Baseline anti-NS4a antibodies in combination with on-treatment quantitative HCV-RNA reliably identifies non responders to peginterferon-ribavirin combination therapy after 4 weeks of treatment.

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Short title: HCV week 4 stopping rule

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Abstract.

BACKGROUND: Early detection of non responders to hepatitis C therapy limits

unnecessary exposure to treatment and its side effects. A recent algorithm combining

baseline anti-NS4a antibodies and on treatment quantitative PCR identified non

responders to a combination of interferon and ribavirin after 1 week of treatment.

AIM: To validate a stopping rule based on baseline anti-NS4a antibody levels and early

on treatment virological response in treatment naive genotype 1 chronic hepatitis C

patients treated with the current standard pegylated interferon and ribavirin

combination therapy.

METHODS: Eighty-nine (89) genotype 1 patients from the DITTO study treated for 48

weeks with standard 180 µg PEG-IFN-α-2a weekly and ribavirin 1000-1200 mg daily,

were analysed. Baseline anti-NS4a antibody ELISA (NS4a AA 1687-1718) was

performed on pre-treatment serum. HCV-RNA was assessed at day 0, 1, 4, 7, 8, 15,

22, 29, week 6, 7, 8, 10, 12 and 6 weekly thereafter until end of treatment. Multiple

regression logistic analysis was performed.

RESULTS: Overall 54/89 (61%) patients achieved sustained viral response. A baseline

anti-NS4a antibody titre <1/1250 correlated with absence of favourable initial viral

decline according to variable response types (p= 0.015). The optimal algorithm was

developed using the combination of the absence of anti-NS4a Ab (<1/1250) at baseline

and the presence of a viral load $\geq$ 100.000 IU/ml at week 4. This algorithm has a

specificity of 43% and negative predictive value of 100% to detect non response to

standard PEG-IFN-α-2a and ribavirin therapy at week 4 of therapy (intention to treat

analysis).

CONCLUSIONS: The decision to stop therapy in genotype 1 chronic hepatitis C patients treated with PEG-IFN-α-2a and ribavirin can be confidently made after 4 weeks of treatment based on the absence of baseline anti-NS4a Ab and a week 4 HCV-RNA above 100.000 IU/ml.

Keywords: hepatitis C; HCV-RNA; peginterferon alfa-2a; ribavirin; stopping rule; anti NS4a antibodies

Introduction.

Pegylated interferon-α-2a and ribavirin (RBV) combination therapy induces a sustained

virological response (SVR) in only half of the genotype 1 infected chronic hepatitis C

patients.[1] Accurate early recognition of those patients who will not clear the HCV

infection despite a full 48 week treatment course allows early treatment discontinuation

limiting unnecessary exposure to potential side effects. The absence of a more than 2

log drop from baseline viral load after 12 weeks of treatment detects 31% of non

responder patients with a negative predictive value (NPV) of 97%.[1, 2] A stopping rule

that would identify more non responder patients earlier on during the treatment would

limit further the side effects and costs of the treatment for these patients.

Recently Desombere et al. developed a stopping algorithm based on the absence or

presence of baseline anti-NS4a antibodies (Ab) and early on treatment viral load that

detects non response with a NPV of 100% in a group of patients treated with standard

interferon and RBV combination therapy.[3]

Our aim was to develop a stopping rule with at least similar NPV and improved

specificity in comparison to the current week 12 stopping rule, based on baseline anti-

NS4a serology status and early on treatment quantitative viral load in patients treated

with peginterferon-α-2a and RBV combination therapy.

Materials and Methods.

