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Short communication

Rapid prediction of sustained virological response in patients chronically infected with HCV by evaluation of RNA decay 48 h after the start of treatment with pegylated interferon and ribavirin

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ABSTRACT

The combination of pegylated interferons (PEG-IFNs) and ribavirin represents the standard of care for the treatment of chronic HCV-infected patients, yet with a success rate around 50% in genotypes 1 and 4, high costs and side effects. Therefore, early prediction of sustained virological response (SVR) is a relevant issue for HCV-patients. We evaluated the association between SVR and decline of HCV-RNA at 48 h in a prospective cohort of 145 HCV-patients treated with PEG-IFNs and ribavirin (males = 69.1%; genotypes 1/4 = 51.0%; HIV-1 coinfected = 6.7%). SVR was obtained in 65.5% of patients, while 16.6% experienced relapse and 17.9% no response. The first-phase of HCV-RNA decline clearly differentiated patients with SVR from relapsers and non-responders, independently of genotype ($P < 0.001$). In univariate and multivariate analyses, different infralogaritmic thresholds of HCV-RNA decay at 48 h were tested, observing the highest predictive potential at 0.5 log: decays above this threshold showed a 76.2% negative predictive value for SVR, whereas decays >0.5 log indicated a 6.8 odds ratio (95% C.I.: 2.0–23.2) for SVR after controlling for genotype, baseline viremia, adherence to therapy and HIV coinfection. Decays beyond the 0.5 log threshold were also strongly associated with and highly predictive of early virological response (95.0% positive predictive value, $P < 0.001$).

The combination of pegylated interferons (PEG-IFNs) and ribavirin (RBV) represents the universal standard of care for the treatment of HCV-infected patients, yet with an overall success rate around 50%, remarkable costs and side effects (Hoofnagle and Seeff, 2006). Therefore, the early prediction of treatment outcome is a relevant issue for HCV-patients, especially those with low a priori chances of success (Berg et al., 2003; Layden-Almer et al., 2006; Boulestin et al., 2006; Shirakawa et al., 2008). Indeed, the rapidity of virological response is now a well-known independent predictor of sustained virological response (SVR, Hoofnagle and Seeff, 2006), which prompted an extensive investigation of the relationship between early kinetics of HCV decay and SVR (Jessner et al., 2001; Berg et al., 2003; Hoofnagle et al., 2009; Arends et al., 2009; Nomura et al., 2009; Durante-Mangoni et al., 2009). The decay of HCV-RNA 48 h after therapy start has been suggested as an independent predictor of SVR (Arends et al., 2009; Nomura et al., 2009; Durante-Mangoni et al., 2009). A decay of HCV-RNA ≤0.8 log at 48 h indicated a 95% negative predictive value (NPV) for SVR in HCV monoinfected patients (Durante-Mangoni et al., 2009). We performed a similar investigation, exploring different HCV-RNA decay thresholds at 48 h for the prediction of SVR and early virological response (EVR) in another Italian prospective cohort.

This investigation started in 2001 at the Infectious Disease Unit, Pescara Hospital, Italy, after approval by the local Ethics Committee. The primary aim was to evaluate the ability of HCV-RNA measurements at 48 h after starting treatment to predict SVR. Patients chronically infected with HCV aged ≥18 years, with detectable HCV-RNA, either previously untreated or relapser to monotherapy with unpegylated interferon, were eligible in accordance with standards of care (Hoofnagle and Seeff, 2006). HIV coinfected patients eligible for treatment of HCV were also offered participation. All participants accepted to return 48 h after the first IFN dose for HCV-RNA measurement. Exclusion criteria were: active abuse of drugs and/or alcohol and autoimmune or thyroid disorders. PEG-IFN alfa-2a (180 mg/w) or alfa-2b (1–1.5 mg/kg/w), were prescribed with ribavirin (800 mg/qd for genotypes 2/3; 1000–1200 mg/qd for

