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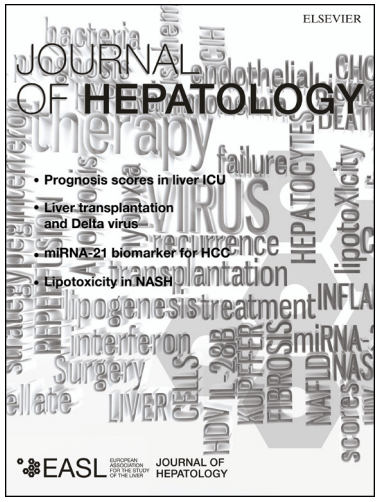

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PII: S0168-8278(13)00446-7
DOI: http://dx.doi.org/10.1016/j.jhep.2013.06.028
Reference: JHEPAT 4782

To appear in: *Journal of Hepatology*

Received Date: 3 April 2013
Revised Date: 30 May 2013
Accepted Date: 26 June 2013

Please cite this article as: Beinhardt, S., Anna Payer, B., Datz, C., Strasser, M., Maieron, A., Dorn, L., Grilnberger-Franz, E., Dulic-Lakovic, E., Stauber, R., Laferl, H., H. Aberle, J., Holzmann, H., Krall, C., Vogel, W., Ferenci, P., Hofer, H., A Diagnostic Score for the Prediction of Spontaneous Resolution of Acute Hepatitis C Virus Infection, *Journal of Hepatology* (2013), doi: http://dx.doi.org/10.1016/j.jhep.2013.06.028

A DIAGNOSTIC SCORE FOR THE PREDICTION OF SPONTANEOUS RESOLUTION OF ACUTE HEPATITIS C VIRUS INFECTION

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Key words: newly acquired HCV; spontaneous clearance; score; IL28B; IP-10.

Abstract word count: 250

Total word count: 4366 (including abstract, - & table legends and references)

Number of tables: 4

Number of figures: 2

Abbreviations: SC: spontaneous clearance; AHC: acute hepatitis C; AUROC: area under the receiver operating curve; HCV: hepatitis C virus; SNP: single nucleotide polymorphism; IP-10: interferon-gamma inducible protein; ALT: alanine aminotransferase; GT: genotype; HIV: human immunodeficiency virus; PPV: positive predictive value; NPV: negative predictive value; DAA: direct acting antivirals

Financial disclosures:

PF is a member of the global advisory board and of the speaker's bureau of ROCHE, Basel CH and Rottapharm-Madaus, Monza, Italy. He is also advisor to Böhringer-Ingelheim, Vertex/Tibotec, Idenix, Achilleon, Glaxo Smith-Kline and MSD and receives an unrestricted research grant from ROCHE Austria and MSD Austria. HH, AM, CD, MS and RS serve as speakers for Roche Austria, MSD Austria, Bristol-Myers Squibb and Janssen Austria. All other authors have no financial disclosures to report.

Author Contributions List:

Sandra Beinhardt: acquisition, analysis and interpretation of data; statistical analysis; drafting of the manuscript.

Berit Anna Payer, Michael Strasser, Emina Dulic-Lakovic, Evelyn Grilnberger-Franz, Hermann Laferl: acquisition of data.

Andreas Maieron: acquisition of data; critical revision of the manuscript for important intellectual content.

Christian Datz: acquisition of data; critical revision of the manuscript for important intellectual content.

Rudolf Stauber: acquisition of data; critical revision of the manuscript for important intellectual content.

Judith H. Aberle: acquisition of data; critical revision of the manuscript for important intellectual content.

Heidemarie Holzmann: critical revision of the manuscript for important intellectual content.

Christoph Krall: statistical analysis.

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Peter Ferenci: critical revision of the manuscript for important intellectual content.

Harald Hofer: study concept and design; analysis and interpretation of data; drafting of the manuscript.

ABSTRACT

BACKGROUND & AIMS: IL28B-polymorphisms, jaundice, decline in HCV-RNA, IP-10 and gender have been proposed to be indicative for spontaneous clearance of acute hepatitis C virus infection. Aim of this study was to define a score enabling the discrimination of patients with spontaneous clearance of HCV from those with development to viral persistence and need for early antiviral treatment.

PATIENTS & METHODS: 136 patients (male:74;35±15years) were analyzed. From variables predictive for spontaneous clearance, calculated by univariate analysis, three scores were built. Analogous cutoffs were evaluated by computing area under the receiver operating characteristic curves. Candidate variables and cutoffs were: (I)presence of IL28B-C/C (P=0.027), (II)age (P=0.031;cutoff:35years), (III)peak-bilirubin (P=0.018;cutoff:6mg/dl), (IV)HCV-RNA-decline within 4 weeks (P<0.001;cutoff:>2.5log), (V)serum IP-10 (P=0.003;cutoff:546pg/ml), (VI)presence of CD4(+)Th1-cells (P=0.024). Each variable was allocated to 0 or 1 point, an HCV-RNA-decline of ≥1log^{10} but <2.5log^{10} 1 point, a decline of ≥2.5log^{10} to 2 points. Three scores were evaluated (Score1:I-IV; Score2:I-V; Score3:I-VI).