The Dynamically Individualized Treatment of hepatitis C Infection and Correlates of

Viral/Host dynamics (DITTO) HCV study group recruited 270 chronic hepatitis C patients

for an open label phase 3 international multicenter trial.[4] Treatment-naive patients

with compensated liver disease and the following characteristics were eligible: positive

test for anti-HCV antibody, HCV-RNA > 1000 IU/ml (Cobas Amplicor HCV Monitor v. 2.0

assay, Roche Diagnostics, lower limit of detection 600 IU/ml), two serum alanine

aminotransferase values above the upper limit of normal within 6 months of treatment

initiation, and a pre-treatment (within previous 12 months) liver biopsy consistent with a

diagnosis of chronic hepatitis C. The major exclusion criteria were previously reported.[4]

Patients were randomised after 6 weeks of combination therapy with peginterferon-α-2a

and weight based RBV combination therapy according to initial viral kinetics of the first 4

weeks to either individualised treatment or continued standard treatment until week 48.

For this analysis the subset of 89 genotype 1 patients who completed at least 4 weeks

of therapy in the standard treatment arm were analysed. All patients were treated with

weekly injections of 180 µg peginterferon-α-2a in combination with daily oral RBV at a

dose of 1000 or 1200 mg depending of bodyweight below or above 75 kg. All patients

were motivated to continue treatment for the 48 week treatment period. Treatment

could be stopped at the discretion of the investigator if HCV-RNA was still positive after

24 weeks of treatment.

Labatory assessments. HCV-genotyping was confirmed centrally at Erasmus MC

University Medical Center Rotterdam by reverse hybridization (Inno LipA HCV II;

Innogenetics, Gent, Belgium). Baseline anti-NS4a ELISA was determined on a pre-

treatment serum sample (NS4a AA 1687-1718, horseradish peroxidise-labelled goat anti-

human IgG antibody; dilutions 1/10, 1/100, 1/250, 1/1250, 1/16250) in the CEVAC lab

in Ghent as described previously.[3] HCV-RNA was determined centrally in Rotterdam with the Cobas Amplicor HCV Monitor v. 2.0 assay (Roche Diagnostics, lower limit of detection 600 IU/ml) at day 0, 1, 4, 7, 8, 15, 22, 29, week 6, 7, 8, 10 and 12 and 6 weekly thereafter until end of treatment. All samples that tested negative with the quantitative assay were retested with the Cobas Amplicor HCV Monitor v2.0 qualitative assay (Roche Diagnostics, lower limit of detection 50 IU/ml).

Definition of response type: In the DITTO study Rapid Viral Response (RVR) was defined as a ≥2 log drop of HCV-RNA during the first 4 weeks and a second-phase decline ≥0.09 log/day. Slow partial response (SPR) was defined as no RVR but a second-phase decline ≥0.09 log/day and (i) a ≥1 log drop of HCV-RNA during the first 4 weeks or (ii) a ≥0.6 log drop of HCV-RNA during the first 4 weeks and a first-phase decline of ≥0.5 log. SVR was defined as negative qualitative HCV-RNA <50 IU/ml at 24 weeks after the end of treatment (week 72).

Study end points and objectives.

The primary objective of this study was to develop a stopping rule that reliably detects non responder patients during the first weeks of treatment based on the absence of pre-treatment anti NS4a Ab and the presence of a rapid on treatment HCV-RNA decline. Two items were considered of paramount importance in the development of the stopping rule: NPV and Specificity. The NPV of a stopping rule reflects the probability that the algorithm indeed predicts a true "non-response", or in other words how sure one can be that no potential SVR patients will be lost by applying the early stopping rule. The specificity (SPEC) depicts the fraction of all non-responding patients that will

indeed be identified by the prediction criteria. The goal is the highest possible

specificity with a NPV of 100%. For this study we aimed to develop a stopping rule that

could detect at least a similar proportion of non responders as the current week 12 2 log

rule, but to detect these patients at an earlier stage during treatment while maintaining

excellent NPV.

Statistical analysis.

Multiple logistic regression analysis was used to explore a possible correlation between

non sustained virological response, baseline anti-NS4a Ab titre and viral load at baseline

and day 0, 1, 4, 7, 8, 15, 22, 29. All analyses were performed as intention to treat

analysis, categorizing patients with missing week 72 data as non sustained virological

patients. Receiver Operating Characteristic curves and Akaike Information Criteria were

used to investigate the non-inferiority of specificity and NPV of a possible new stopping

algorithm with the current week 12 2 log rule specificity and NPV. All analyses were

performed using SPSS$^{®}$ 12.0 statistical software (SPSS Inc., Chicago, Illinois, US).

