-week time-point.

response appeared to be due to the existence of complex multiphasic decay patterns in some patients. Therefore, as for the early viral kinetics, the rate of viral decay in the first 4 weeks of treatment did not appear to determine the rate of viral decay in the following phase (the next 20 weeks of treatment).

# DISCUSSION

This study provides the first detailed viral kinetic data following tenofovir treatment of patients with drug-resistant HBV mutants. Previous modelling studies in chronic-infected HBV patients have demonstrated that a biphasic pattern of viral response occurs during the first 4 weeks of antiviral treatment with nucleoside analogues.[19] In the study reported here, the viral decay in patients treated with tenofovir showed a similar biphasic pattern of early viral response. However, after 4 weeks, treatment response was less predictable and a variety of patterns of viral decay were observed, a finding that is similar to the patterns of viral decay previously found following adefovir treatment.[18]

The effectiveness of tenofovir, as calculated with the individual fit, was 0.926. This is much lower than the reported efficacy of adefovir in treatment-naive patients, which was 0.993 ± 0.008 (mean ± SE; median: 0.996),[24] but was comparable with the efficacy of 0.928 (±0.015 SE) for lamivudine.[19]

Also of note is that the duration of the first-phase is <7 days, which means that the transition from the first-to the second-phase occurs in the first week. This is significant because Tsiang et al.[24] conducted the first HBV-DNA measurement after 1 week, while we measured on day 1 (t = 0 and t = 8 h), and on days 2, 4 and 7 during the first week.

Another difference in methodology is that Tsiang et al.[24] calculated the efficacy over a period of 12 weeks. By contrast, we calculated the efficacy, as in the study of Wolters et al.[19], over 28 days.

To determine the effects of different sampling frequency and of sampling over different periods of time, we applied individual nonlinear fitting to the biphasic exponential model to describe the viral decay of the first 12 weeks in our tenofovir-treated patients. HBV-DNA measurements were used from weeks 1, 2, 4, 8 and 12. A median efficacy of 0.996 was found (if $\eta = 0$) and a median efficacy of 0.995 was found, if $\eta = 1$. These values are comparable with the values found in adefovir-treated patients (0.993) and show that outcome of the calculation depends on a combination of the sampling frequency and duration of the sampling period.

Tsiang et al.[24] assumed that generation of new productively infected cells during therapy is completely inhibited ($\eta = 1$). By contrast, Neumann et al.[18] set $\eta = 0$, based on the hypothesis that the major effect of standard interferon-$\alpha$ is to block viral production or

release. Although we cannot rule out a possible effect of tenofovir on blocking infection ($\eta$ varying between 0 and 100%), the viral kinetic data for tenofovir could be fitted assuming both effects ($\eta = 1$ and $\eta = 0$).

The HBV-DNA levels can fluctuate even in untreated patients. However, pretreatment levels of HBV-DNA in the patients in our study were similar to t = 0. This suggests that the rapid decrease in HBV-DNA levels after t = 0 could be attributed to treatment with tenofovir, and was not the consequence of a spontaneous decrease.

In our study, 4 weeks after addition of tenofovir to the treatment regimen, a mean log HBV-DNA decline of 2.54 ± 0.91 (median 2.34; range: 1.33 4.02) could be observed. This is comparable with the 2.42 log HBV-DNA decline found in a study with tenofovir in five HIV/HBV coinfected resistant patients,[12] and is higher than the 0.9 log HBV-DNA decline in a study performed in 12 HIV/ HBV co-infected patients who were treated with tenofovir.[11]

Taken together, the data which showed a mean log HBV-DNA decline of 4.95 ± 0.90 log HBV-DNA (median 5.05; range: 3.64 5.94) after 24 weeks of tenofovir in our study and the data which showed a mean log decline of 3.4 copies/ mL after 24 weeks treatment with adefovir in lamivudineresistant HIV/HBV co-infected patients,[27] suggests that tenofovir may have an important role to play in patients who experience breakthrough viraemia on lamivudine therapy.



The second-phase decline in viral levels reflects the death rate of productively infected cells. The death of these cells is thought to require a host immune response. A possible marker of the strength of host immune response is the level of ALT, which is an indicator of the level of cell damage and death.

Previously, authors have observed a positive correlation between the decay rate of infected cells and the pretreatment ALT level among chronic HBV patients who were treated with lamivudine therapy.[25] Another study, which analysed the influence of lamivudine dose and baseline ALT on the viral dynamics of the HBV, confirmed that higher baseline ALT levels were significantly related to the slope of the second-phase of viral decay.[28]

Nevertheless, in another study, in which patients were treated with either lamivudine monotherapy or with a combination of lamivudine and famciclovir, the investigators found no association between the slope of the second-phase and baseline ALT.[29] This is in agreement with our study, in which kinetic parameters $\lambda_1$, $\lambda_2$ and e were not associated with the pretreatment ALT levels. This discrepancy with some other studies may be explained by the selection of patients in our study, which included patients with only moderate elevation of ALT. We speculate that the ALT levels were too low to produce a detectable association with the slope of viral decay.

Our data demonstrate that direct comparison of the efficacies given by different mathematical models is not always possible. As we have demonstrated, variations between the models with respect to sampling frequencies and duration of follow up result in different outcomes. In addition, our data show that tenofovir is capable of effectively blocking viral replication in patients with lamivudine-induced mutant viruses in both HBV and HBV/ HIV co-infected patients. However, for effective treatment of patients, the first goal should be to totally inactivate disease by completely blocking virion production. In terms of modelling this will mean an antiviral efficacy 'ε' equivalent to 1. Our results show that, in patients with lamivudine-induced drug-resistant mutants, we can reach an efficacy of 0.99. Therefore, despite the drug having an excellent effect, our data also show some low-grade viral replication remains. We suggest that the residual replication may present a risk for genotypic succession during tenofovir therapy.

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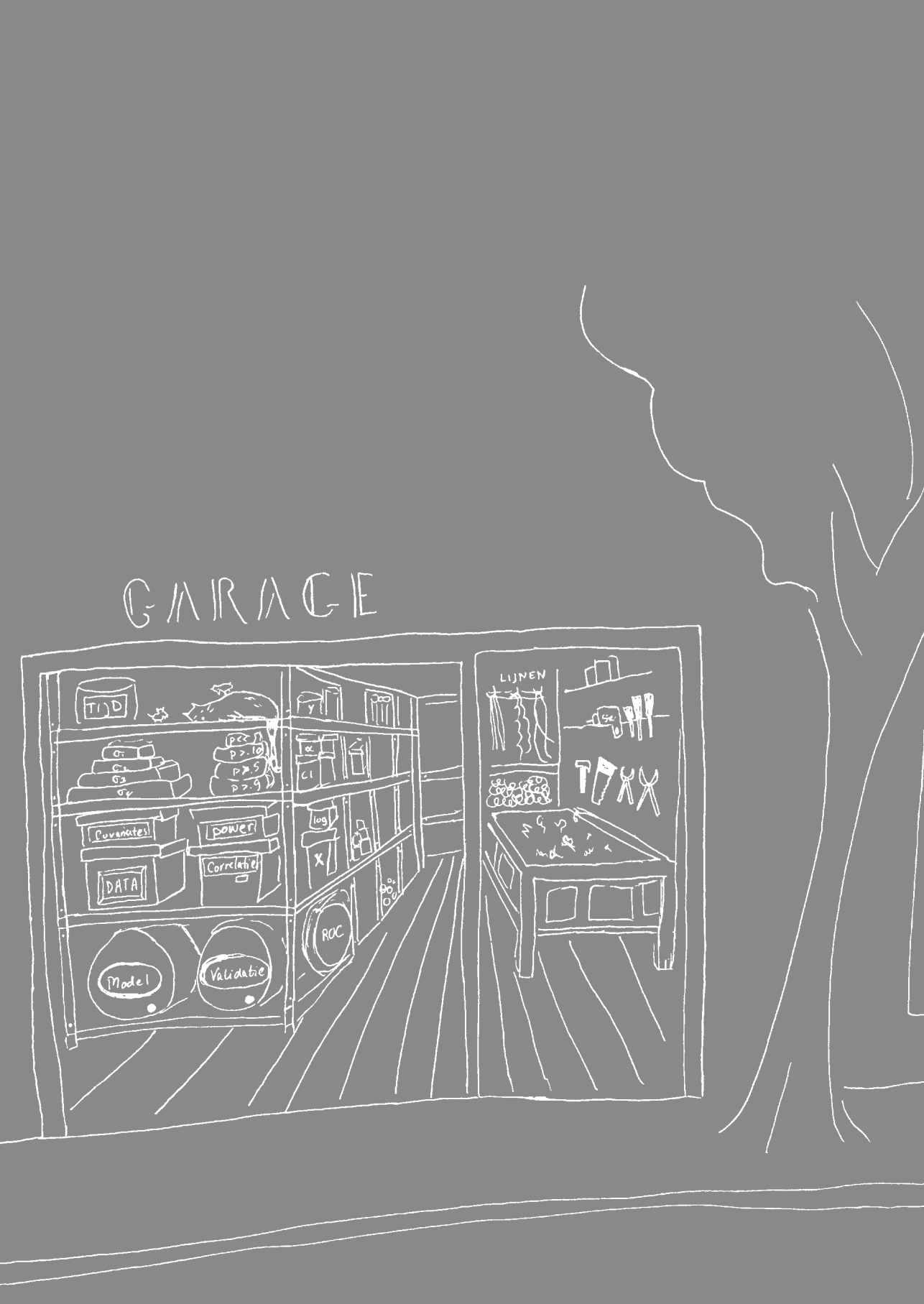
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# CHAPTER 4.3

# Modelling of early viral kinetics and pegylated interferon-alpha-2b pharmacokinetics in patients with HBeAg-positive chronic hepatitis B

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## ABSTRACT

Treatment with pegylated interferon (PEG-IFN) α-2b is effective for HBeAg-positive chronic hepatitis B although its mechanism of action remains unclear. HBeAg loss is achieved in 36% of patients after one year of PEG-IFN α-2b treatment and combination therapy with lamivudine is not superior to PEG-IFN α-2b monotherapy. In this study, we analyzed early pharmaco- and viral kinetics in patients treated for 52 weeks with PEG-IFN α-2b with or without lamivudine. After 4 weeks of treatment, there was a median viral decline of 2.94 $\log_{10}$ copies/ml in those treated with PEG-IFN α-2b and lamivudine and only 0.45 $\log_{10}$ copies/ml in the PEG-IFN α-2b monotherapy group. Peak IFN levels were reached approximately one day after administration and subsequently declined exponentially consistent with a viral load rebound near to baseline levels at the end of the dosing period in most patients receiving PEG-IFN α-2b monotherapy. Modelling of pharmaco- and viral kinetics data in this group revealed that viral load was minimal 3.6 days after PEG-IFN α-2b administration, the mean maximal and mean antiviral effectiveness was 70% and 48% with a mean infected cell loss rate of 0.07 per day, while no significant biphasic decline was observed. We conclude that PEG-IFN α-2b induces a sustained response in a considerable number of patients despite limited direct antiviral activity during the first weeks of antiviral therapy.

# INTRODUCTION

Patients with HBeAg-positive chronic hepatitis B often have high levels of circulating virus and immune responses directed against the virus cause inflammation which in turn may lead to cirrhosis and hepatocellular carcinoma.[1] Although treatment with nucleos(t)ide analogues, like lamivudine, adefovir and entecavir, is effective for viral load reduction, long-term treatment is often necessary and carries the risk of viral resistance.[2-4] Using interferon therapy, a durable treatment response can be achieved in 35-45% of HBeAg-positive and HBeAg-negative chronic hepatitis B patients.