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genotypes 1/4) by attending physicians. Standard duration of treatment was 48 weeks for genotypes 1/4, 24 weeks for genotypes 2/3. Treatment was discontinued after 12 weeks in the absence of ≥2 log HCV-RNA drop or in the presence of detectable HCV-RNA after 24 weeks. PEG-IFN and/or ribavirin were reduced when necessary in accordance with current recommendations (Hoofnagle and Seeff, 2006). Dose or duration reductions for causes other than stopping rules were recorded in accordance with the 80/80/80 compliance threshold (McHutchinson et al., 2002). SVR was defined as undetectable (<50 UI/mL) HCV-RNA 24 weeks after treatment (Hoofnagle and Seeff, 2006). Age, gender, genotype (INNO-LiPA; Innogenetics), ALT and gGT levels were recorded at baseline. Samples collected at the same hour both on the day of therapy start and after 48 h were quantified by using Cobas® Amplicor HCV-Monitor2.0 until May 2007 and by Cobas® TaqMan HCV Test thereafter. Until May 2007, negative samples were further checked by the qualitative Cobas® Amplicor HCV-RNA Test, with lower limit of detection 50 UI/mL. For each patient, paired baseline and 48 h samples were processed in the same analytic session to minimize the potential influence of inter-assay variation. Further samples were evaluated at 90 and 180 days, at the end of and 24 weeks after treatment. Early HCV-RNA decay was calculated as the logarithmic difference between baseline and 48 h HCV-RNA values for each patient. The degree of HCV-RNA decline (Δ(0–48 h) log 10 HCV-RNA values) was evaluated according to treatment outcomes (EVR, SVR, relapse, and non-response), stratifying by HCV-genotype, using Wilcoxon rank-sum test. Multiple logistic regression models were performed to evaluate potential predictors of SVR and EVR, including all variables significantly associated in univariate analyses. Serial infralogaritmic cut-offs of Δ(0–48 h) Log 10 HCV-RNA decline from 0.5 to 1 log, increasing by 0.1 log (log transformation based upon Shapiro–Wilk test) were evaluated for their positive and negative predictive values (PPV and NPV, respectively) for SVR and EVR and included in the final multivariate models. Collinearity among included covariates was checked using Spearman correlation coefficient. All analyses used a two-tailed P-value = 0.05 and were performed using Stata10.1 (Stata Corp., Texas, 2007).

One hundred ninety-two patients were enrolled. Twenty patients did not return at 48 h; only qualitative assays were performed at 48 h for 23 additional patients; four patients were lost during follow-up. Therefore, 145 patients were available for SVR prediction: mean age was 42.9 ± 12.4 years, 69.1% were males and 51.0% with genotype 1/4 (only 4 patients had genotype 4); 6.7% were HIV coinfected. SVR was obtained in 65.5% of patients, whereas 16.6% experienced relapse, and 17.9% showed no response. Median baseline HCV-RNA values (5.62 log IU/ml, [IQR 5.13–6.11]) were very similar across groups, being 5.6 [4.95–6.23] for patients with SVR, 5.5 [4.95–6.11] for relapsers and 5.7 [5.2–6.2] for non-responders. The first-phase of HCV-RNA decline from 0 to 48 h, however, clearly differentiated patients with SVR from relapsers and non-responders. Indeed, SVR patients experienced a median HCV-RNA decline of 1.8 [1.1–2.5], while the median viremia drop in both relapsers and non-responders was significantly lower (1.1 [0.5–1.8] and 0.6 [0.2–1.5], respectively; P < 0.001, Fig. 1A). Similarly, we found that patients who achieved either complete or partial EVR had a significantly greater log decline in HCV-RNA level at 48 h (median [IQR] = 1.7 [1.0–2.5] and 1.6 [0.7–2.1], respectively) than patients not reaching EVR (0.3 [0.2–0.8], P < 0.001, Fig. 1B). Importantly, significantly different Δ(0–48 h) log 10 HCV-RNA values were observed in responders versus non-responders/relapsers independently of genotype (Fig. 2). Serial infralogaritmic cut-offs of Δ(0–48 h) Log 10 HCV-RNA decline were evaluated for their PPV and NPV of SVR. The highest NPV (76.2%) was observed at the 0.5 log cut-off, the lowest at 1 log (52.9%, Table 1). PPVs were rather stable across the series, around 75% (Table 1). Logistic regression analyses for predictors of SVR were performed for all Δ(0–48 h) Log 10