RESULTS: A cutoff of ≥3 points out of 5 in Score1 (AUROC:0.82; DeLong95%CI:0.76-0.93) predicted spontaneous clearance with a sensitivity of 71% (95%CI:0.53-0.86) and specificity of 87% (95%CI:0.73-0.95). PPV and NPV were 79% and 82%. Corresponding findings for Score2 including IP-10 (AUROC:0.93; DeLong95%CI:0.86-0.93) at a cutoff ≥4 were: sensitivity 81%, specificity 95% (PPV:100%;NPV:77%). A cutoff of ≥5 in Score3 (AUROC:0.98; DeLong95%CI:0.95-1.0) predicted spontaneous resolution with a sensitivity of 75% and specificity of 100% (PPV:100%;NPV:88%).

CONCLUSION: The scores enable a reliable discrimination between AHC-patients with high potential for spontaneous clearance from candidates for early therapeutic intervention due to marginal chance of spontaneous resolution.

INTRODUCTION

Chronic infection with hepatitis C virus (HCV) remains a major global health burden. Hepatitis C virus infection commonly runs asymptomatic with only a minority of patients presenting with symptomatic, acute hepatitis C (AHC). Spontaneous viral resolution occurs in about 20-40% of infected patients. Considerable evidence has accumulated that particularly those patients with an icteric, symptomatic course of AHC have higher chance to resolve HCV infection spontaneously [1,2,3,4,5,6,7,8]. In this respect, it was shown that a fast decline of HCV-RNA during early stages of infection is indicative for viral clearance [9]. As a genetic marker a single nucleotide polymorphism (SNP) on chromosome 19 (rs12979860) near the IL28B-region was found to be associated with spontaneous resolution of AHC [8,10,11,12]. Moreover it was reported, that in the early stages of hepatitis C infection the appearance of HCV specific CD4(+)Th1 cells is more common in patients with consecutive viral clearance [13]. Recently, high serum levels of interferon-gamma inducible protein 10 (IP-10) were also shown to be associated with spontaneous resolution of acute HCV infection [8,14,15].

Taken together, spontaneous clearance of HCV is dependent on both, host as well as pathogen related factors [16,17,18,19]. Nevertheless, a proportion of patients even with acute symptomatic presentation, develop chronic infection and therefore optimal therapy strategies to prevent this evolution are still under debate. Early antiviral therapy for AHC has been shown to exert a favorable outcome [20,21] but is associated with substantial toxicity and costs and is dispensable in patients who would clear the virus spontaneously during the course of the disease.

However, discussion is ongoing if delayed treatment-initiation impairs response to antiviral treatment [22]. A study performed in Germany suggested comparable outcome for both, the early treatment intervention and the delayed initiation of antiviral therapy [23].

Hence, aim of this study was to develop a simple and reliable score, based on clinical and laboratory parameters, found to be predictive for spontaneous viral resolution, to discriminate patients in need of early antiviral treatment from those who could be monitored by watchful waiting due to high probability of SC.

PATIENTS AND METHODS

Patients

This retrospective study included 136 Caucasian patients (male: 74 [54.4%]; mean age at time of infection: 35±15 years) with proven acute hepatitis C virus infection, diagnosed in Austrian tertiary referral centers. AHC was defined either by an elevation of alanine aminotransferase (ALT) serum-levels of more than 5 times upper limit of normal (ULN) with or without jaundice in anti-HCV antibody and HCV-RNA positive patients (N=103) with exclusion of chronic hepatitis C and exclusion of other causes of transient hepatitis. If ALT serum-levels were less than 5 times ULN documented sero-conversion was present in all cases. History of other liver diseases was excluded in all patients (patients characteristics are shown in table 1).

Spontaneous clearance was defined as undetectable HCV-RNA and anti-HCV antibody sero-positivity; persistence of HCV infection was defined as anti-HCV antibody sero-positivity and detectable HCV-RNA during a follow-up period of more than 6 months. Due to low serum HCV-RNA at diagnosis HCV-genotyping was not feasible in all patients. The study was approved by the local ethics committee.

Specimen Collection

Serum samples obtained were stored at -80°C until analysis. For genetic testing, patients were recalled in some cases and, if they agreed, EDTA blood was drawn. EDTA blood was centrifuged at 1500g for 20 minutes at room temperature within 4 hours of venipuncture, and aliquots were immediately frozen after centrifugation at -80°C until testing was performed.