Pegylated interferons induce HBeAg seroconversion in approximately one third of HBeAg-positive patients.[5-9] In a recent trial, a durable loss of HBeAg was achieved in 36% of patients after a 52 week course of PEG-IFN α-2b treatment with a 26 week follow-up period.[6] The decline in viral load during PEG-IFN α-2b therapy was not uniform and different patterns of viral decline could be recognized both during treatment and follow-up.[10] Remarkably, a marked viral decline between weeks 4 and 32 of treatment resulted in the highest rate of HBeAg-loss.[10] In general, there was only minimal decline in viral load in the first month of treatment. Until now, no viral kinetics data are available during PEG-IFN treatment in HBeAg-positive chronic hepatitis B.[11] Therefore, we analyzed the relation between viral kinetics and pharmacokinetics of PEG-IFN α-2b in HBeAg-positive chronic hepatitis B. To our knowledge, this is the first analysis fitting data from both pharmacokinetics and viral kinetics during treatment in patients with chronic hepatitis B.

# MATERIAL AND METHODS

## Patients

A total of 96 patients who participated in an international multicenter randomized double-blinded study reported previously[6], underwent frequent blood sampling in the first month of therapy. Eligible patients were men and women over 16 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over six months, and positive serum HBV DNA levels. All patients were HBeAg-positive and had ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization. Patients received PEG-IFN α-2b 100 µg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN α-2b was reduced to 50 µg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and followed for 6 months post-treatment.

## HBV DNA quantification

HBV DNA levels were measured frequently during the first month of therapy (at days 0, 1, 2, 3, 4, 7, 14, 21 and 28) in a randomly selected subgroup of 38 patients (19 patients in the monotherapy group and 19 patients in the combination therapy group) using an in-house developed TaqMan real-time PCR test with a dynamic range of $4x10^2$-$10^{10}$ copies/ml.[12] Monthly HBV DNA measurements were available in all 96 patients.

## PEG-IFN α-2b concentration

PEG-IFN α-2b serum concentrations were also measured at days 0, 1, 2, 3, 4, 7, 14, 21 and 28 using a quantitative sandwich interferon enzyme-linked immuno-sorbent assay (ELISA, Bender MedSystems Diagnostics GmbH, Vienna, Austria) in all 96 patients. Binding of (pegylated) interferon to a murine monoclonal antibody directed against interferon adsorbed onto micro wells was detected by an HRP-conjugated monoclonal anti-interferon antibody. Following 2 hours of incubation unbound complexes were removed by washing (three times) after which tetramethyl-benzidine was used to determine the amount of interferon in the sample. Absorbency was read using a spectro-photometer using 450nm as the primary wave length. Standards were prepared from diluted series of pegylated interferon in normal human serum obtained from healthy volunteers. Patient sera and standards were tested in triplicate, on the same plate. Although optical densities obtained were related to a standard of pegylated interferon, the ELISA also may detect free recombinant interferon-2b molecules and natural interferon. The detection limit of the assay is 35 pg/ml and is linear up to a concentration of 2000 pg/ml.



## Modelling of pharmacokinetics

For modelling of the pharmacokinetics of PEG-IFN α-2b we used the absorption and elimination model recently applied by Powers et al. and Talal et al. [13-14] This model describes the concentration of drug in the blood (*C*) following a single injection at time *t=0* as follows:

$$C(t) = \frac{k_a D(F/V_d)}{(k_e - k_a)}\left(e^{-k_a t} - e^{-k_e t}\right) \quad (1)$$

where *t* is the time after injection, $k_a$ is the rate of absorption, $k_e$ is the rate of elimination, *F* is the bioavailability, D is the drug dose and $V_d$ is the volume of distribution. We used

a more general model for multiple weekly injections of PEG-IFN α-2b that accounts for random variability effects between subjects. The PEG-IFN α-2b concentration in the blood for individual *i* at the time point *t* is then described as the sum of the individual contributions of each injection *d* until time t, i.e. $t_d < t$ is the injection day (i.e. $t_d$ = 0, 7, 14, 21, …,) and $D_d$ is the dose per injection d:

$$C_i(t) = \sum_{d:\, t_d < t} \frac{(FD_d)_i}{V_{di}} \frac{k_{a,i}}{(k_{e,i} - k_{a,i})} (e^{-k_{a,i}*(t-t_d)} - e^{-k_{e,i}*(t-t_d)}) \quad (2)$$

$k_{a,i}$, $k_{e,i}$ *and* $(F/V_d)_i$ consist of both a fixed-effect as well as a individual random effect parameter. Using this formula, the area under the curve (AUC) of the PEG-IFN α-2b concentration could be calculated. Furthermore, these changes in PEG-IFN α-2b concentration over time have an effect on the effectiveness of PEG-IFN in contrast to a constant effect. Assume that the effectiveness of PEG-IFN α-2b for individual *i* is given by:

$$\varepsilon_i(t) = \frac{C_i(t - t_0)^n}{IC_{50}{}^n + C_i(t - t_0)^n} \quad (3)$$

where $IC_{50}$ is the concentration at which the drug's effectiveness is half its maximum, and *n* is the Hill coefficient, a parameter that determines the steepness of the rise of the effectiveness with increasing PEG-IFN concentration, and $t_0$ is a possible time delay.[14]

## Modelling of viral kinetics

Using the pharmacodynamic efficacy model (3), the viral kinetics for the first week of PEG-IFN α-2b monotherapy can be described by a model originally applied by Nowak et al. [15] and modified by Sypsa et al.[16] and Powers et al.[13]. In our approach the constant $\varepsilon_i$ is substituted by $\varepsilon_i(t)$ in the differential equation system modelling viral kinetics:

$$\frac{d}{dt} V_i(t) = (1 - \varepsilon_i(t)) p_i \, I_i(t) - c_i \, V_i(t) \quad (4)$$

and

$$\frac{d}{dt} I_i(t) = \beta_i \, V_i(t) \, T_i(t) - \delta_i \, V_i(t) \quad (5)$$

The resulting model function $V_i(t)$ describes the viral load of individual *i* at time point *t* and depends on the virion clearance rate $c_i$ and the infected cell loss rate $\delta_i$. The total number of cells (i.e. infected target cells, $I_i$, and uninfected target cells, $T_i$) is assumed to remain constant in each individual during treatment motivated by a fast liver regeneration. As usual, the infection rate $\beta_i$ and the viral production rate $p_i$ were substituted by the other parameters assuming that they remained unchanged from the steady state situation.

## Modelling and data fitting

The PROC NLMIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) was used to fit the first month pharmacokinetic data of all 96 patients with a non-linear mixed modelling approach. The NLME procedure of R (R Foundation for Statistical Computing) yield highly comparable results (data not shown). The prediction of the PEG-IFN α-2b concentration (equation 2) and the model for effectiveness (equation 3) was thereafter incorporated in the model of the viral load as solution from equations 4 and 5 of the patients treated with PEG-IFN α-2b monotherapy. The viral load was hereafter fitted with non-linear mixed modelling with the NLME procedure of R including the ordinary differential equation solver LSODA from the ODESOLVE package in a nested way to estimate the infected cell loss rate δ, the baseline levels of viral load as well as the $IC_{50}$ levels and the time delay $t_0$. Because interindividual variation could already be modelled by baseline viral load and $IC_{50}$ levels, the other parameters were set constant between patients (fixed effects). Furthermore, relatively few data points can lead to biased estimates of the viral clearance rate *c*.[16] Therefore, we fixed *c* to 1.3 per day. Different Hill coefficients (n=1, n=2, n=3 and n=4) were checked and we used a coefficient of 1 because this gave the best results.

SPSS (version 14.0.1, SPSS Inc., Chicago, IL) was used for further data analyses. All tests for significance and resulting P values were two-sided, with a level of significance of 0.05.



# RESULTS

## Patient characteristics

Demographic and baseline characteristics of the 96 included patients in this study are shown in table 1. Forty-eight patients received PEG-IFN α-2b monotherapy; the other 48 patients received combination therapy consisting of PEG-IFN α-2b and lamivudine. There were no significant differences between the two groups with respect to ALT, viral load, age, sex, weight and race. PEG-IFN α-2b concentration was measured in all 96

patients whereas frequent HBV DNA measurements were obtained in a representative subset of 38 patients (19 in each treatment arm).

**Table 1:** Baseline characteristics

| | PEG-IFN + lamivudine (n=48) | PEG-IFN + placebo (n=48) |
|---|---|---|
| Age (years)* | 33 ± 12 | 32 ± 12 |
| Sex M/F (% male) | 32/16 (67%) | 37/11 (77%) |
| Weight (kg)* | 72 ± 16 | 71 ± 13 |
| Race (%) | | |
| Caucasian | 42 (88%) | 43 (90%) |
| Asian | 2 (4%) | 3 (6%) |
| Other | 4 (8%) | 2 (4%) |
| Genotype (%) | | |
| A | 13 (27%) | 15 (31%) |
| B | 1 (2%) | 2 (4%) |
| C | 2 (4%) | 2 (4%) |
| D | 31 (65%) | 29 (61%) |
| E | 1 (2%) | 0 (0%) |
| ALT (U/L)* | 175 ± 193 | 167 ± 130 |
| HBV DNA ($log_{10}$ copies/mL)* | 9.2 ± 1.1 | 9.3 ± 0.7 |

* Mean ± standard deviation.

## Viral kinetics

In the PEG-IFN α-2b monotherapy group (n=19), the median viral decline after one month of treatment was 0.45 $log_{10}$ copies/ml (range, -0.03 – 1.56) (Figure 1) and 0.40 $log_{10}$ copies/ml (range, -0.28 – 2.30) at week 8 of treatment. The median viral decline was 0.028 $log_{10}$ copies/mL per day (range, -0.069 – 0.165) for the first week and 0.017 $log_{10}$ copies/mL per day (range, -0.006 – 0.046) between week 1 and 4. In the first week of treatment, there was a median decline in viral load of 0.20 $log_{10}$ copies/ml (range, -0.48 – 1.15). There was an initial decline in viral load until 4 days after drug administration in all patients in the PEG-IFN α-2b monotherapy group. Thereafter there was a rebound towards the end of the week. The median slope of viral rebound at the end of the first week (day 4 to day 7) was 0.060 $log_{10}$ copies/ml per day (range, -0.117 – 0.393). There was no effect of the baseline viral load level on the amount of viral decline in the first month of treatment.

When viral decline was analyzed in the PEG-IFN α-2b and lamivudine combination therapy group (n=19) on the other hand, a median decline in viral load of 2.94 $log_{10}$ copies/ml (range, 0.55 – 5.02) after one month of treatment was observed (Figure 1). There was a viral decline of 3.43 $log_{10}$ copies/ml (range, 0.71 – 6.25) at week 8 of treatment. The median viral decline was 0.228 $log_{10}$ copies/ml per day (range, -0.037 – 0.337) for the first week of treatment and 0.055 $log_{10}$ copies/ml per day (range, 0.010 – 0.127) between

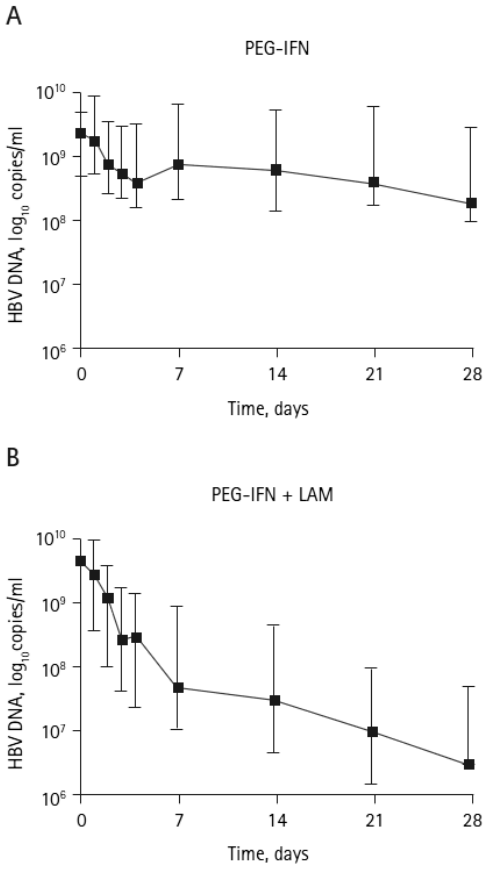


**Figure 1.** Median HBV DNA ($log_{10}$ copies/ml) in patients with HBeAg-positive chronic hepatitis B in the first month of treatment with PEG-IFN alone (A) or in combination with lamivudine (B).

week 1 and 4. All patients treated with combination therapy showed a biphasic HBV DNA decline pattern. The median decline in viral load was 1.59 $log_{10}$ copies/ml (range, -0.26 – 2.36) in the first week of treatment. The median slope of viral decline at the end of the first week (day 4 to day 7) was 0.083 $log_{10}$ copies/ml (range, -0.297 – 0.250) per day in the combination therapy group.