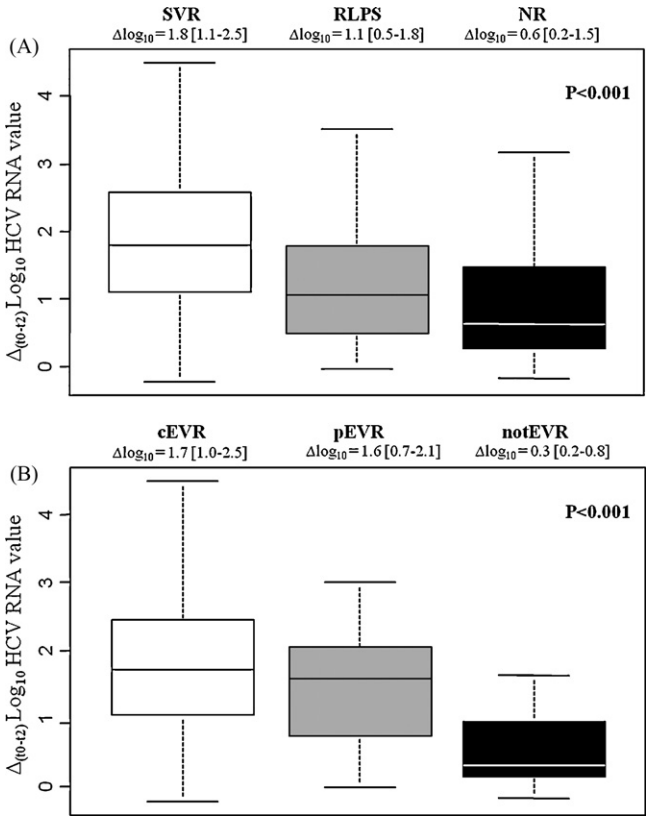

Fig. 1. Box plot representing the logarithmic decline in HCV-RNA levels 2 days after treatment start ($\Delta_{(t0-t2)}$ Log_{10}), according to treatment outcome. Colored boxes represent $\Delta_{(t0-t2)}$ Log_{10} HCV-RNA value distribution among the 6 study groups classified according to treatment outcome, with the relative confidence intervals. Horizontal lines inside boxes indicate the median $\Delta_{(t0-t2)}$ Log_{10} HCV-RNA value. (A) After 2 days of treatment, patients who subsequently developed a SVR had a significantly greater log decline in HCV-RNA level than those who experienced a relapse or those who experienced a non-response (P < 0.001). (B) After 2 days of treatment, patients who subsequently achieved a cEVR or pEVR had a significantly greater log decline in HCV-RNA level than those who did not experience an EVR (P < 0.001). P-values were calculated through Kruskal–Wallis test. SVR, sustained virological responders; RLPS, relapsers; NR, non-responders: cEVR, complete early virological responders; pEVR, partial early virological responders; notEVR, not early virological responders.

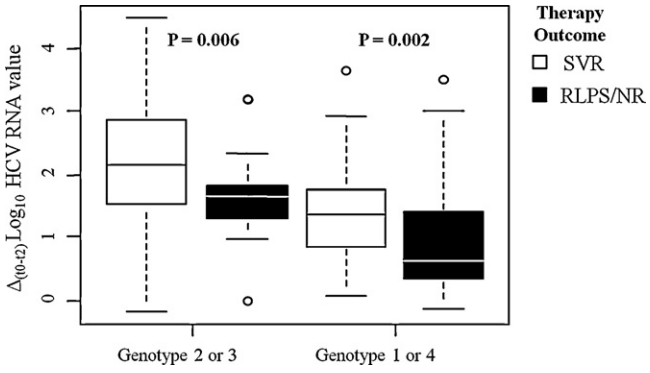

Fig. 2. Box plot showing the comparison among HCV genotype and $\Delta_{(t0-t2)}$ Log_{10} HCV-RNA value (logarithmic decline in HCV-RNA level 2 days after treatment start), according to final treatment outcome. Colored boxes represent $\Delta_{(t0-t2)}$ Log_{10} HCV-RNA value distribution for each HCV genotype, with the relative confidence intervals. Empty dots represent outlier values. Horizontal lines inside boxes indicate the median $\Delta_{(t0-t2)}$ Log_{10} HCV-RNA value. SVR, sustained virological responders; RLPS, relapsers; NR, non-responders. P-values were calculated through Wilcoxon rank-sum test.

Table 1
Negative and positive predictive values for SVR and EVR at different cut-off values of $\Delta_{(0-48\,h)}$ Log_{10} HCV-RNA decay.