Results.

Patient characteristics. The study population (n=89) consisted genotype 1 infected

chronic hepatitis C patients with a mean age of 41 years (20-66), predominantly of

Caucasian origin. Sixty-four percent of patients (57/89) had a high baseline viremia of

≥ 0.8 x 10^6 IU/ml and the median viremia at baseline in this study group was 1.38 x 10^6

IU/ml. Ten percent of patients had a baseline viremia $< 10^5$ IU/ml. Cirrhosis, defined

as an Ishak score of 5 or 6, was present in 10% of the patients (Table 1). SVR in this

study group with a high baseline viremia was 61% (54/89) when treated with a

standard 48 weeks peginterferon-α-2a and RBV combination therapy (intention to treat

analysis).. SVR was achieved in 39/47 (83%) and 14/30 (47%) of the patients showing

RVR and SPR according to the DITTO response types, respectively. 35/89 patients had

no SVR at week 72. Of these 9/89 (10%) discontinued treatment prematurely prior to

week 48. 15/89 (17%) did not achieve week 48 end of treatment virological response.

Relapse occurred in the remaining 11/89 (12%) at week 72.

Baseline anti-NS4a antibody levels. Logistic regression analysis showed a

correlation between baseline anti-NS4a Ab levels and the initial viral kinetics. Patients

with a baseline anti-NS4a Ab titre of <1/1250 displayed significantly fewer rapid viral

responses or slow partial responses compared to patients with a baseline anti-NS4a Ab

titre ≥ 1/1250 (p= 0.015 Chi-square) (Fig. 1). There was no statistically significant

correlation between baseline anti-NS4a Ab levels and HCV-RNA negativity at any time

point of treatment or with the SVR status at end of follow-up.

HCV-RNA at Week 4. A single HCV-RNA value exceeding 10^5 IU/ml at week 4 was

superior in detecting non responding patients early on during treatment (sensitivity

96.3%, specificity 48.6%, PPV 74.3%, NPV 89.5%) than a 2 log HCV-RNA decline at this

time point. This parameter however lacked adequate NPV prohibiting its clinical

application as stopping rule , since therapy would be incorrectly stopped in 10.5% of

patients.

HCV-RNA at week 12. Evaluation of the current week 12 2 log stopping rule in this group showed a sensitivity of 100%, a specificity of 31%, a PPV of 69% and a NPV of 100%, confirming previously reported values.[1, 2] In this group of 89 patients, a stopping rule based on the absence of a week 12 HCV-RNA below 1600 IU/ml had the best specificity of 43% while maintaining a NPV of 100% to predict non response.

Algorithm based on early HCV-RNA determination and baseline anti-NS4a antibody titer. Multiple logistic regression analysis with the variables baseline anti-NS4a Ab titre, HCV-RNA level and treatment duration was performed (Fig.2). An algorithm could be developed based on a baseline anti-NS4a Ab titre of <1/1250 and the presence of a HCV-RNA $\geq 10^5$ IU/ml after 4 weeks of treatment. Patients who had week 4 HCV-RNA of $\geq 10^5$ IU/ml and a baseline anti-NS4a Ab titre of < 1/1250 had no chance of achieving SVR with continued treatment. This algorithm had a sensitivity of 100%, specificity of 43%, PPV of 73% and NPV of 100% to detect non response to standard peginterferon-α-2a RBV combination therapy after 4 weeks of therapy (intention to treat analysis). The performance of this stopping rule is similar in the group of 80/89 patients with a baseline HCV-RNA $\geq 10^5$ IU/ml as all patients with a week 4 HCV-RNA $< 10^5$ IU/ml are to continue therapy with this rule (sensitivity 100%, specificity 43%, PPV 69%, NPV 100%). Our week 4 stopping rule categorised 7 of the 9/89 drop out patients as week 4 responders. The week 4 algorithm has a sensitivity of 100%, specificity of 50%, PPV of 81% and NPV of 100% in the 80/89 patients who

continued treatment until week 48. It is hence fair to assume that the drop-out rate did not favour a positive bias towards the week 4 algorithm.