## Pharmacokinetics of pegylated interferon-alpha-2b

In a first attempt to understand why HBV DNA levels showed a minimal decline during the first month, we analyzed PEG-IFN α-2b levels in all 96 patients. Maximum levels of PEG-IFN α-2b concentration were reached one day after administration. Thereafter, a decline in the PEG-IFN α-2b levels was seen in all patients (Figure 2A). No significant

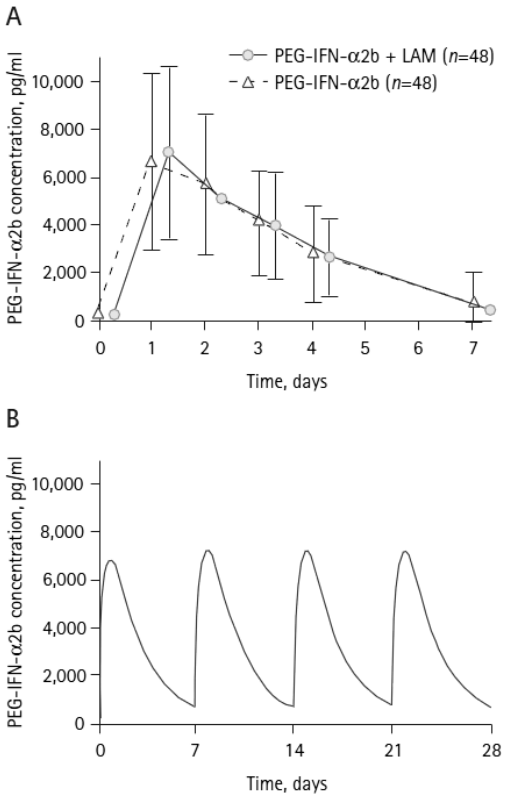


**Figure 2.** Pharmacokinetics of PEG-IFN in patients treated with PEG-IFN with or without lamivudine in the first week (A) and the modelled pharmacokinetics in the first month (B) of treatment.

differences in PEG-IFN α-2b levels between patients treated in the PEG-IFN α-2b monotherapy and the PEG-IFN α-2b plus lamivudine combination therapy group were observed. In 52 out of 96 patients (54%), the PEG-IFN α-2b concentration had returned to undetectable levels 7 days after drug administration; this was still the case in 24/96 (25%) patients at day 28, 7 days after the fourth injection. In those with detectable PEG-IFN α-2b levels at day 7 and 28, these concentrations were in general low with a mean of 1175 pg/mL and 1645 pg/mL, respectively.

The pharmacokinetics were modelled using a non-linear mixed model. The fitted non-linear mixed model resulted in a population mean of the pharmacokinetic parameters $k_a$, $k_e$ and $F/V_d$ of all 96 patients as well as an individual fit of these parameters (Figure 2B). The estimated population mean of $k_a$ was 2.363 $d^{-1}$(SE 0.461), of $k_e$ 0.420 $d^{-1}$ (SE 0.029) and of $F/V_d$ 1.023 pg/mL (SE 0.084) (Table 2 gives per patient data). The modelled interval between PEG-IFN α-2b administration and the maximum modelled drug concentration ($t_{max}$) was 0.89 day (0.71-1.24). There was a significant negative correlation between the

**Table 2:** Pharmacokinetic and viral kinetic parameters for the first week of treatment for the 19 patients treated with PEG-IFN monotherapy.

| | Pharmacokinetic parameters | | | | | | | Viral kinetic parameters[1] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | $k_e$ ($day^{-1}$) | $k_a$ ($day^{-1}$) | $F/V_d$ (pg/mL) | $t_{max}$ (days) | $C_{max}$ (pg/mL) | $EC_{50}$ | AUC (pg.wk/ mL) | $v_0$ ($log_{10}$cp/mL) | decl. wk 1 ($log_{10}$cp/mL) | $c$ ($day^{-1}$) | ε |
| 1 | 0.42 | 0.60 | 1.30 | 1.98 | 5630 | 5852 | 26415 | 9.30 | 0.20 | 0.97 | 0.49 |
| 3 | 0.48 | 2.73 | 1.80 | 0.77 | 12410 | 6485 | 35918 | 9.49 | 0.35 | 0.96 | 0.66 |
| 5 | 0.48 | 1.02 | 1.55 | 1.40 | 7940 | 6713 | 30267 | 10.04 | -0.48 | 0.90 | 0.54 |
| 8 | 0.47 | 4.47 | 1.62 | 0.56 | 12430 | 1400 | 33228 | 9.06 | 0.30 | 0.80 | 0.89 |
| 9 | 0.55 | 2.93 | 1.86 | 0.70 | 12610 | 1480 | 32823 | 8.68 | 0.34 | 0.91 | 0.89 |
| 11 | 0.46 | 0.91 | 1.43 | 1.52 | 7160 | 3782 | 28890 | 8.69 | 0.53 | 0.77 | 0.65 |
| 12 | 0.43 | 1.34 | 1.31 | 1.25 | 7630 | 3969 | 28079 | 8.36 | 0.24 | 0.87 | 0.66 |
| 22 | 0.56 | 4.29 | 1.63 | 0.54 | 12020 | 276 | 28295 | 9.57 | 1.15 | 0.97 | 0.98 |
| 24 | 0.50 | 2.07 | 1.93 | 0.91 | 12300 | 2017 | 37259 | 8.65 | 0.71 | 0.55 | 0.86 |
| 27 | 0.37 | 2.08 | 0.88 | 1.01 | 6020 | 5881 | 21372 | 9.03 | -0.28 | 1.43 | 0.50 |
| 29 | 0.55 | 1.14 | 2.43 | 1.24 | 12320 | 3717 | 42477 | 8.36 | 0.10 | 1.58 | 0.77 |
| 32 | 0.58 | 0.69 | 3.37 | 1.57 | 13500 | 9634 | 54171 | 10.26 | 0.07 | 1.09 | 0.58 |
| 39 | 0.39 | 4.43 | 0.65 | 0.60 | 5140 | 570 | 15541 | 9.30 | 0.69 | 1.05 | 0.90 |
| 41 | 0.36 | 1.09 | 0.76 | 1.52 | 4420 | 3844 | 18654 | 9.94 | -0.07 | 1.09 | 0.54 |
| 43 | 0.45 | 1.23 | 1.43 | 1.29 | 8030 | 12684 | 29735 | 9.53 | 0.11 | 1.08 | 0.39 |
| 45 | 0.37 | 1.56 | 0.87 | 1.21 | 5540 | 1078 | 21063 | 9.13 | 0.42 | 1.26 | 0.84 |
| 50 | 0.58 | 3.34 | 1.83 | 0.64 | 12660 | 5325 | 31017 | 9.91 | 0.03 | 0.89 | 0.70 |
| 51 | 0.58 | 0.71 | 3.29 | 1.56 | 13360 | 5282 | 53428 | 8.85 | -0.16 | 1.03 | 0.71 |
| 73 | 0.43 | 4.09 | 0.87 | 0.61 | 6690 | 3228 | 19105 | 10.03 | 0.17 | 0.87 | 0.67 |
| Median | 0.47 | 1.56 | 1.55 | 1.21 | 8030 | 3844 | 29735 | 9.30 | 0.20 | 0.97 | 0.67 |
| Q 25 | 0.42 | 1.02 | 0.88 | 0.64 | 6020 | 1480 | 21372 | 8.69 | 0.06 | 0.87 | 0.54 |
| Q 75 | 0.55 | 3.34 | 1.86 | 1.52 | 12430 | 5881 | 35918 | 9.91 | 0.39 | 1.09 | 0.86 |

decl. wk 1 = the decline in viral load in the first week of treatment, Q = quartile

[1]Identical estimates for all patients (fixed effects) were obtained for the pharmacokinetic time delay $t_0$ (0.9 day), the infected cell loss rate δ (0.07 per day) and the Hill coefficient (n=1).

per patient AUC of the PEG-IFN α-2b concentration for the first week of treatment and the body mass index (BMI) (p=.024) as well as a significant relation between the AUC and sex; AUC was higher in females than in males (p=.002).

### Modelling of viral kinetics and its relation to pharmacokinetics and response

Using the non-linear mixed model it was possible to fit the first month viral kinetics data in the PEG-IFN α-2b monotherapy arm (n = 19) using the results of the modelled pharmacokinetics for the first month of treatment with ε dependant on time (Figure 3, Table 2). Thus, a clear biphasic viral load decline is not observed using PEG-IFN α-2b monotherapy in HBeAg-positive chronic hepatitis B patients.

In the first week, the modelled viral load was minimal at 3.6 days (2.8-4.5) after administration of PEG-IFN α-2b. The mean and maximum estimated population antiviral effectiveness $\varepsilon_{mean}$ and $\varepsilon_{max}$ in patients receiving PEG-IFN α-2b monotherapy 48% and 70% (24-80% and 39 - 98%), respectively. The infected cell loss rate δ was estimated as 0.07 per day and the time delay of pharmacokinetics $t_0$ as 0.9 days. No clear association was found between the estimated maximum antiviral effectiveness and baseline HBV DNA levels, ALT levels, sex and BMI. Maximal effectiveness but not mean effectiveness was significantly smaller in older patients (p=0.046).

HBeAg loss at the end of follow-up was observed in 9 out of 19 patients. Despite the correlation between the AUC of the PEG-IFN α-2b concentration and BMI and sex, no significant difference was observed between the AUC in relation to treatment response (HBeAg loss at the end of follow-up) or viral decline at the end of treatment and follow-up. Furthermore, viral decline in the first month of treatment was 0.45 $\log_{10}$ copies/ml (range -0.12 – 1.56) in patients with a lower than median AUC and also 0.45 $\log_{10}$ copies/ml (range -0.03 – 1.87) in those with a higher than median AUC of the PEG-IFN α-2b concentration.