$\Delta_{(0-48\,h)}$ Log_{10} HCV-RNA decay	Sensitivity	Specificity	PPV	NPV	Adjusted OR (95% CI)[a]	*P*
Outcome: SVR						
>0.5	94.7	32.0	72.6	76.2	6.8 (2.0–23.2)	0.002
>0.6	91.6	42.0	75.0	72.4	5.6 (2.0–15.7)	0.001
>0.7	89.4	48.0	76.6	70.6	4.7 (1.9–12.5)	0.002
>0.8	86.3	50.0	76.6	65.8	3.6 (1.5–9.2)	0.006
>0.9	82.1	52.0	76.5	60.5	2.6 (1.1–6.4)	0.036
>1.0	74.7	54.0	75.5	52.9	2.1 (0.9–4.9)	0.084
Outcome: EVR						
>0.5	92.6	62.5	95.0	52.6	19.6 (4.6–83.2)	<0.001
>0.6	87.7	68.8	95.5	42.3	12.4 (2.9–52.2)	0.001
>0.7	84.4	75.0	96.3	38.7	13.3 (2.9–61.2)	0.001
>0.8	81.1	75.0	96.1	34.3	10.3 (2.3–46.8)	0.002
>0.9	77.9	75.0	96.0	30.8	9.0 (2.0–40.7)	0.004
>1.0	72.1	75.0	95.6	26.1	5.9 (1.5–24.0)	0.023

NPV, negative predictive value; PPV, positive predictive value; OR, odds ratio; CI, confidence interval.
From: http://faculty.vassar.edu/lowry/clin1.html.
[a] Logistic regression models including logarithmic baseline HCV viremia, genotype, premature treatment interruption, HIV coinfection, type of pegilated interferon.

HCV-RNA decline cut-offs, each including all significant variables in the logistic evaluation, that is age, genotype, Log 10 baseline HCV viremia, receiving ≥80% of prescribed doses and HIV status (Table 1 and Tables 1 and 2a). All decline cut-offs were independently related to SVR, with the exception of the 1 log decline model ($P = 0.084$); the strongest prediction of SVR among independent predictors, however, was provided at the 0.5 cut-off (OR 6.8, 95% C.I.: 2.0–23.2, $P = 0.002$, Table 1 and Table 2a). We also evaluated the ability of the 0.5 log cut-off and other thresholds to predict EVR (partial + complete, Table 1). Interestingly, the PPV of ≥0.5 log Δ(0–48 h) Log 10 HCV-RNA decline for EVR was 95.0% (113/119 patients, Table 1); its prediction of EVR was independent of baseline viremia and genotype in the final multivariate logistic regression model (Table 2b).

Over the past years, a number of studies have evaluated the possible relationships between early kinetics of HCV-RNA decline and SVR (Jessner et al., 2001; Hoofnagle et al., 2009; Nomura et al., 2009; Arends et al., 2009; Durante-Mangoni et al., 2009). HCV-RNA decline at 24 h after the start of Interferon was studied as a tool for the prediction of SVR in 2001 by Jessner et al., showing that the decline in viral load 24 h after one dose of 5 MU Interferon alfa was a significant predictor of SVR in 29 consecutive patients. Indeed, none of those with <70% viral load decline at 24 h responded after 6 months of standard antiviral combination therapy. The other studies confirmed that early viral kinetics may predict achievement of SVR. In the study by Hoofnagle et al., SVR was far more frequent among patients with a decrease in HCV-RNA ≥2 log by day 28, although in the same study EVR was a far more reliable predictor of SVR than early decline. Nomura et al. showed that the 24 h decline of viral load for patients reaching SVR was significantly higher compared with that of unresponsive patients and suggested the use of 2 complex indices to differentiate responders from non-responders. SVR rates were reported as 90% in patients with a ≤1 ratio for viral load at week 1 over viral load at 24 h, and 93% for patients a <0.7 ratio for viral load at week 2 over viral load at 24 h. Arends et al. looked at the prediction power of 48 h HCV-RNA decline in 23 patients treated with combination therapy, 14 monoinfected and 9 coinfected; they also investigated multiple early time points. They suggested that the entity and steepness of HCV-RNA decline at 48 h could predict RVR (rapid virological response) but not SVR in HCV-monoinfected patients in such a limited setting. Finally, the evaluation of HCV-RNA decline at 48 h appeared easy to perform and able to yield a remarkable amount of information for SVR in the paper by Durante-Mangoni et al. (2009), which had a similar design in comparison with our prospective study. We used, however, PCR-based rather than branched-DNA amplifica-

Table 2
Logistic regression models predicting (a) sustained virological response (SVR) and (b) early virological response (EVR). OR = odds ratio; CI = confidence interval.