Virological testing & testing for IL28B rs12979860

HCV-RNA was tested with the qualitative and quantitative COBAS Amplicor Monitor 2.0® (Roche Molecular Systems, Branchburg, NJ; lower limit of detection 50 IU/ml; lower limit of quantification 600 IU/ml) and since 2007 with COBAS AmpliPrep™/COBAS TaqMan® (Roche Diagnostics, Pleasanton, CA; lower limit of detection 15 IU/ ml). Retesting of available sera with COBAS AmpliPrep™/COBAS TaqMan® did not affect diagnostic performance of the score. HCV genotype was determined using the VERSANT HCV Genotype [GT] 2.0 Assay (LiPA; Siemens Medical Solutions Diagnostics, Tarrytown, NY). For IL28B rs12979860 determination genomic DNA was isolated from peripheral blood according to the QIAmp DNA Blood Mini Kit from Qiagen (Hilden, Germany) and SNP rs12979860 in the region of the IL28B gene was analyzed as described before [8]. All assays were performed according to manufacturer´s instructions.

Testing for IP-10 and HCV-specific CD4(+)Th1 Cells

IP-10 concentrations in serum samples were measured by a commercially available enzyme-linked immune-sorbent assay (BD OptEIA Set Human IP-10; Becton Dickinson Biosciences, San Diego, USA). Each sample was tested both undiluted as well as diluted 1:10 and 1:50. As described previously [8], the relationship between diluted and undiluted samples was not linear, but piecewise linear, with increasing deviation from undiluted values with increasing concentration. The cut-off points for the pieces of linearity were 350 pg/ml and 1625 pg/ ml, respectively.

For samples with IP-10 concentrations above 350 pg/ml or 1625 pg/ ml, 1:10 or 1:50 dilutions were used to estimate the undiluted concentration from the reverse function. In

50 (37%) out of 136 patients (male: N=32 [64%]) IP-10 was available at initial presentation. HCV-specific CD4(+)Th1-cell responses were assessed in unfractionated whole blood by cytokine-production (TNF-α) upon re-stimulation with recombinant NS3 and NS4 proteins derived from GT1a, as described previously [13]. In 40 (29%) mono-infected patients HCV-specific antiviral CD4(+)Th1 cells could be determined (male: 24 [60%]) at first presentation.

Statistical Methods

Database management and statistical analyses were performed using commercially available software systems (Microsoft Office Excel 2010; Microsoft Corp, Redmond, WA; SPSS 2012 for Mac; version 20, SPSS Inc., Chicago, IL) as well as the open source program R2.10.1. (http://www.r-project.org). Continuous variables were expressed either as mean (±SD) for Gaussian distributions or median (range) for non-Gaussian distributions. Kolmogorov-Smirnov test was applied to determine whether continuous variables were normally distributed. Continuous variables were analyzed by using the Mann-Whitney-*U-test or the Student´s T* test where appropriate. Categorical variables were given as absolute and relative (in percent) frequencies. Relationship between categorical variables was investigated by Chi-square tests. All statistical tests were two-sided and used a significance level of $P<0.05$. For model building to predict spontaneous resolution univariate analysis for variables, thought to be associated with SC, was performed.

These variables were: age at infection, sex, IL-28B rs12979860 C/C genotype, peak bilirubin (mg/dl) and alanine aminotransferase (ALT; U/l), HCV-genotype (1,4,6 vs. 2,3), IP-10 (pg/ml; log^{10}), CD4(+)Th1 cells responding to HCV-AGs as well as HCV-RNA decline within the first 4 weeks (IU/ml log^{10} from time point of first presentation till week 4). Furthermore AUROCs were computed to identify analogous cutoffs for each continuous variable predictive for SC as indicated by univariate analysis (table 2A; figure1).

Consecutively three different scores by adding 0 or 1 point according to computed cutoff values (table 3; A-C) were built. Patient's aged ≤35 years receive 1 point, patients older than 35 years 0 points. Patients with a serum bilirubin ≥6 mg/dl receive1 point, <6 mg/dl are assigned 0 points. Patients with an HCV-RNA decline of ≥1log^{10} but <2.5log^{10} within 4 weeks after diagnosis were scored with 1 point and 1 additional point for a HCV-RNA decline of ≥2.5log^{10}. Patients with an increase of HCV-RNA or an HCV-RNA decline <1log^{10} were assigned to 0 points respectively. For the binary variables "IL28B C/C" and "detectability of HCV-specific CD4(+)Th1 cells" 1 additional point was allocated as well (table 3; B-C). For calculated scores again AUROCs were computed (table 2B). These were compared to AUROCs of each single significant parameter for SC by means of DeLong test.[24] Sensitivities, specificities and corresponding 95% Clopper-Pearson confidence intervals for each score as well as positive and negative predictive values were calculated accordingly (table 4).