## DISCUSSION

In this study, we analyzed early pharmacokinetics and HBV viral kinetics in HBeAg-positive chronic hepatitis B patients during the first 4 weeks of treatment with PEG-IFN α-2b and used the PEG-IFN α-2b pharmacokinetics to model viral decline. We observed only a minimal decline in viral load during the first month of PEG-IFN α-2b monotherapy, without a clear biphasic pattern. Given the fact that a significant number of patients are able to control the infection after 52 week of PEG-IFN α-2b treatment, immunomodulatory

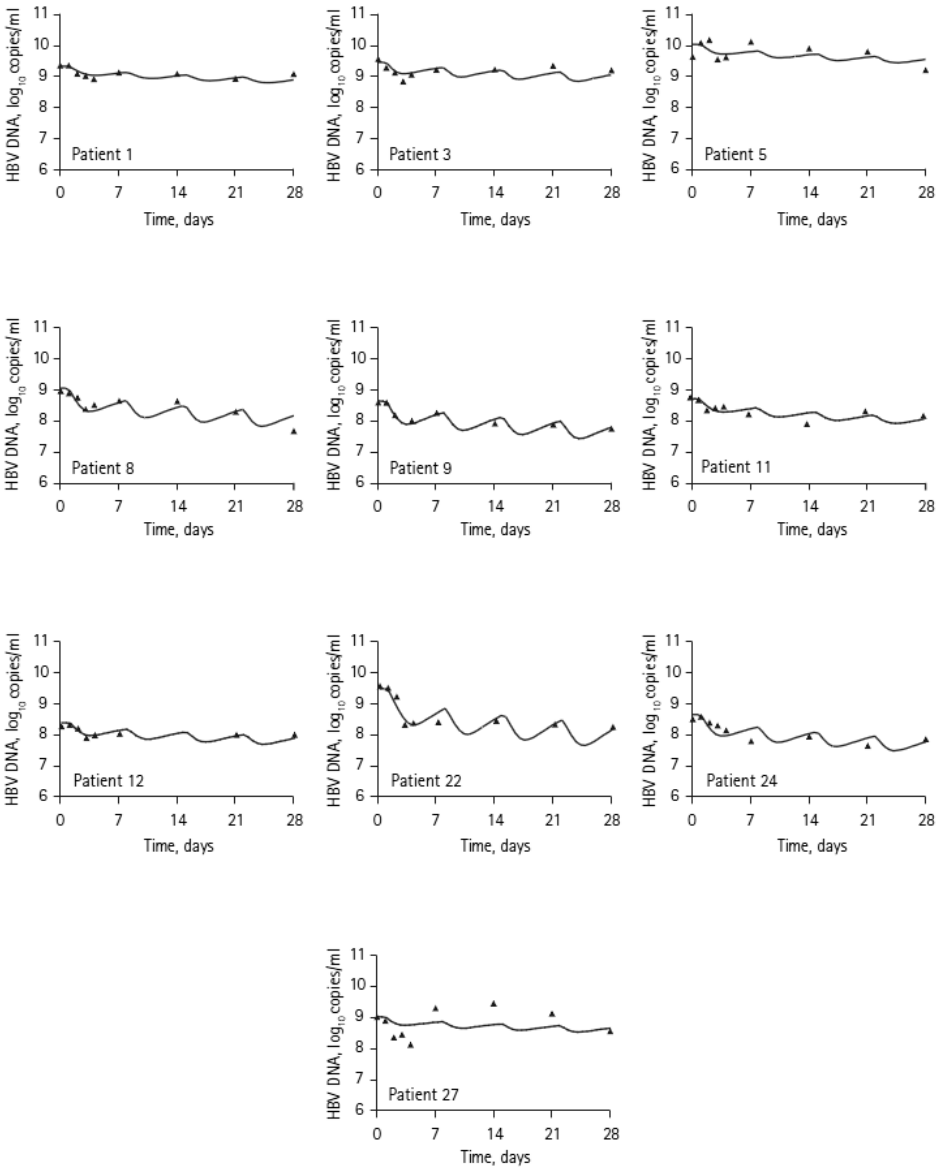


**Figure 3.**

effects rather then direct antiviral activities of PEG-IFN-2b may explain its beneficial effect.

In the first week of PEG-IFN α-2b treatment, we found highest drug concentrations one day after drug administration followed by a pronounced decline over time until the end of the week. At the end of the week, the PEG-IFN α-2b concentration returned

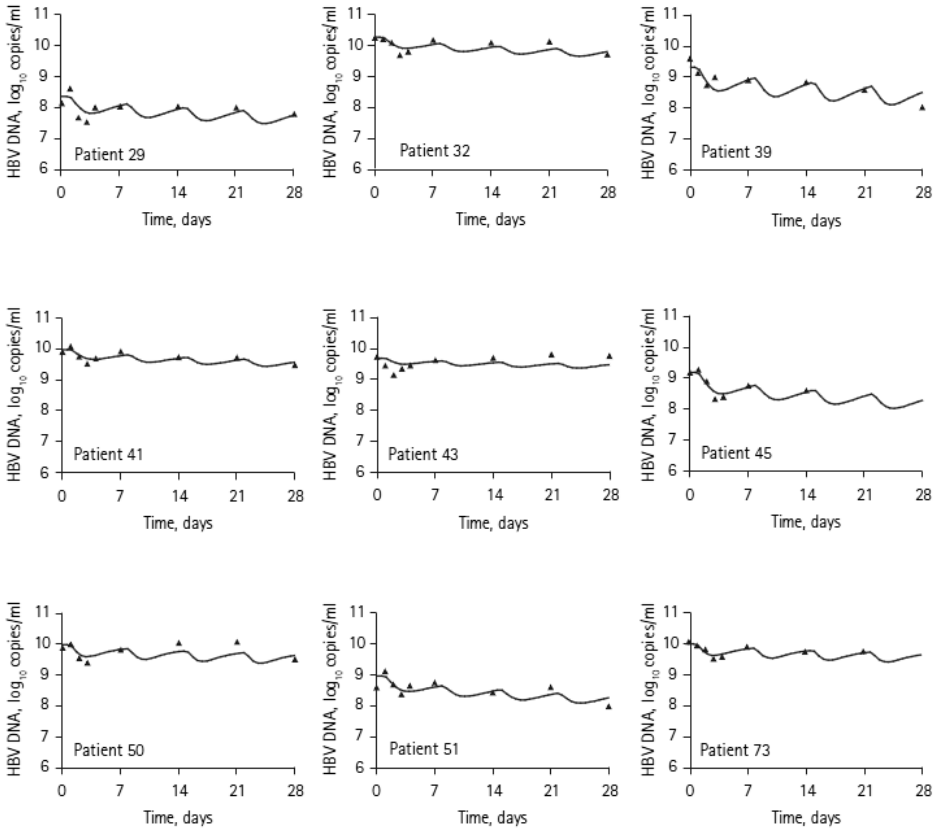


**Figure 3.** Modelled viral decline and observed viral load in all 19 patients treated with PEG-IFN monotherapy in the first month of treatment.

to undetectable levels in the majority of patients. This is in accordance with previous PEG-IFN α-2b pharmacokinetic studies in patients with chronic hepatitis C.[14, 17-19] Based on these pharmacokinetic data, one could consider twice-weekly administration of PEG-IFN α-2b. In chronic hepatitis C patients treated with twice weekly administration for 28 days, there were high PEG-IFN α-2b concentrations in the blood at all days during the week and there was no rebound in HCV-RNA at the end of the week as was seen with once weekly injections.[18] Nevertheless, despite these suboptimal pharmacokinetic characteristics for PEG-IFN α-2b, the end of treatment and follow-up results of PEG-IFN α-2b and PEG-IFN α-2a - which has a prolonged higher concentration in blood - are comparable in chronic hepatitis B.[6-7, 9]

We analyzed the pharmacokinetics during PEG-IFN α-2b therapy in all 96 patients using a model proposed by Powers et al. and Talal et al. for chronic hepatitis C infection.

[13-14] This model takes the decreasing efficacy of PEG-IFN α-2b at the end of the week into account during once-weekly administration. We observed a significant correlation between the AUC of the PEG-IFN α-2b concentration and body mass index (BMI) and a correlation between sex and the AUC of PEG-IFN α-2b. Based on these findings, weight-based PEG-IFN α-2b dosing should also be considered in the treatment of chronic hepatitis B to optimize drug availability as is the standard in hepatitis C treatment.[20-21] However, despite the influence of BMI on the pharmacokinetic constants of PEG-IFN α-2b, no clear effect of the PEG-IFN α-2b concentration was observed on treatment outcome or decline in viral load, as previously shown for PEG-IFN α-2a.[22] Furthermore, treatment of chronic hepatitis B patients with escalating doses of both PEG-IFN α-2a and α-2b did not lead to a better treatment outcome in chronic hepatitis B.[5, 16]

Next we incorporated the pharmacokinetic model for multiple weekly PEG-IFN α-2b injections proposed recently [13-14] in a combined pharmacokinetic-pharmacodynamic model. Viral kinetics were modelled using equations 3-5 We were able to use per patient PEG-IFN α-2b pharmacokinetics as well as viral kinetics data in 19 patients of the PEG-IFN α-2b monotherapy group. With this approach, it was possible to fit the viral decline during the first month of PEG-IFN α-2b monotherapy in patients with HBeAg-positive chronic hepatitis B. The maximum antiviral effectiveness of PEG-IFN α-2b monotherapy, $\varepsilon_{max}$, was 70% and this is slightly lower than the antiviral effectiveness (83%) of PEG-IFN α-2b 100/200 µg in HBeAg negative chronic hepatitis B patients in the study by Sypsa et al., probably due to the lower PEG-IFN dose given.[16] There was no clear association between the antiviral effectiveness and several baseline factors, only older patients showed a slightly reduced maximal antiviral effectiveness (p=0.046). This antiviral effectiveness is lower compared to the estimated antiviral effectiveness of approximately 92-99% for nucleos(t)ide analogues.[23-26] In the combination therapy group, viral load showed a biphasic decline pattern as a result of the addition of lamivudine. This pattern has already been extensively described in chronic hepatitis B patients treated with nucleos(t)ide analogues and therefore we did not model viral decline in the combination therapy group.[15, 24-26]

In the first week, there was a pronounced decline in viral load in the combination therapy group and after one month of treatment there was a 2.94 $\log_{10}$ copies/ml decline in viral load. In the monotherapy group, probably as a result of the decline in drug concentration associated with once-weekly administration of PEG-IFN α-2b, we observed only a minimal decline in viral load with a rise towards the end of the week as also recently reported by Sypsa et al. in HBeAg-negative chronic hepatitis B [16] Therefore, there was only a limited decrease in viral load at the end of the first week of treatment in the monotherapy group and no clear biphasic decline pattern was observed as seen during

PEG-IFN α-2a treatment in HBeAg-negative chronic hepatitis B.[27] After one month of PEG-IFN α-2b monotherapy there was still only a marginal decline of 0.45 $\log_{10}$ copies/ml in viral load. Regardless of this minimal decline in viral load early during treatment, treatment outcome was comparable in both treatment arms.[6] This emphasizes that a rapid early antiviral effect of PEG-IFN α-2b is not necessary for a sustained response 24 weeks post-treatment in HBeAg-positive chronic hepatitis B as it is in chronic hepatitis C infection. In line with these results, we previously showed that patients with a delayed rather than with an early viral load decline pattern exhibited the highest rates of HBeAg loss after PEG-IFN α-2b treatment.[10]

In conclusion, the pharmacokinetics during the first week of therapy with PEG-IFN α-2b alone showed a peak one day after the administration with a rapid decline thereafter. Concurrently, after an initial decline an increase in HBV DNA was found during the second half of the week. Using the PEG-IFN α-2b pharmacokinetic data it was possible to model the HBV viral dynamics during the first month of treatment. Despite the minimal viral decline in the first weeks of PEG-IFN α-2b treatment, a sustained HBeAg-response was achieved in a considerable proportion of patients