Variables	SVR		
	OR	(95% CI)	P^a
(a)			
Logarithmic baseline HCV viremia (1-log increase)	0.37	(0.14–0.97)	0.043
Genotype 1	0.43	(0.15–0.8)	0.014
HCV-RNA ≥0.5 log drop at 48 h	6.8	(2.0–23.2)	0.002
Premature treatment interruption	0.2	(0.1–0.7)	0.007
HIV coinfection	0.13	(0.02–0.83)	0.031
Alfa1a PEG-IFN	1.6	(0.6–3.6)	0.3
Variables	EVR		
	OR	(95% CI)	P^b
(b)			
Logarithmic baseline HCV viremia (1-log increase)	0.43	(0.1–2.4)	0.33
Genotype 1	0.47	(0.1–2.2)	0.34
HCV-RNA ≥0.5 log drop at 48 h	19.6	(4.6–83.2)	<0.001
Premature treatment interruption	1.1	(0.2–6.0)	0.93
HIV coinfection	0.1	(0.01–0.6)	0.02
Therapy with alfa1a PEG-IFN	1.1	(0.3–4.7)	0.88

[a] Logistic model with 145 observations; Hosmer–Lemeshow *P*-value for the goodness of fit = 0.3; area under the ROC curve = 0.83.
[b] Logistic model with 138 observations; Hosmer–Lemeshow *P*-value for the goodness of fit = 0.55; area under the ROC curve = 0.88.

tion methods and collected a larger Caucasian sample including few HIV-coinfected patients. Our results confirm the predictive potential of 48 h measurements, supporting an infralogaritmic threshold of RNA decay for SVR prediction and indicating the best threshold at 0.5 log. At this threshold, which approximately corresponds to a 70% reduction of baseline viremia (as in the pivotal work by Jessner at al.), the 48 h RNA decay reveals a predisposition to SVR independent of genotype and viral load (Pascu et al., 2004; Shirakawa et al., 2008). Although a 7-fold higher chance of SVR for patients experiencing a decay across this threshold was demonstrated independent of genotype, baseline viremia, adherence to therapy and HIV coinfection, the NPV for SVR was only 75%, lower than what announced by Durante-Mangoni et al. (95%), a prediction power not allowing per se the introduction of an early stopping rule in clinical practice. Differences in NPV and PPV estimates may be related to the larger size of our sample and to the use of different quantification systems, as most other features, including the proportion of difficult genotypes, females and patients of Caucasian descent were similar in the 2 studies. Interestingly, we found that obtaining a >0.5 log RNA decay at 48 h was strongly associated with and predictive of EVR, considered as the sum of partial and complete EVR. None of the recent studies investigating the early phases of HCV-RNA decay to predict SVR focused also on the prediction of EVR, which indeed may turn out to be remarkably useful (Hoofnagle et al., 2009; Arends et al., 2009; Nomura et al., 2009; Durante-Mangoni et al., 2009). Identical results for both predictions were obtained when HIV-coinfected patients and/or unpegylated INF pre-exposed patients were excluded from the final models (data not shown). A limitation of our study was that it was performed at a single site and on an entirely Caucasian cohort. As a consequence, our results should be validated for different populations. Another limitation may be represented by the fact that in 2001 our experimental model did not include RNA assays at 4 weeks to evaluate RVR, which was performed only in 31 patients in later years, thus not allowing any direct comparison with the 48 h RNA decay as to SVR prediction power. In conclusion, NPVs and PPVs for both EVR and SVR in our study are too low to warrant discontinuation of treatment after 48 h. They confirm, however, that measurement of HCV-RNA at 48 h after starting treatment may identify patients with poor a-posterior chances of SVR, in greater need for novel therapeutic options in the anti-HCV pipeline (Pereira and Jacobson, 2009). We propose that obtaining >0.5 log RNA decay at 48 h may be referred to as very rapid virological response (VRVR).

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