The week 4 rule identified at least a similar proportion of non sustained virological responders as the current week 12 2 log rule (specificity 43 vs 31 respectively, Receiver Operating Characteristic curves: Akaike Information Criteria 91.2 and 98.3 and Area Under the Curve 0.724 and 0.657 for the new week 4 and current week 12 algorithm respectively) and a NPV of 100% at an 8 week earlier time point. Analysis of concordant and discordant results showed that all adherent week 12 2 log non sustained virological responders were identified by the week 4 algorithm. Consecutive application of the new week 4 algorithm and by the current week 12 2 log rule would detect 51 % of non responders (18/35) after 12 weeks of treatment in this study group (Fig.3).

Discussion.

The aim of this study was to develop a clinical applicable stopping rule that would allow earlier detection of non response compared to the current week 12 2 log stopping rule while maintaining a reliable NPV and detecting at least a similar proportion of non responder patients. The combination of a low anti-NS4a Ab titer at baseline, correlating with a low likelihood of favourable initial viral kinetics, and HCV-RNA determination at week 4, constitutes a stopping rule with adequate NPV and specificity. This stopping rule detects at least a similar proportion of non responders as the week 12 2 log rule, but does so 8 weeks earlier.

Careful examination of the three variables used in this analysis, namely HCV-RNA level, treatment duration and baseline anti NS4a Ab titre, revealed the following trends. The earlier the time point, the better the specificity and PPV but the worse the sensitivity and NPV for a given HCV-RNA cut-off or log decline value. This translates into the clinical knowledge that the presence of early on treatment HCV-RNA negativity favours subsequent SVR but is not an absolute requirement since a number of patients who achieve HCV-RNA negativity later than week 4 still become sustained responders with continued treatment. This prevents the development of a clinically relevant stopping algorithm based on a single qualitative HCV-RNA level alone before week 12. In this cohort 96.3% of responder patients had a week 4 HCV-RNA below 10^5 IU/ml, the cut-off put forward in Desombere's algorithm.[3] In patients treated with pegylated interferon this cut-off has clinical relevance after 4 weeks treatment duration whereas in Desombere's study in patients treated with short acting standard interferon this cut-off

value appeared to be useful already after one week of therapy. The NPV of 90% of the

week 4 HCV-RNA cut-off of 10^5 IU/ml is insufficient to be used as a reliable stopping

rule. We were able to increase the NPV of an early on treatment qualitative HCV-RNA

level by adding a baseline parameter to our algorithm. Our findings corroborate those

of Desombere et al.[3] in that decreasing baseline anti-NS4a Ab levels correlate with the

absence of a favourable initial viral response to peginterferon and RBV combination

treatment. The mechanism underlying this association is unclear at present. It has

recently been shown that the HCV NS3/4a protease interferes with the innate immune

response against HCV infection by abrogation of the RIG1 and TLR3 pathway.[5-7] It has

also been shown that interferon non responders have high baseline Interferon

Stimulated Genes status (ISGs) without any further increase upon interferon

administration compared to interferon responders.[8] We speculate that the counteractive

interaction of the NS3/4a protease with the innate immune system and the ISGs

response is abrogated in the presence of a high titer of anti-NS4a specific antibodies,

resulting in both better innate response and more favourable viral response to

exogenous administered interferon.