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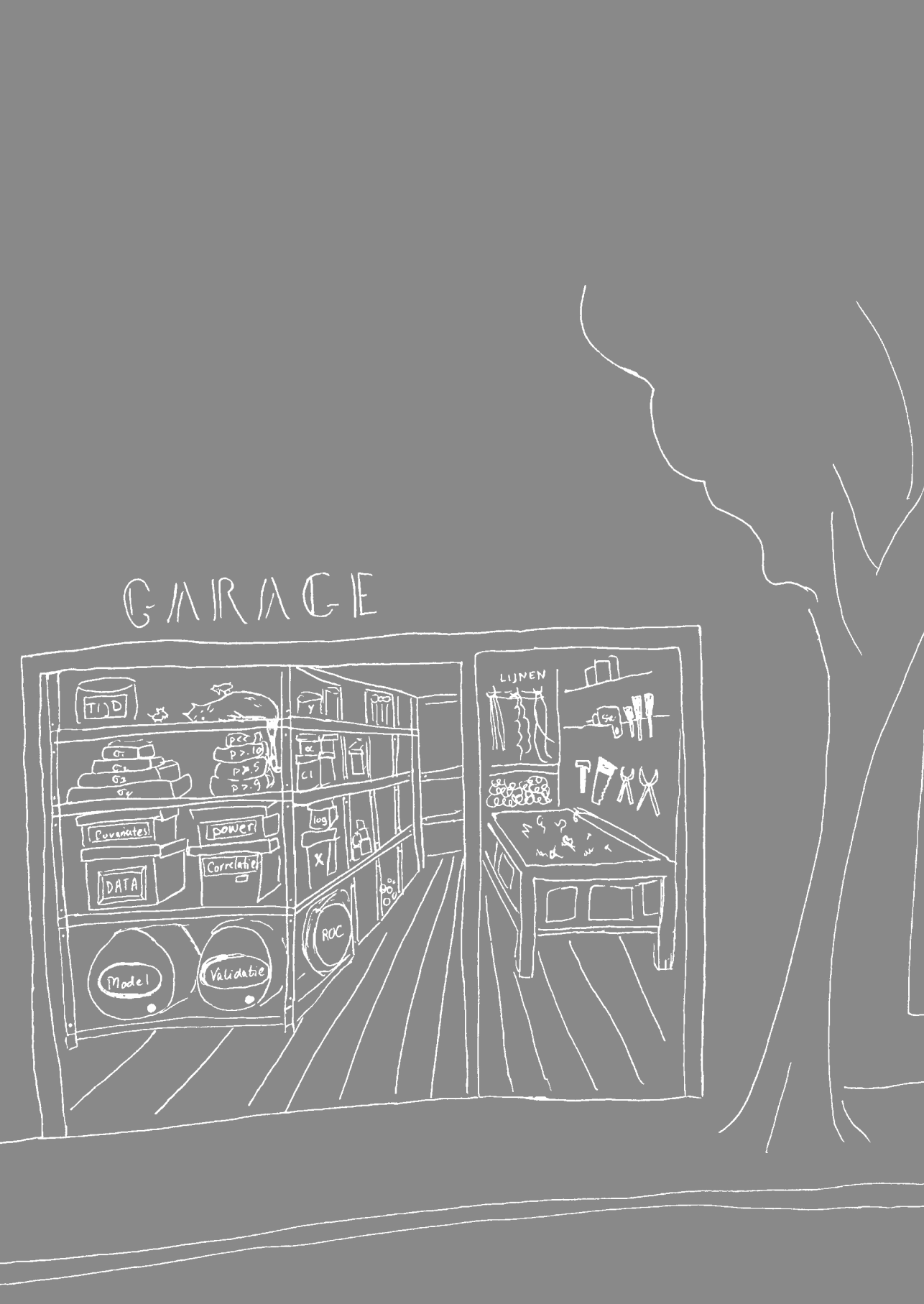
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# CHAPTER 5

Summary and conclusion

# SUMMARY AND CONCLUSION

This research focuses on development and application of statistical models to analyse the treatment effects in chronic hepatitis B and C.
Treatment options for both hepatitis B and C are expanding and there is thus a growing demand for individual first-line treatment recommendation. This can only be achieved by studying the effects of treatment in detail. Which patients benefit from treatment, in which should treatment be stopped and what are the early and long-term effects of treatment?
With advanced statistical models the results of clinical studies of treatment of chronic hepatitis B and C were analysed, achieving more insight into how the individual patient reacts to treatment.

# PREDICTION OF RESPONSE TO TREATMENT

The decline of HBV DNA during peginterferon (PEG-IFN) therapy and the spontaneous fluctuations in viral load in placebo-treated patients with HBeAg-positive chronic hepatitis B were compared in **Chapter 2.1**.
A total of 136 HBeAg-positive patients who participated in a randomized trial were treated with PEG-IFN alfa-2b for 52 weeks. This group was compared with 167 HBeAg-positive patients who received placebo for 48 weeks using linear mixed regression analysis. Response was defined as negative HBeAg at end of treatment (EOT).
Overall, decline of HBV DNA at EOT was larger in the PEG-IFN group compared with placebo and varied according to HBV genotype. Viral suppression was stronger in the PEG-IFN group compared with placebo starting at week 4 and throughout the entire treatment period. Among responders, HBV DNA decline was larger for PEG-IFN than placebo. ALT levels were significantly related to HBV DNA decline at the next visit and ALT flares (≥5 times the upper limit) during PEG-IFN therapy were associated with a stronger HBV DNA decline compared with placebo.
In conclusion PEG-IFN therapy resulted in a larger HBV DNA decline compared with placebo. Furthermore, the decline of HBV DNA was stronger in HBeAg-positive patients who lost HBeAg or who exhibited a flare during PEG-IFN therapy compared with spontaneous HBeAg loss or flares occurring during placebo therapy.

## Baseline prediction model

In **Chapter 2.2** the baseline prediction of response of HBeAg positive chronic hepatitis B to PEG-IFN is studied.

Therapy with PEG-IFN results in sustained response in a minority of chronic HBV infected patients and has considerable side-effects. We combined data from individual patients (n=721) from the 2 largest global trials of HBeAg positive chronic hepatitis B to determine which patients are most likely to respond to PEG-IFN therapy.
A sustained response was defined as HBeAg loss and HBV DNA $<2.0x10^4$ IU/ml at 6 months post treatment. Logistic regression analysis was used to identify predictors of sustained response and a prediction model was constructed.
HBV genotype, high ALT (≥2 x ULN) , low HBV DNA ($<2.0 \times 10^8$ IU/ml) and absence of previous IFN therapy predicted sustained response. Genotype A patients with either high ALT and/or low HBV DNA had a high (>30%) predicted probability of sustained response. High ALT was the strongest predictor in genotype B and low HBV DNA level was the strongest predictor in genotype C. Genotype D patients had a low chance of sustained response, irrespective of ALT or HBV DNA.
The final prediction model, corrected for overfitting is applicable for a new patient with HBeAg positive chronic hepatitis B, considering PEG-IFN treatment. We designed a webpage to easily obtain the patient specific prediction of sustained response: www.liver-gi.nl/peg-ifn



## Dynamic prediction models

Dynamic prediction of response of individual patients to PEG-IFN in chronic hepatitis B is analysed in **Chapter 2.3**
Baseline predictors of response to PEG-IFN include HBV-genotype, pre-treatment HBV DNA levels and ALT. The aim of this study was to develop a model, which enables improved baseline prediction of response to PEG-IFN for individual patients by adding early HBV DNA measurements during treatment. Furthermore, early indications for cessation of treatment were sought.
One hundred and thirty six patients treated with PEG-IFN were included in the study. Response was defined as loss of HBeAg and HBV DNA<10,000 copies/ml at 26 weeks post-treatment. Logistic regression analysis techniques were used to develop a dynamic prediction model with HBV DNA during the first 32 weeks of therapy. An early clinically useful rule for discontinuation of treatment was identified with a grid of cut-off values of HBV DNA decline during treatment.
Adding HBV DNA decline to baseline prediction significantly increased the c-statistics at week 4, 12 and 24. A HBV DNA decline of at least 2log10 within 24 weeks was significantly associated with response when added to the baseline prediction model: OR 6.62(95%CI, 1.94-22.6; p=0.002).

The model strongly supports individual decision making on treatment discontinuation in patients with HBeAg positive chronic hepatitis B. It is recommended to stop PEG-IFN treatment by 24 weeks if HBV DNA declined less than 2log10.

In **Chapter 2.4** statistical methods are presented that enable dynamic updates of the prediction of a significant clinical event.
If biomarkers change during follow-up, the clinical prognosis changes along. Our aim was to incorporate longitudinal profiles of these markers in a dynamic model to repeatedly update the individual prediction of the event. The general concept is presented specifically in the setup when the clinical event has a bivariate outcome.
First a direct approach is proposed, extending the usual logistic regression of baseline variables with the observed repeated measurements of the markers. The model is designed to update the prognosis of the outcome each time new information becomes available. Instead of entering the observed marker values the behaviour of the markers can also be used. Proceeding this way first linear mixed modelling is applied to fit the subject specific patterns of the markers and afterwards entering the random effects in the logistic regression while adjusting for the estimation error of the random effects.
Secondly an indirect prediction method using multivariate mixed effects models is applied. The patterns of the markers are allowed to vary depending on the outcome variable. Thereafter, the empirical Bayes estimates are used to obtain posterior probabilities that are subsequently used to update the probability of the outcome variable each time new information becomes available.
The different methods were applied to data on treatment of chronic hepatitis B patients. We conclude that the prediction of response obtained at baseline can be significantly improved with all of the above mentioned methods and may be useful tools to update the prognosis for the individual patients.

PEG-IFN alfa-2a results in a sustained response in a minority of HBeAg-negative chronic hepatitis B patients. In **Chapter 2.5** the role of on-treatment quantitative HBsAg and HBV DNA levels in the prediction of sustained response in HBeAg-negative patients receiving PEG-IFN alfa-2a is assessed.
HBV DNA and HBsAg were quantified at baseline, during treatment and follow-up in the sera from 107 patients who participated in an international multicenter trial. Overall, 22% of patients achieved sustained response (serum HBV DNA <10,000 copies/mL and normal ALT level at week 72). Starting at week 12, a marked on-treatment HBsAg decline was observed in sustained responders, in contrast to a modest decrease in non responders. HBV DNA levels decreased from week 4 on, more pronounced in patients

who developed sustained response. However, a substantial on-treatment HBV DNA decline was observed in non responders as well. At week 12, patients without a HBV DNA decline ≥2 log copies/mL combined with a HBsAg decline ≥0 log IU/mL from baseline were non responders (NPV 100%). Quantitative serum HBsAg in combination with HBV DNA may enable on-treatment adjustment of PEG-IFN therapy in HBeAg-negative chronic hepatitis B.
A solid stopping rule at week 12 is suggested using a combination of declines in serum HBV DNA and HBsAg level from baseline.

## STATISTICAL MODELS OF LONG-TERM TREATMENT EFFECTS

In **Chapter 3.1** the long term effects of glycyrrhizin treatment on the incidence of hepatocellular carcinoma (HCC) in chronic hepatitis C patients not responding to interferon were studied.
Data of all consecutive patients treated with interferon, who showed no sustained response after interferon treatment in 12 major Japanese hospitals between 1990 and 1995 were analysed.
During a mean follow-up of 6.1±1.8 years, 107 of 1093 included patients developed HCC. Cox regression with time dependent variables showed that older age, male sex, higher ALT and higher fibrosis stage were significantly associated with a higher risk for developing HCC. Response to glycyrrhizin, defined as ALT<1.5 x upper limit of normal, was significantly associated with a decreased incidence of HCC: hazard ratio 0.39 (95%CI 0.21-0.72; p<0.01). G-estimation, used to correct for ALT as confounder, showed no significant benefit of glycyrrhizin in the overall study population.
There is some evidence that interferon non-responder patients with chronic hepatitis C and fibrosis stage 3 or 4 may have a reduced incidence of HCC when ALT levels are normalized due to glycyrrhizin therapy.

In **Chapter 3.2,** a method was developed to longitudinally classify subjects into two or more prognostic groups using longitudinally observed values of markers related to the prognosis. The proposed method proceeds in two steps. First, multivariate linear mixed models are fitted in each prognostic group to model the dependence of markers on time and possibly other covariates. Secondly, fitted mixed models are used to develop a discrimination rule for future subjects. Our method improves upon existing approaches by relaxing the normality assumption of random effects in the underlying mixed models. Namely, we assume a heteroscedastic multivariate normal mixture for random effects.