RESULTS

Patient characteristics and clinical presentation

Seventy-four (54%) of all patients and twenty-nine (55%) of patients with SC were male. HCV-genotyping could be performed in 109 patients (80%) as follows: HCV-GT 1: 67 (61%); GT2: 6 (6%); GT3: 30 (28%); GT4: 4 (3%), GT6: 2 (2%), mixed GT (2/4): 1 (1%; [1]). Spontaneously clearing patients were younger than patients with development of viral persistence (31±13 [17-81] vs. 37±16 [16-81] years; mean±SD [range]; P=0.031). Quantitative HCV-RNA levels at first presentation were higher in patients developing chronic HCV infection than in patients with SC (5.2±1.4 vs. 4.3±1.7 $\log^{10}$ IU/ml; mean±SD; P=0.002). HCV-RNA decline at week 4 after first presentation was stronger in patients with spontaneous resolution of AHC (2.7±2.3 vs. 0.7±1.6 HCV-RNA decline $\log^{10}$ in IU/ml; mean±SD; P<0.001). Peak levels of bilirubin were higher (6.6±6.3 vs. 4.0±5.0 mg/dl; mean±SD; P=0.018) and IP-10 levels were lower in spontaneous clearers compared to those with development of chronic HCV infection (417 [113 - 2232] vs. 1355 [141 - 4412] pg/ml; median [range]; P=0.002). HCV-specific antiviral CD4(+)Th1 cell response was detectable in 87.5% of patients spontaneously resolving AHC but only in 50% of patients developing viral persistence (P=0.02). Mean ALT elevation of included patients was 27.2 ± 25.1 (range: 0.8 – 125.1) and ALT levels were not different between patients who cleared the virus spontaneously and patients with evolution of chronicity.

Thirteen (9.6%) patients were co-infected with human immunodeficiency virus (HIV), two (1.5%) patients with hepatitis B virus. Patient's demographic data are given in table 1.

Predictive Parameters for spontaneous clearance & Cutoffs

Variables predictive for SC computed by univariate analysis were: IL28B (rs12979860) C/C-genotype (P=0.027), age at infection (P=0.031), detectability of HCV-specific CD4(+)Th1 cells (P=0.024) and serum IP-10 levels (pg/ml; P=0.003) at time of first presentation as well as peak bilirubin levels (mg/dl; P=0.018) and HCV-RNA decline till week 4 after diagnosis (log^{10}; P<0.001) as well. By calculating predictive parameters for SC separately for HCV mono-infected patients, no difference could be detected.

Female sex, ALT serum-levels as well as HCV-genotype did not show any significances (table 1). Appropriate cut-offs for variables found to be predictive for SC were: age at infection <35 years, peak bilirubin >6 mg/dl, HCV-RNA decline till week 4 after diagnosis ≥2.5 (log^{10}) as well as serum IP-10-levels <546.0 pg/ml (binary variables excluded; table 2A; Supplementary material, figure 1).

Development of Scores

According to computed cutoff-levels, evaluated to be predictive for SC, Score1 includes following parameters: IL28B rs12979860 C/C-genotype, age at infection, peak bilirubin and HCV-RNA decline till week 4. Although IP-10 and detectability of HCV-specific CD4(+)Th1 cells are not routinely determined variables, but nevertheless strengthened statistically significance, we added them up to build Score2 and Score3 in a stepwise manner. Score1 is built at week 4 after diagnosis by 1 point for each criterion (see table 3 A, B and C). For Score2 an additional point is added if IP-10 serum levels are <546 pg/ml; for Score3 an additional point is added if HCV-specific CD4(+)Th1 cells were detectable at first presentation as well (see table 3C).

Accordingly AUROCs could be computed as follows: Score1: 0.863, Score2: 0.914 and Score3: 0.973 respectively (figure 2). Comparing areas under the curve of all evaluated scores, increased predictability for spontaneous resolution of AHC, compared to individual AUROCs of variables found to be significant by means of univariate analysis (figure 1 and 2). The “simple” diagnostic score (Score1) with a cutoff of ≥3 points reveals a sensitivity for SC of 71% and a specificity of 88% with a PPV of 79% and NPV of 82% (table 4), allowing to recognize patients with high probability for development of chronicity (2 points – NPV: 92%; 1 point – NPV: 100%).

The more comprehensive scores, including IP-10 (Score2) and HCV-specific CD4(+)Th1 cell-response (Score3) and their cutoffs of ≥4 and ≥5 points increased PPV for spontaneous clearance up to 100% (table 4).

DISCUSSION

In view of high sustained virologic response (SVR) rates reached by early initiation of antiviral therapy in patients with acute hepatitis C virus infection, identification of patients probably not clearing HCV spontaneously, is of particular importance, as a delayed start of treatment may diminish efficacy of antiviral therapy [5,19,25,26,27,28]. Thus optimal timing and an appropriate patient selection are crucial in the treatment of patients with acute hepatitis C and still cause controversy to some extent [29]. As symptomatic presentation of acute hepatitis C virus infection is infrequent and hence it is difficult to conduct adequately powered prospective randomized clinical trials; a recent study addressed this issue by building a mathematical modeling [30].

In our study we aimed to develop a simple score (Score1) to facilitate a diagnostic tool for the differentiation of patients with high probability for SC from those in need for early antiviral treatment. Patients with 3 and more points could be managed by watchful waiting, as they showed a high likelihood to resolve AHC infection and an unnecessary therapy, causing costs and side effects, could be avoided. On the other hand, patients with less than 3 points should be considered for early antiviral therapy, as there is a marginal chance that AHC resolves spontaneously. As calculation of the proposed scores is already available at week four after first diagnosis of AHC, antiviral treatment could be started early in these patients, which might have beneficial effects on HCV eradication rates. Moreover, patients often prefer the “more active approach” of beginning antiviral therapy than just follow the natural course of the disease [23]. Thus, patients might be lost to follow-up by a prolonged observational period before treatment even can be initiated.