Many baseline factors have been described that correlate with a lower probability to

obtain SVR in genotype 1 patients with peginterferon and RBV treatment. Amongst

these high viral load, advanced fibrotic stage, afro-american descendance, high BMI and

presence of steatosis are recognised baseline factors that make SVR less likely.[9, 10] Low

IP10 interferon gamma inducible protein[11, 12], baseline homozygosity for the promoter

diploy of ACC-IL10 in non-hispanic caucasian patients[13] , and IL28B CC genotype

regardless of descendance[14, 15] correlate with a higher probability to achieve SVR in adherent patients. The presence of baseline factors statistically in disfavour for SVR does not obviate the possibility to achieve SVR in a given patient completely. Withholding therapy on the basis of any of these parameters is thus unjustified and the need for early accurate on treatment detection of non response remains of paramount importance. Our new week 4 stopping rule is based on the presence of a baseline factor that correlates with favourable initial viral kinetics on the one hand, baseline level of anti-NS4a Ab, and on treatment HCV-RNA on the other hand as direct reflection of interferon response and indirect reflection of patient adherence. Our new paradigm will be easy to apply in current practice since week 4 HCV-RNA determinations have become custom management in most centres. This algorithm has a higher specificity and hence detects a larger proportion of non responding patients early on during treatment compared to a recently proposed stopping rule that is based on the absence of a 2 log viral decline and week 4 HCV-RNA >5.5 log IU/ml[16]. It is important that the new, week 4 stopping rule has a 100% NPV and will thus stop therapy only in true non responders. All patients in this study were offered treatment for 48 weeks of treatment, irrespective of the week 12 HCV-RNA results, with the possibility to stop treatment if week 24 HCV-RNA was positive. The new stopping rule is therefore valid for the true occurrence of SVR or no SVR.

Our findings are based on data of a moderate size group and ideally are to be validated in the framework of larger patient groups. It is likely that our stopping rule will need adaptations with the advent of new future direct antiviral agent treatment regimes ,

since the majority of genotype 1 patients treated with these regimes have undetectable

HCV-RNA after 4 weeks of combination therapies with these new agents.[17, 18]

In conclusion, a baseline anti-NS4a Ab titer of < 1/1250 in combination with a week 4

PCR $\geq 10^5$ IU/ml reliably detects non response to 48 weeks of peginterferon and RBV

therapy with a negative predictive value of 100% and does so 8 weeks earlier than the

current week 12 2 log rule.

<u>Appendix:</u>

Steering Committee members of the DITTO-HCV Study are: Carlo Ferrari (Divisione di Malattie Infettive, Azienda Ospedaliera di Parma, Parma, Italy), Avidan U. Neumann (Faculty of Life Sciences, Bar-Ilan UniversityRamat-Gan , Israel), Jean-Michel Pawlotsky (Department of Bacteriology and Virology, Hopital Henri Mondor - Université Paris XII, Creteil, France), Solko W. Schalm and Stefan Zeuzem (Medizinische Klinik II, Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt A.M., Germany).

Tables.

Table 1: Patient characteristics.

Male/Female (%)	70/30
Mean age in years (range)	41 (20-66)
Caucasian (%)	97
Mean baseline ALT (xULN)	2.9
Median HCV-RNA (IU/ml)	1.380.000
# of pts with HCV-RNA >800.000 IU/ml	57/89 (64 %)
# of pts with HCV-RNA <100.000 IU/ml	9/89 (10 %)
# of pts with Ishak fibrosis stage 5-6	7/77 (9%)
# of pts with SVR	54/89 (61%)

Figure Legends.

Figure 1: Baseline anti-NS4a antibody titer (1/n) and early on treatment response type.

A baseline anti-NS4a Ab titer ≥1/1250 correlates with the occurrence of more RVR/SPR versus absence of RVR/SPR in patients with a baseline anti-NS4a Ab <1/1250 (RVR: Rapid Viral Respnose, SPR Slow Partial Response) (p= 0.015).

Figure 2: Multiple logistic regression analysis.

Negative Predictive Value and Specificity of a new algorithm combining early PCR and baseline anti-NS4a serology: the addition of ascending baseline anti-NS4a antibody titer <1/1250 to a PCR cut-off of 100.000 IU/ml at week 4 creates a stopping rule with a specificity of 43% and a NPV of 100% (ITT analysis).