The inference is performed in the Bayesian framework using the Markov chain Monte Carlo methodology. The methodology is applied to data from the Dutch primary biliary cirrhosis study.

## STATISTICAL MODELS OF EARLY TREATMENT EFFECTS

Viral dynamics during and after entecavir therapy in patients with chronic hepatitis B are described in **Chapter 4.1.**

Nucleoside analogues inhibit HBV replication. Entecavir, a guanine nucleoside, has also been shown to reduce covalently closed circular DNA (cccDNA) to undetectable levels in woodchucks chronically infected with hepatitis virus.

Mathematical description of changes in viral load during and after therapy may help to understand the process that takes place during nucleoside analogue treatment.

Ten chronic hepatitis B patients were evaluated with a mathematical model during treatment with and after withdrawal of four doses of entecavir. Blood was drawn for HBV DNA measurement at frequent intervals. Decay and rebound of viral concentration during and after entecavir therapy, respectively, showed a biphasic pattern. Non-linear modelling was used to fit individual patient data.

The median effectiveness in blocking viral production was 96% and the median half-life of viral turn-over was 16 h. The median half-life of infected hepatocytes was 257 h. Data on levels of cccDNA in the liver in these patients could be helpful in supporting the parameters as calculated with the model.

Tenofovir, an antihuman immunodeficiency virus (HIV) drug, has activity against lamivudine-resistant HBV mutants. In **Chapter 4.2** the efficacy of tenofovir is described in patients with lamivudine-resistant hepatitis B. Two investigative approaches based on mathematical models of viral dynamics were applied: the individual nonlinear fitting and the mixed-effect group fitting approaches.

Eleven chronic HBV patients on lamivudine for a median of 176 weeks (range: 72–382) with YMDD mutation-related HBV-DNA breakthrough received 'add-on' tenofovir 300 mg once-daily, while maintaining their existing therapy. Sequential sera assessing HBV DNA levels were frequently taken during the first 4 weeks of treatment and every 4 weeks thereafter.

Median baseline log HBV-DNA was 8.62. Tenofovir treatment resulted in a mean log HBV-DNA decline of 1.37 in the first phase, 2.54 after 4 weeks, and 4.95 log HBV-DNA after 24 weeks. The median effectiveness of blocking viral replication in the individual

fit model was 93%. There was only a small difference between the efficacy parameter of the individual nonlinear fitting and mixed-effect group fitting on the biphasic exponential model.
These data show that tenofovir has good efficacy in blocking viral replication in HBV patients with lamivudine induced drug-resistant HBV mutants. Both models can be used to describe viral decay during tenofovir therapy.

Treatment with PEG-IFN α-2b is effective for HBeAg-positive chronic hepatitis B although its mechanism of action remains unclear. In **Chapter 4.3** early pharmaco- and viral kinetics in patients treated for 52 weeks with PEG-IFN α-2b with or without lamivudine were analysed. After 4 weeks of treatment, there was a median viral decline of 2.94 $\log_{10}$ copies/ml in those treated with PEG-IFN α-2b and lamivudine and only 0.45 $\log_{10}$ copies/ml in the PEG-IFN α-2b monotherapy group. Peak IFN levels were reached approximately one day after administration and subsequently declined exponentially consistent with a viral load rebound near to baseline levels at the end of the dosing period in most patients receiving PEG-IFN α-2b monotherapy. Modelling of pharmaco- and viral kinetics data in this group revealed that viral load was minimal 3.6 days after PEG-IFN α-2b administration, the mean maximal and mean antiviral effectiveness was 70% and 48% with a mean infected cell loss rate of 0.07 per day, while no significant biphasic decline was observed. We conclude that PEG-IFN α-2b induces a sustained response in a considerable number of patients despite limited direct antiviral activity during the first weeks of antiviral therapy.

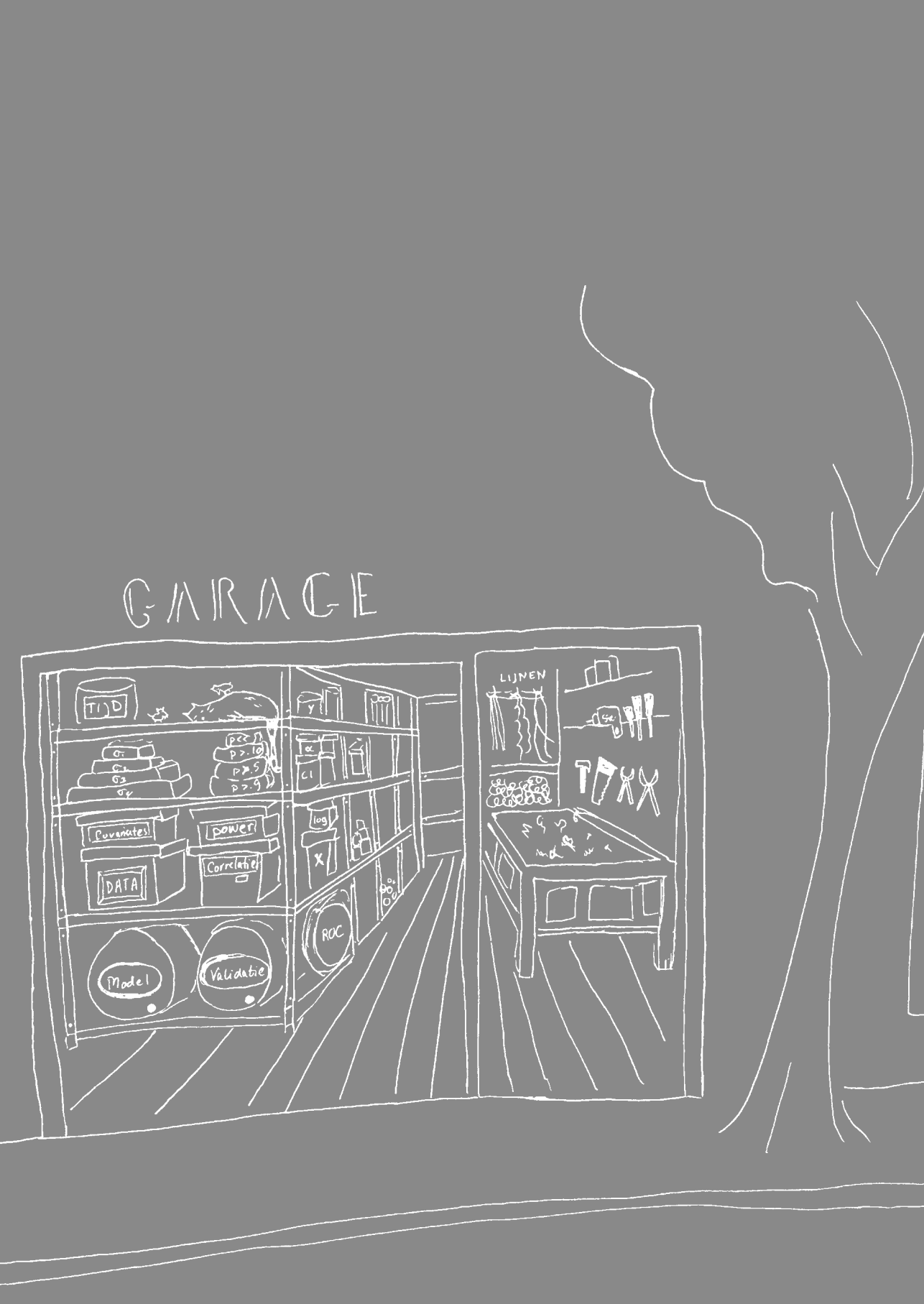



# CONCLUSION

For the sustained response 6 months after end of peginterferon (PEG-IFN) treatment of HBeAg positive chronic hepatitis B patients a prediction model of baseline factors was designed. This model offers a practical tool to calculate the individual patient prediction to sustained response and can easily be used in clinical practice to select optimal candidates for PEG-IFN therapy.
To update the individual response prediction during PEG-IFN therapy in HBeAG positive chronic hepatitis B patients with the repeated measurements of HBV DNA, assessed at each visit, dynamic prediction models were developed and different approaches were compared. The prediction of response to PEG-IFN obtained at baseline can be significantly improved with these new methods of dynamic prediction. These methods may be used to update the prognosis for the individual patients.

Early stopping rules for treatment with PEG-IFN in HBeAg positive and negative chronic hepatitis B patients were designed to identify patients who do not benefit from therapy. For the long-term effect of glycyrrhizin in chronic hepatitis C on the hepatocellular carcinoma-free period a Japanese cohort was studied. G-estimation was applied to offer a good solution to the problem of estimating the crude treatment effect when treatment is given on indication.

For the early treatment response pharmacokinetic modelling of the viral load decline during the first weeks of treatment with entecavir, tenofovir and PEG-IFN in chronic hepatitis B patients was studied. Available mathematical models were reformulated to fit into the framework of non-linear mixed regression modelling of repeated measurement. The estimated parameters describe the pattern of viral decline the first 4 weeks well. Both entecavir and tenofovir have a good efficacy in blocking the viral replication. The viral kinetics for PEG-IFN was more complicated but despite the limited antiviral activity during the first weeks PEG-IFN induces a sustained response in a considerable number of patients.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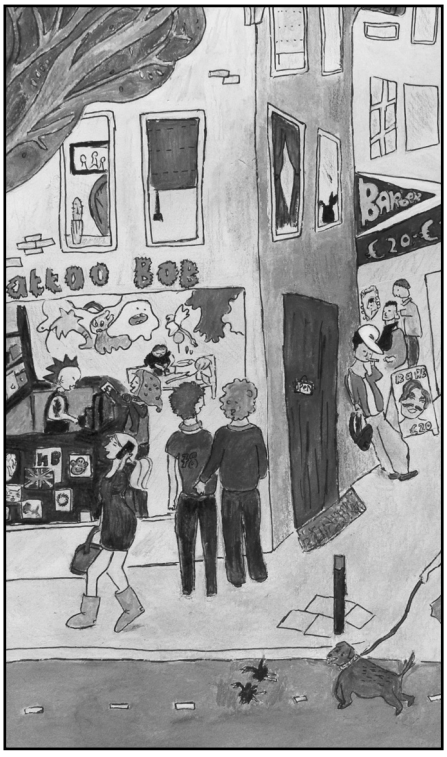
# CHAPTER 6

Samenvatting en conclusie



# SAMENVATTING EN CONCLUSIE

Dit proefschrift richt zich op de ontwikkeling en toepassing van statistische modellen van behandelingseffecten in chronische hepatitis B en C. De laatste jaren is er een snelle toename van het aantal behandelingsmogelijkheden voor patiënten met hepatitis B en C. Hierdoor onstaat er in toenemende mate de behoefte aan eerste lijns behandel adviezen voor individuele patiënten. Dit kan alleen worden bewerkstelligd door de effecten van behandelingen tot in detail te analyseren en bestuderen. Welke patiënten hebben baat bij hun behandeling, bij wie moet de behandeling worden gestopt en wat zijn de korte en lange termijn effecten van behandeling?
Resultaten van klinische studies betreffende de behandeling van chronische hepatitis B en C patiënten werden met behulp van geavanceerde statistische modellen geanalyseerd om inzicht te verkrijgen in hoe de individuele patiënt op behandeling reageert.