All scores, whether they are preferentially clinically orientated (Score1) or more scientific (Score2 and Score3) can be calculated very early during follow-up period at week 4 and therefore might improve patients concealing as well as compliance.

Although IP-10 is not yet a routinely tested variable, it´s role as a marker for the prediction of spontaneous resolution of AHC becomes more and more evident [8,14,15]. By adding IP-10 as additional variable (Score2) specificity and PPV for SC could be increased up to 95% and 100%, favoring an watchful waiting strategy in patients with an high aggregate score. By adding HCV-specific CD4(+)Th1 cells the performance could be even more improved. However determination of HCV-specific CD4(+)Th1 cells remains a time consuming and costly procedure and is therefore hardly useful in everyday clinical practice.

One major drawback of this study is the retrospective character as well as the relatively low number of patients within subgroup analysis, reflecting the rarity of acute hepatitis C as discussed above. Thus, our data should be prospectively validated in other large patient groups.

Our scoring system does not include HCV-genotype, as it apparently does not influence rates of spontaneous clearance in our cohort. Nevertheless, HCV-genotypes carry substantial impact on the clinical approach to patients with acute hepatitis C virus infection: in view of high sustained virological response rates reported in chronically infected HCV-genotype 2 and 3 patients, one could postulate that there is no need to treat these patients during the acute phase of infection. And even in chronic HCV-genotype 1 patients advances in antiviral treatment are fast, with SVR rates similar to the high HCV eradication-rates reported during acute hepatitis C.

So far no data about the use of direct acting antivirals or interferon-sparing regimens in acute hepatitis are available. In the recently presented pivotal German HEP-NET acute HCV-III study Deterding et al. confirmed that early treatment with (peg)IFNalpha-2b alone has similar efficacy as delayed combination treatment with interferon and ribavirin [23]. One could speculate that triple therapy could increase the response rates in future, however, in view of increased toxicity and costs a careful selection of patients who are in need of antiviral therapy is even more important.

In summary we developed an easily to compute scoring system for daily routine clinical practice, which may help to differentiate patients with high probability of spontaneous clearance, from those who could benefit from an early antiviral treatment approach.

FIGURE-LEGENDS

Fig. 1.: AUROCs of variables predictive for spontaneous clearance in AHC

AUROCs for the predictive variables: age at infection, peak bilirubin, HCV-RNA-decline (IU/ml log^{10}) till week 4 after first presentation and IP-10 (pg/ml) as well.

Fig. 2.: AUROCs of the different Scores for predictability of spontaneous clearance in AHC

SUPPLEMENTARY MATERIAL

Fig. 1.: Plots of cutoffs of variables predictive for spontaneous clearance in AHC

Plots of the Euclidean distance of the AUROCs of figure 1 against all cutoff values for all variables. x-axes: variable predictive for SC; y-axes: Euclidean distances; Peak Bilirubin (mg/dl); Log Drop WE4: HCV-RNA (IU/ml; log^{10}) decline from first presentation till week 4; IP-10: serum levels of IP-10 in pg/ml (log^{10}).

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Figure 1:

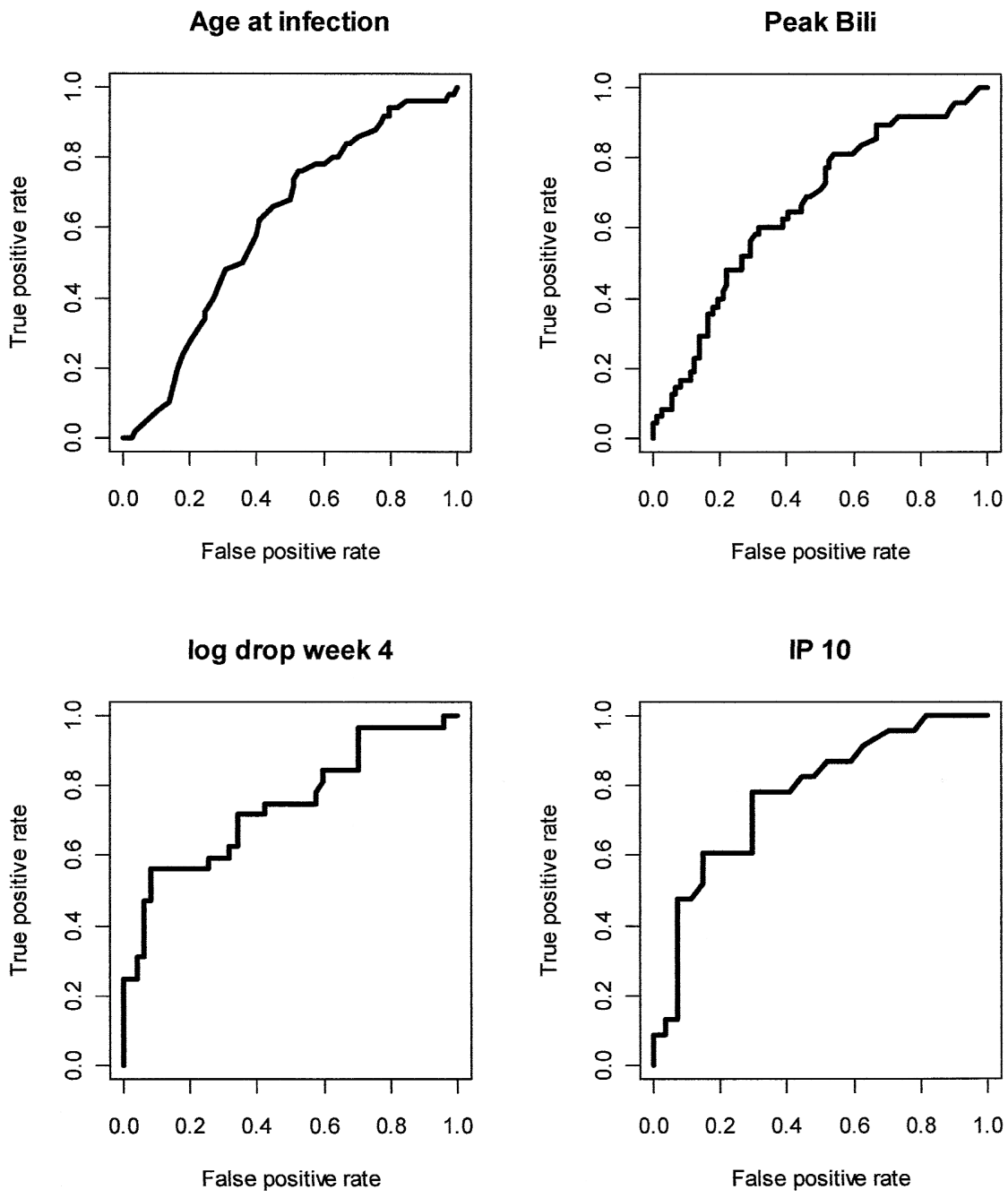


Figure 2: AUROCs of the different Scores for predictability of spontaneous clearance in AHC