Figure 3: Week 4 stopping rule:

Patients with a baseline anti-NS4a antibody titer <1/1250 and a week 4 viremia ≥100.000 IU/ml have no chance of obtaining SVR with continued treatment and can be advised to stop therapy.

Figure 1: Baseline anti-NS4a antibody titer and early on treatment response type.
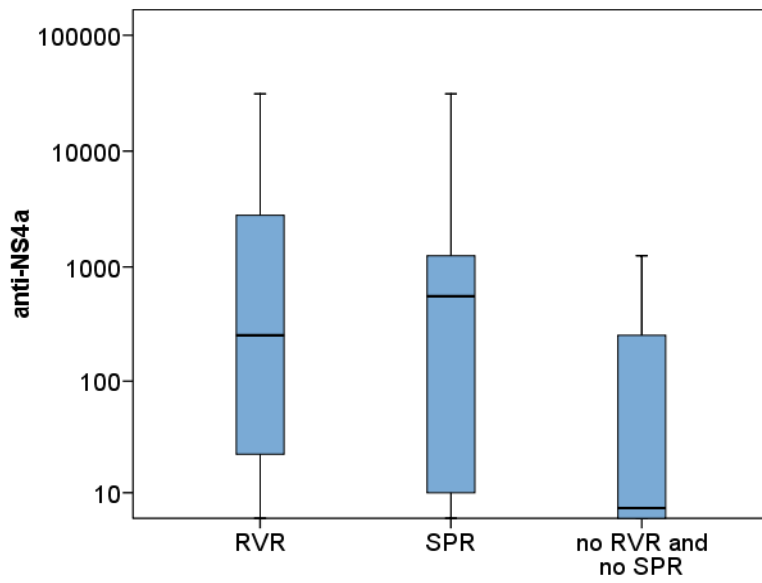
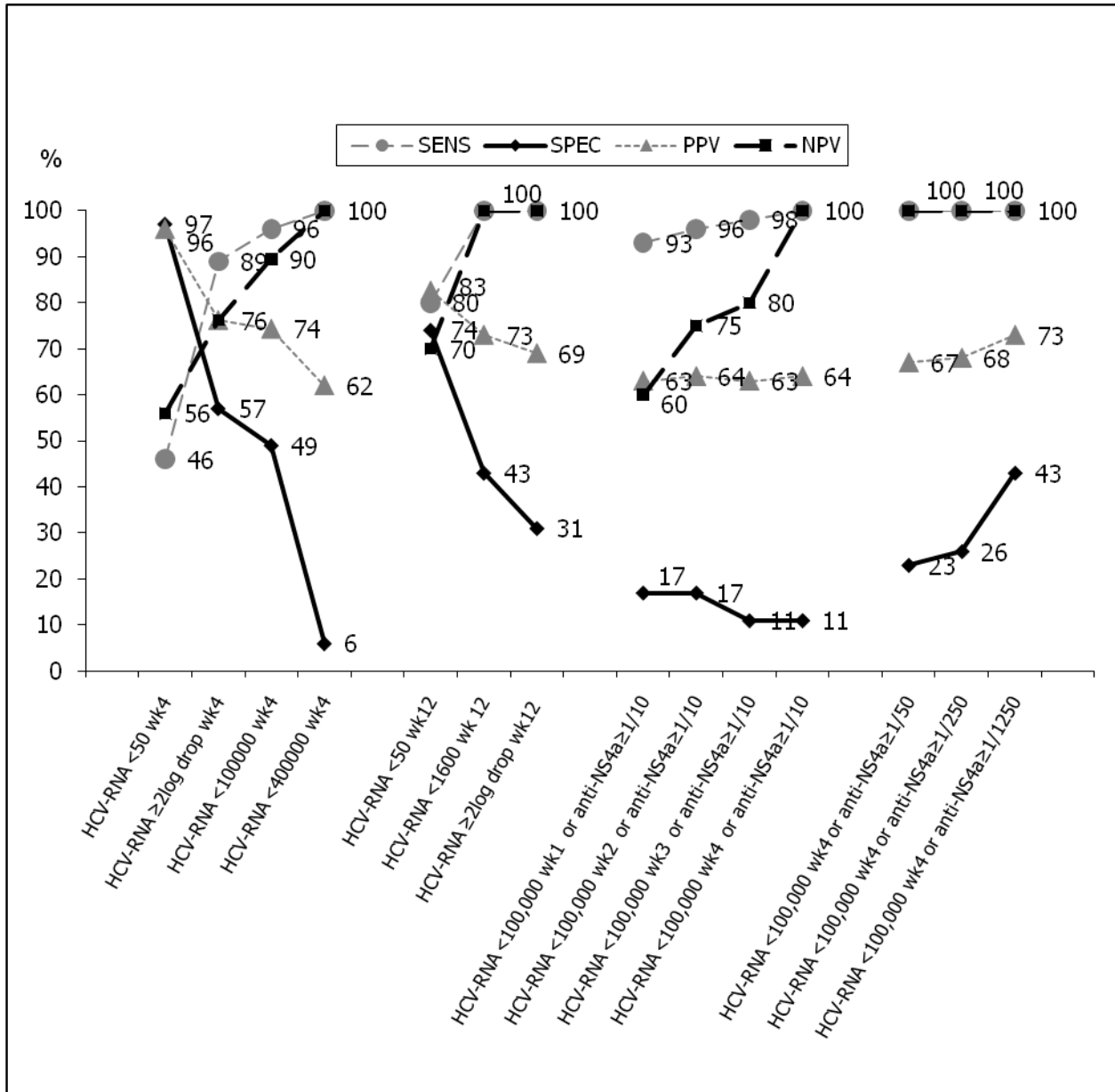**Figure 2**: Multiple logistic regression analysis.