# VOORSPELLEN VAN RESPONS OP BEHANDELING

De daling van HBV DNA gedurende peginterferon (PEG-IFN) behandeling en de spontane fluctuaties van de hoeveelheid virus in het bloed van met placebo behandelde HBeAg-positieve chronische hepatitis B patiënten werden met elkaar vergeleken in **Hoofdstuk 2.1.**
Honderd zesendertig HBeAg-positieve patiënten die deelgenomen hadden aan een gerandomiseerd onderzoek werden gedurende 52 weken behandeld met PEG-IFN alfa-2b. De groep werd met behulp van lineaire mixed regressie analyse vergeleken met 167 HBeAg-positieve patiënten die gedurende 48 weken behandeld waren met een placebo. Respons werd gedefinieerd als HBeAg negativiteit aan het einde van de behandeling.
Over het geheel genomen was de daling van HBV DNA sterker in de PEG-IFN groep dan in de placebo groep en gerelateerd aan het HBV genotype. Virus onderdrukking was sterker in de PEG-IFN groep vergeleken met de placebo groep vanaf week 4 en gedurende de verdere behandelingsduur. Voor de responders was de HBV DNA daling groter voor de PEG-IFN behandelden dan voor de met placebo behandelden. ALT nivo's waren significant gerelateerd aan een daling van HBV DNA gedurende het volgende polikliniek bezoek. En zgn. ALT flare (waarde meer dan 5x de hoogste normaal waarde) was geassocieerd met een sterkere HBV DNA daling gedurende PEG-IFN behandeling dan bij behandeling met een placebo.
Concluderend resulteerde PEG-IFN behandeling in een grotere HBV DNA daling dan behandeling met een placebo. Bovendien was de HBV DNA daling sterker in HBeAg

positieve patiënten met verlies van HBeAg positiviteit dan wel een ALT flare wanneer patiënten behandeld werden met PEG-IFN dan met een placebo.

## Predictiemodel op baseline

In **hoofdstuk 2.2** wordt de voorafkans op respons van HBeAg positieve chronische hepatitis B op PEG-IFN behandeling bestudeerd en een predictie model ontwikkelt.
Behandeling met PEG-IFN resulteert slechts in een minderheid van de patiënten in een blijvende respons en is geassocieerd met aanzienlijke bijwerkingen. Wij voegden de onderzoeksgegevens samen van 721 patiënten uit de 2 grootste internationale studies naar HBeAg positieve chronische hepatitis B en onderzochten bij welke patiënten een blijvende respons op PEG-IFN behandeling het grootst is. Een blijvende respons werd gedefinieerd als HBeAg verlies en HBV DNA < $2.0x10^4$ IE/ml 6 maanden na behandeling. Logistische regressie analyse werd toegepast om voorspellers van een blijvende respons te identificeren.
Het HBV genotype, een verhoogd ALT (≥2xULN), een laag HBV DNA (<$2.0 \times 10^8$ IE/ml), en geen eerdere IFN behandeling waren in combinatie met het geslacht en leeftijd voorspellers van een blijvende respons.
Genotype A patiënten met een verhoogd ALT en/of een laag HBV DNA hadden een hoge kans (30%) op een respons. Een verhoogd ALT was de sterkste voorspeller in patienten met genotype B en een laag HBV DNA was de sterkste voorspeller in patiënten met genotype C. Genotype D patiënten hadden een lage kans op een blijvende respons onafhankelijk van hun ALT of HBV DNA.
De predictiemodel van een blijvende respons, gecorrigeerd voor overfitting, is gerealiseerd in een webpage, die de berekening direct uitvoert via een simpel invoer scherm: www.liver-gi.nl/peg-ifn

## Dynamische predictiemodellen

In **hoofdstuk 2.3** wordt een dynamische predictie model op een blijvende respons van individuele patiënten met HBeAg positieve chronische hepatitis B op PEG-IFN behandeling ontwikkeld. Dit hoofdstuk is een directe voortzetting van hoofdstuk 2.2.
Het doel van deze studie was het ontwikkelen van een model waarin de vroege bepaling van kwantitatief HBV DNA tijdens de behandeling een herhaaldelijke nieuwe individuele voorspelling van respons op PEG-IFN behandeling geeft. Tevens werd gezocht naar een klinische beslissingsregel om de behandeling voortijdig te beëindige van patiënten die een ongunstige respons kans hadden.

Er werden 136 patiënten geïncludeerd. Een blijvende respons werd gedefiniëerd als een verlies van HBeAg en een afname van HBV DNA < 10000 kopieën/ml (< $2.0x10^4$ IE/ml) op week 26 na behandeling. Logistische regressie analyse technieken werden gebruikt om een dynamisch voorspellings model te ontwikkelen met behulp van HBV DNA metingen gedurende de eerste 32 weken van behandeling. Een vroege klinische stopregel voor het beëindigen van behandeling werd geïdentificeerd met behulp van een rooster van afkapwaarden van de afname van de kwantitatieve HBV DNA tijdens de behandeling.

Toevoeging van de daling van kwantitatief HBV DNA aan de predictiemode in hoofdstuk 2.2 leidde tot een verbetering van de c-statistiek op week 4-24. Een daling van het HBV DNA van 2log10 of meer binnen 24 weken was significant geassocieerd met een blijvende respons (OR 6.62 (95% CI, 1.94-22.6; p=0.002)).

Dit model geeft een sterke aanbeveling voor individuele besluitvorming over het beëindigen van de behandeling bij patiënten met HBeAg-positieve chronische hepatitis B. We adviseren de PEG-IFN behandeling te staken na week 24wanneer het HBV DNA met minder dan 2log10 is afgenomen.

In **hoofdstuk 2.4** worden statistische methoden behandeld die in staat stellen om een dynamische voorspelling te geven op een belangrijke klinische gebeurtenis. Wanneer biomarkers veranderen gedurende de follow-up van een behandeling, verandert de prognose van de ziekte. Het doel van deze studie was het includeren van longitudinale profielen van deze markers in een dynamisch model om de individuele voorspelling van een gebeurtenis te kunnen bijstellen. Het concept wordt specifiek gepresenteerd wanneer de klinische gebeurtenis een bivariate uitkomst heeft, zoals respons op therapie.

Eerst wordt een directe methode geïntroduceerd waarbij de logistische regressie met uitgangswaarden uitgebreid wordt met herhaalde meetresultaten van markers. Dit model is ontwikkeld om de prognose van behandeling bij te stellen wanneer nieuwe informatie beschikbaar komt. Behalve de waarden van de markers kan ook het gedrag worden ingevoerd. Met deze methode wordt eerst lineaire mixed modellen toegepast om de individuele specifieke patronen van de markers te schatten. Vervolgens worden de geschatte parameters hiervan geincludeerd in de logistische regressie, rekening houdend met de schattingsfout.

Vervolgens wordt een indirecte voorspellingsmethode, gebruik makend van lineaire mixed modellen, gepresenteerd. De specifieke patronen van de markers worden geschat apart voor elk uitkomstgroep. Hierna worden empirische Bayesiaanse schattingen gebruikt om de voorspelling van de uitkomsten bij te stellen wanneer nieuwe informatie beschikbaar komt.

De verschillende methoden zijn toegepast op de gegevens van patiënten met chronische hepatitis B.
Wij concluderen dat het vooraf voorspellen van de respons op behandeling significant verbeterd kan worden door gebruik te maken van de bovenbeschreven technieken. Ook kan hiermee de prognose van individuele patiënten worden bijgesteld.

PEG-IFN-alfa-2a leidt in de minderheid van de HBeAg negatieve patiënten tot blijvende respons. In **hoofdstuk 2.5** wordt de voorspellende waarde van het kwantitatieve HBsAg en HBV DNA bepalingen met het oog op aanhoudend respons tijdens de behandeling van HBeAg negatieve patiënten met PEG-IFN alfa-2a onderzocht.
HBV DNA en HBsAg werden kwantitatief bepaald voorafgaande en tijdens de behandeling en gedurende de follow-up in de sera van 107 patiënten die deelnamen aan een internationaal multicentre onderzoek.
In het totaal werd bij 22% van de patiënten een blijvende respons op behandeling waargenomen (serum HBV DNA < 10000 kopieën/ml en normale ALT waarden op week 72). Vanaf week 12 van de behandeling werd een duidelijke daling van HBsAg waargenomen in patiënten met een blijvende respons. Slechts een geringe daling van het HBsAg werd geobserveerd bij patiënten zonder respons. HBV DNA niveaus daalden vanaf week 4 van de behandeling meer uitgesproken bij patiënten met een blijvende respons, echter een daling was ook aanwezig bij de non-responders.
Alle patiënten zonder een HBV DNA daling van ≥ 2 log kopieën/ml in combinatie met het ontbreken van een HBsAg daling ≥ 0 log IE/ml van de uitgangswaarde waren non-responders (NPV 100%).
Kwantitatieve serum HBsAg bepaling in combinatie met kwantitatieve HBV DNA bepaling kan gedurende de behandeling met PEG-IFN bij patiënten met HBeAg-negatieve chronische hepatitis B bijstelling van de voorspelde respons faciliteren. Stoppen van de behandeling op week 12 wordt gesuggereerd gebruikmakend van de gecombineerde daling van het kwantitatieve HBV DNA en het kwantitatieve HBsAg ten op zicht van tijdstip 0.

## STATISTISCHE MODELLEN VAN LANGE TERMIJNEFFECTEN VAN BEHANDELING

In **hoofdstuk 3.1** wordt het lange termijn effect van glycyrrhizine op de incidentie van hepatocellulair carcinoom (HCC) bepaald in chronische hepatitis C patiënten die niet reageren op IFN behandeling.

Data van opeenvolgende patiënten zonder respons na IFN behandeling in 12 grote Japanse ziekenhuizen in de periode 1990-1995 werden geanalyseerd. Gedurende een gemiddelde follow-up periode van 6.1 ± 1.8 jaar ontwikkelden 107 van de 1093 patiënten een HCC. Cox regressie analyse met tijdsafhankelijke variabelen toonde aan dat een oudere leeftijd, het mannelijke geslacht, verhoogde ALT niveaus en een hoger fibrose stadium geassocieerd waren met een verhoogd risico op het ontwikkelen van HCC. Respons op glycyrrhizine behandeling, gedefinieerd als ALT< 1.5xULN was significant geassocieerd met een verlaagde incidentie van HCC; HR 0.39 (95%CI 0.21-0.72; p<0.01). De G schatting, welke wordt gebruikt om te corrigeren voor ALT als confounder, toonde geen beschermende werking aan van glycyrrhizine voor de studie populatie als geheel. In patiënten met chronische hepatitis C met fibrose stadium 3 of 4, die niet reageren op IFN behandeling, lijkt de incidentie van HCC te reduceren in patiënten waar de ALT waarden normaliseren na glycyrrhizine therapie.

In **hoofdstuk 3.2** wordt een methode besproken om op longitudinale wijze patiënten te verdelen in twee of meer prognostische groepen, gebruik makend van longitudinaal geobserveerde waarden van markers die gerelateerd zijn aan de prognose. Deze methode heeft 2 opeenvolgende stappen, waarbij eerst de multivariate lineaire mixed modellen worden geschat voor elke prognostische groep om de afhankelijkheid van markers over de tijd en/of andere mogelijke covariaten te bepalen. Vervolgens worden de modellen gebruikt om toekomstige patiënten van elkaar te onderscheiden. Onze methode verbetert de bestaande methoden door de veronderstelling van de normale verdeling van random effecten van het onderliggende mixed model te versoepelen. Wij veronderstellen namelijk een heteroscedastische multivariate normale mix van random effecten. De schattingen zijn verricht in het Bayesiaanse kader gebruikmakend van de Markov keten Monte Carlo methodologie. De methode is toegepast op de gegevens van de Nederlandse Primaire Biliaire Cirrhose studie.