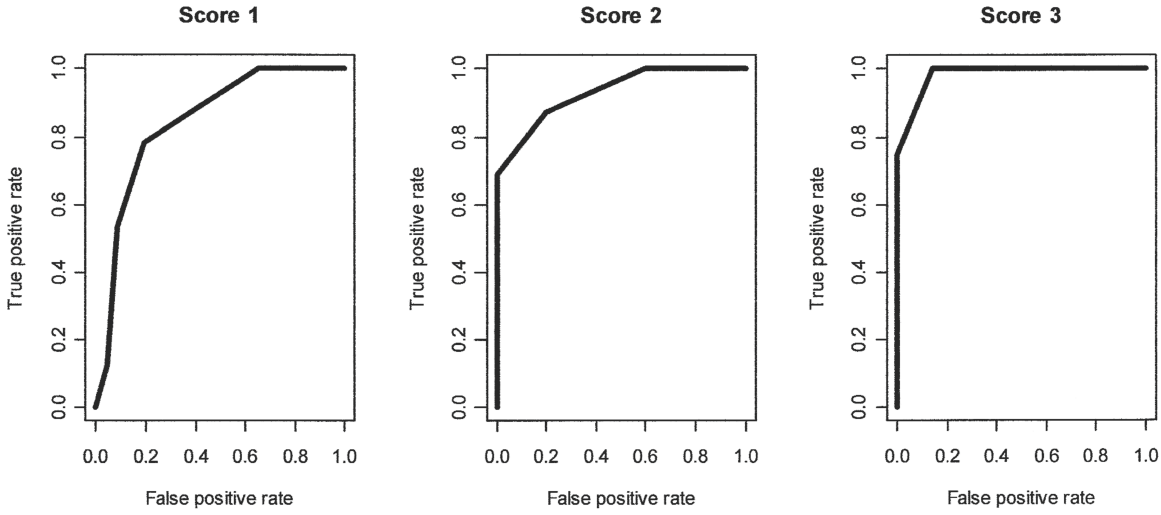
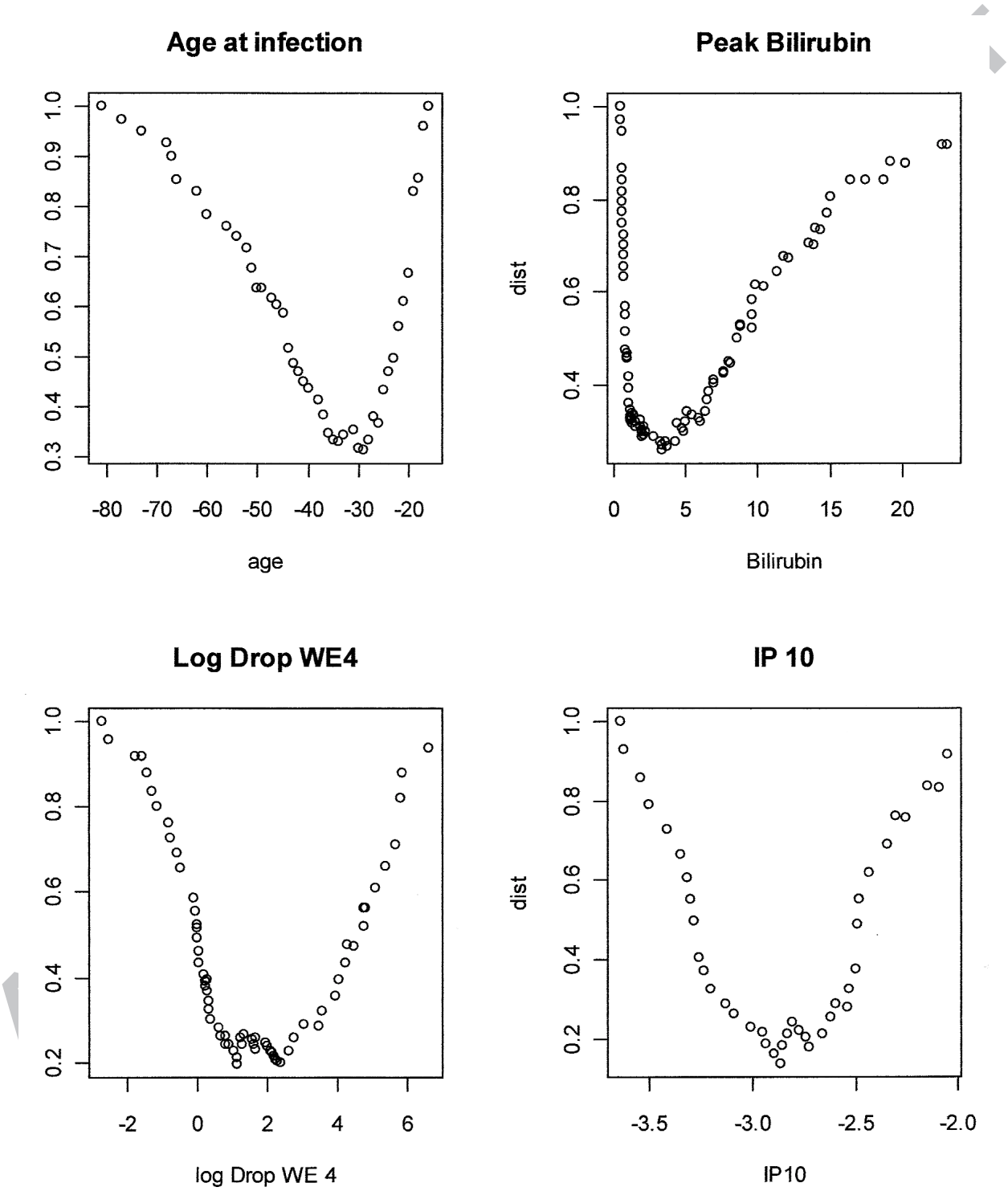


Figure 3:



TABLES

Table 1.

Patients Characteristics	All	SC	no SC
N (%)	136	53 (39)	83 (61)
Male, N (%)	74 (54)	29 (55)	45 (54)
Age at infection (years), mean±SD (range)[1]	35 ± 15 (16-81)	31 ± 13 (17-81)	37 ± 16 (16-81)[$]
Source of infection; N (%)			
IVDA/nasal DA	62 (46)	28 (53)	34 (41)
unknown	32 (24)	12 (23)	20 (24)
iatrogenic	23 (17)	6 (11)	17 (20)
sexual transmission	17 (13)	7 (13)	10 (12)
plasma donation	1 (1)	0 (0)	1 (1)
tatoo/piercing	1 (1)	0 (0)	1 (1)
HCV genotype; N (%)			
determined	109 (80)	37 (70)	72 (87)
1	67 (61)	26 (70)	41 (57)
2	6 (6)	2 (5)	4 (6)
3	30 (28)	8 (22)	22 (31)
4	3 (3)	1 (3)	2 (3)
6	2 (2)	0 (0)	2 (3)
mixed (2a/4)	1 (1)	0 (0)	1 (1)
IL28B rs12979860; C/C-genotype, N (%)	87 (64)	40 (75)	47 (53)
HCV-RNA (IU/ml; log[10])*			
at presentation[2]	4.9 ± 1.6	4.3 ± 1.7	5.2 ± 1.4[¢]
logDrop week 4[3]	1.5 ± 2.2	2.7 ± 2.3	0.7 ± 1.6[¥]
Bilirubin x ULN[+] (mg/dl; [range])*;[4]	4.2 ± 4.8 [0.3 - 19.3]	5.5 ± 5.2 [0.4 - 19.3]	3.3 ± 4.4 [0.3 - 19.0]
ALT x ULN[-] (IU/l)*;[5]	27.2 ± 25.1 [0.8 - 125.1]	34.5 ± 29.0 [0.8 - 125.1]	22.5 ± 21.1 [1.0 - 103.8]
IP-10 (pg/ml; median [range])[6]	718 (113 - 4412)	417 (113 - 2232)	1355 (141 - 4412)[§]
HCV-specific CD4(+)Th1Cells at diagnosis[7]			
detectable	26 (65)	14 (87.5)	12 (50)[€]
not detectable	14 (35)	2 (12.5)	12 (50)