Figure 3: Week 4 stopping rule.

References

1.   Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon Alfa-2a plus Ribavirin for Chronic Hepatitis C Virus Infection. N Engl J Med 2002;347(13):975-82.

2.   Lee S, Heathcote E, Reddy K, et al. Prognostic factors and early predictability of sustained viral response with peginterferon alfa-2a (40KD). J Hepatol 2002;37(4):500.

3.   Desombere I, Van Vlierberghe H, Weiland O, et al. Serum levels of anti-NS4a and anti-NS5a predict treatment response of patients with chronic hepatitis C. J Med Virol 2007;79(6):701-13.

4.   Zeuzem S, Pawlotsky JM, Lukasiewicz E, et al. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol 2005;43(2):250-7.

5.   Li XD, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. Proc Natl Acad Sci U S A 2005;102(49):17717-22.

6.   Li K, Foy E, Ferreon JC, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci U S A 2005;102(8):2992-7.

7.   Meylan E, Curran J, Hofmann K, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 2005;437(7062):1167-72.

8.   Sarasin-Filipowicz M, Oakeley EJ, Duong FH, et al. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci U S A 2008;105(19):7034-9.

9.   Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol 2008;49(4):634-51.

10.   Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49(4):1335-74.

11.   Lagging M, Romero AI, Westin J, et al. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. Hepatology 2006;44(6):1617-25.

12.   Romero AI, Lagging M, Westin J, et al. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and

ribavirin for chronic hepatitis C virus infection. J Infect Dis 2006;194(7):895-903.

13.   Morgan TR, Lambrecht RW, Bonkovsky HL, et al. DNA polymorphisms and response to treatment in patients with chronic hepatitis C: results from the HALT-C trial. J Hepatol 2008;49(4):548-56.

14.   Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461(7262):399-401.

15.   Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461(7265):798-801.

16.   Neumann AU, Pianko S, Zeuzem S, et al. Positive and negative prediction of sustained virologic response at weeks 2 and 4 of treatment with albinterferon alfa-2b or peginterferon alfa-2a in treatment-naive patients with genotype 1, chronic hepatitis C. J Hepatol 2009;51(1):21-8.

17.   McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360(18):1827-38.

18.   Hezode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360(18):1839-50.