## STATISTISCHE MODELLEN VAN DE EERSTE BEHANDELINGSEFFECTEN

In **hoofdstuk 4.1.** wordt de virale dynamiek gedurende en na Entecavir behandeling in patiënten met chronische hepatitis B beschreven.
Nucleoside analogen remmen HBV replicatie. Van entecavir, een guanine nucleoside, is beschreven dat dit leidt tot een reductie van het covalent gesloten circulaire DNA (cccDNA) tot niet detecteerbare waarden in bosmarmotten met een chronische HBV. Wiskundige beschrijving van de veranderingen van de hoeveelheid virus gedurende

en na de behandeling met entecavir kan inzicht geven in de virale dynamiek die zich voordoet tijdens therapie.
Tien patiënten met een chronische hepatitis B werden met behulp van een wiskundig model bestudeerd gedurende de toediening van 4 doses entecavir en na het beëindigen hiervan. Bloed werd regelmatig afgenomen om het HBV DNA te meten. De daling en stijging van de virusconcentratie gedurende en na het beëindigen van entecavir behandeling toonde een bifasisch patroon. Een niet-lineair modellen werden toegepast op de individuele patiënt gegevens.
De mediane effectiviteit in het remmen van de virusproduktie was 96% en de mediane halfwaarde tijd van virusturnover was 16 uur. De mediane halveringstijd van geïnfecteerde hepatocyten was 257 uur. Data betreffende de niveaus van cccDNA in de lever van deze patiënten zouden de parameters kunnen ondersteunen in het kader van het gebruikte model.




Tenofovir, een medicament wat gebruikt wordt bij HIV, is werkzaam bij lamivudine-resistente HBV mutanten. In **hoofdstuk 4.2** wordt de effectiviteit van tenofovir beschreven in patiënten met lamivudine resistente hepatitis B. Twee onderzoeksstrategieën gebaseerd op wiskundige modellen van de virale dynamiek werden toegepast: de individuele niet-lineaire schatting en de niet-lineaire mixed groeps schatting.
Elf patiënten met chronische HBV, behandeld met lamivudine gedurende een mediane periode van 176 weken (72-382 weken) met een YMDD mutatie gerelateerde HBV DNA doorbraak kregen additioneel tenofovir (1 x daags 300mg). Opeenvolgende bloedmonsters om het kwantitatieve HBV DNA te bepalen werden regelmatig afgenomen gedurende de eerste 4 weken van de additionele behandeling en vervolgens elke 4 weken.
De mediane uitgangswaarde van log HBV-DNA bedroeg 8.62. Behandeling met tenofovir leidde tot een gemiddelde log HBV-DNA daling van 1.37 gedurende de eerste fase van behandeling, een daling van 2.54 op week 4 en een daling van 4.95 log HBV-DNA op week 24. De mediane effectiviteit van virus replicatieremming in het individuele geschatte model bedroeg 93%. Er was slechts een klein verschil tussen de effectiviteits parameter $\varepsilon$ volgens de individuele niet-lineaire schatting en de mixed groeps schatting van het bifasische exponentiële model.
De data tonen aan dat tenofovir effectief is in het remmen van virus replicatie in patiënten met HBV met lamivudine geïnduceerde resistente HBV mutanten. Beide schattings methoden zijn geschikt om de afname van het virus gedurende tenofovir behandeling te bestuderen.

Behandeling met PEG-IFN-α-2b is een effectieve therapie voor patiënten met HBeAg positieve chronische hepatitis B hoewel het werkingsmechanisme niet geheel duidelijk is. In **hoofdstuk 4.3** worden de vroege farmacologische en virale kinetiek bestudeerd in patiënten gedurende een behandeling van 52 weken met PEG-IFN-α-2b al dan niet in combinatie met lamivudine. Na 4 weken behandeling was er een mediane kwantitatieve HBV-DNA daling van 2.94 $\log_{10}$ kopieën/ml in patiënten behandeld met PEG-IFN-alfa-2b en lamivudine versus een daling van 0.45 $\log_{10}$ kopieën/ml in patiënten behandeld met PEG-IFN-alfa-2b monotherapie. Maximale IFN levels werden na ongeveer één dag behandeling bereikt en daalden vervolgens exponentieel , consistent met een afname van het virus tot uitgangswaarden aan het einde van de behandel periode bij patiënten behandeld met PEG-IFN α2b monotherapie. Onderzoek van de farmacologische en virale kinetiek in deze groep toonden aan dat de hoeveelheid virus het laagst was 3.6 dagen na PEG-IFN-alfa-2b toediening. De gemiddelde maximale en minimale antivirale effectiviteit was 70% en 48% respectievelijk met een gemiddeld geïnfecteerde cel verlies van 0.7 per dag terwijl er geen bifasische daling werd geobserveerd. Wij concluderen dat PEG-IFN-alfa-2b een blijvende respons induceert in een groot aantal patiënten ondanks de beperkte antivirale activiteit gedurende de eerste weken van de antivirale behandeling.

## CONCLUSIE

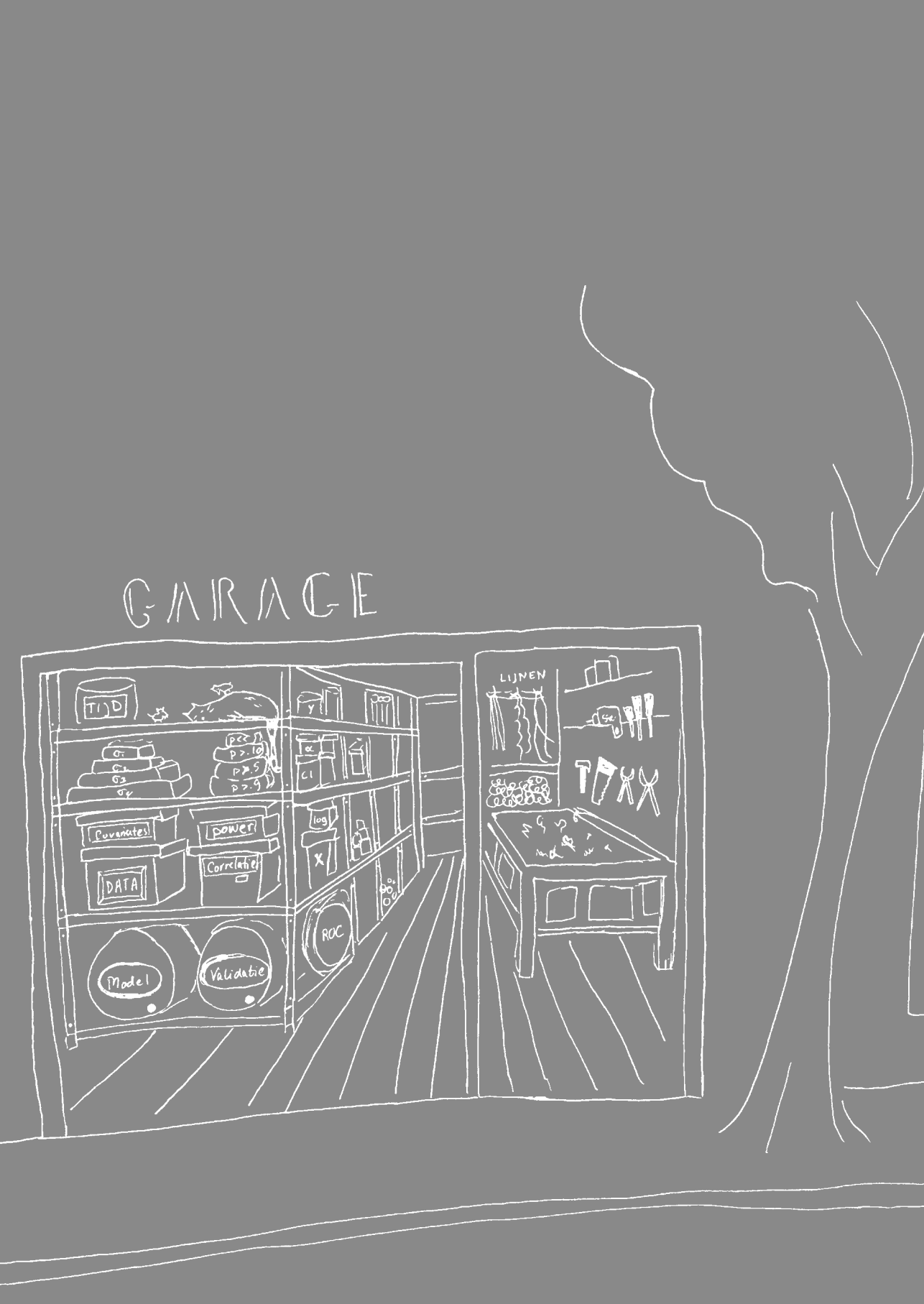
Een predictiemodel van uitgangswaarden werd ontworpen voor het eindpunt van blijvende respons 6 maanden na het beëindigen van de PEG-IFNbehandeling van HBeAg positieve patiënten met chronische hepatitis B. Met dit praktische model is het mogelijk de kans op respons van de individuele patiënt te berekenen en het model is bruikbaar om de juiste patiënten te selecteren voor behandeling met PEG-IFN.

Om de individuele respons voorspelling gedurende PEG-IFN behandeling in chronische hepatitis B te optimaliseren, werd bij HBeAg positieve patiënten met herhaalde HBV DNA metingen gedurende opeenvolgende polikliniek controles dynamische predictie modellen ontworpen. Tevens werden verschillende benaderingen vergeleken. De voorspelling van respons op PEG-IFN ten tijde van het begin van de behandeling kan significant verbeterd worden met de nieuwe methoden die gebruikt kunnen worden om de prognose van individuele patiënten bij te stellen.

Vroege stopregels voor de behandeling met PEG-IFN in HBeAg positieve en negatieve hepatitis B patiënten werden ontwikkeld om patiënten te identificeren die geen baat ondervinden van behandeling.

In een Japans cohort van chronische hepatitis C patiënten die gedurende een lange periode behandeld waren met glycyrrhizine werd de heptocellulair carcinoom vrije periode bestudeerd. Vanwege de cohort structuur van de data werd de G schattingsmethode toegepast. Deze geavanceerde methode biedt een bevredigende oplossing voor het probleem van het schatten van zuivere behandel effecten wanneer de behandeling gegeven wordt op indicatie.

Ten behoeve van de vroege termijn respons werd in een pharmacokinetiek model de virusafname in het bloed van chronische hepatitis B patiënten de eerste weken gedurende de behandeling met entecavir, tenofovir en PEG-IFN bestudeerd. Beschikbare mathematische modellen werden herschreven om te gebruiken in het kader van niet-lineaire mixed regressie modellering van herhaalde metingen. De geschatte parameters beschrijven duidelijk het patroon van virusdaling gedurende de eerste 4 weken. Zowel entacavir en tenofovir zijn effectief in het blokkeren van de virusreplicatie. De pharmacokinetiek voor PEG-IFN is meer gecompliceerd. Ondanks de beperkte antivirale activiteit gedurende de eerste weken induceert PEG-IFN een blijvende respons in een aanzienlijk aantal patiënten.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# Dankwoord

# DANKWOORD

Met opluchting begin ik aan het dankwoord van dit proefschrift. Promoveren is teamwork, vele mensen zijn betrokken geweest. Ik wil jullie hiervoor allemaal bedanken.
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# CURRICULUM VITAE

Bettina Elisabeth Hansen was born in Gentofte in Denmark on the 25th of November 1964. After finishing secondary school at N. Zahles Gymnasium in Copenhagen in 1983, she started studying statistics at the Institute of Mathematical Statistics at the University of Copenhagen. During her study she worked as a research statistician at the Statistical Research Unit at the University of Copenhagen. From 1990 she moved to the Netherlands where she continued her study at the University of Delft at the department of Statistics. In 1991 she obtained her MSc degree in Statistics with Mathematics as secondary subject.

From 1991-1995 she worked as a biostatistician at the department of Medical Statistics at the University of Leiden. In 1995 she started working at the Erasmus MC in Rotterdam as a biostatistician at the department of Gastroenterology & Hepatology and at the department of Epidemiology & Biostatistics. In 2000 she was registered as a VVS biostatistician. She started this PhD project in 2005 under the supervision of Prof.dr. Harry Janssen.

She is married to Hans van Overhagen and has two children Katrine and Rasmus.

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