Tab. 1.: SC: spontaneous clearance; IVDA: intravenous drug abuse; DA: drug abuse; *: mean ± SD; [+]: ULN Bilirubin 1.2 mg/dl; [-]: alanine aminotransferase (ULN: females 34 U/ml, males 45 U/ml); 1: N=128; 2: N=121; 3: N=79; 4: N=120; 5: N= 122; 6: N=50; 7: N=40; ***$:*** P=0.031 no SC vs. SC; ***£:*** P=0.002 no SC vs. SC; ***¥:*** P<0.001 in SC vs. no SC; ***€:*** P=0.018 in patients with SC vs. non SC; ***§:*** P=0.002 SC vs. non SC; **¢:** P=0.02 SC vs. non SC.

Table 2.

A
AUROCS & Cutoffs - variables predictive for SC in AHC

Variables	AUROC (95%DeLong CI)	Cutoff	$(1\text{-spec})^2 + (1\text{-sens})^2$
Age at Infection (years)	0.610 (0.511 - 0.708)	35	0.313
Bilirubin (mg/dl)	0.660 (0.561 - 0.760)	6	0.367
HCV-RNA logDrop at Week4	0.744 (0.629 - 0.859)	2.5	0.199
IP-10 (pg/ml)	0.772 (0.639 - 0.905)	546	0.175

B
AUROCs for different Scores

Score	AUROC (95%DeLong CI)
Score 1	0.86 (0.75 - 0.93)
Score 2	0.91 (0.82 - 1.00)
Score 3	0.97 (0.92 - 1.00)

Tab. 2.: ***A & B.:*** SC: spontaneous clearance; AHC: acute hepatitis C; AUROC: area under the receiver operating curve; CI: confidence interval; HCV-RNA logDrop: HCV-RNA decline IU/ml $\log^{10}$ from diagnosis till week 4.

Table 3.

A

Score1 for SC in AHC

Variable	Cutoff	Points
IL28B rs12979860	C/C	1
Age at Infection	≤ 35	1
Bilirubin (mg/dl)	≥ 6	1
HCV-RNA logDrop from diagnosis till Week 4		
	increase - logDrop ≤ 1.0	0
	logDrop ≥ 1.0 - < 2.5	1
	logDrop ≥ 2.5	2
Maximum		5

B

Score2 for SC in AHC including IP-10

Variable	Cutoff	Points
IL28B rs12979860	C/C	1
Age at Infection	≤ 35	1
Bilirubin (mg/dl)	≥ 6	1
HCV-RNA logDrop from diagnosis till Week 4		
	increase - logDrop ≤ 1.0	0
	logDrop ≥ 1.0 - < 2.5	1
	logDrop ≥ 2.5	2
IP-10 (pg/ml)	< 546	1
Maximum		6

C

Score3 for SC in AHC including IP-10 & CD(4+) Th1 Cells

Variable	Cutoff	Points
IL28B rs12979860	C/C	1
Age at Infection	≤ 35	1
Bilirubin (mg/dl)	≥ 6	1
HCV-RNA logDrop from diagnosis till Week 4		
	increase - logDrop ≤ 1.0	0
	logDrop ≥ 1.0 - < 2.5	1
	logDrop ≥ 2.5	2
IP-10 (pg/ml)	< 546	1
HCV-specific CD4(+) Th1 cells	detectable	1
Maximum		7

Tab. 3.: ***A – C.:*** SC: spontaneous clearance; AHC: acute hepatitis C; HCV-RNA logDrop: HCV-RNA decline IU/ml $\log^{10}$ from diagnosis till week 4.

Table 4.

Sensitivity & Specificity; Positive & Negative Predictive Values of different Scores

Score	Cutoff	Sensitivity	Exact 95% CI*	Specificity	Exact 95% CI*	PPV	Exact 95% CI*	NPV	Exact 95% CI*
Score 1	≥ 3	71%	53% - 86%	87%	73% - 95%	79%	60-92%	82%	68% - 91%
Score 2	≥ 4	81%	54% - 96%	95%	75% - 100%	100%	-	77%	66% - 97%
Score 3	≥ 5	75%	35% - 97%	100%	-	100%	-	88%	62% - 98%

Tab. 4.: *: Clopper Pearson confidence interval.