

Prediction of Post-Treatment Relapse Using Transcription Mediated Amplification (TMA) at the End of Peg-IFN plus Ribavirin Therapy in HIV/HCV Coinfected Patients

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CONCLUSIONS

In HCV/HIV coinfectd patients, a positive TMA after 48 weeks of therapy may help identify patients with EOT response by PCR who are at high risk of relapse.

TMA performed in earlier periods of treatment does not seem useful to predict sustained virological response.

INTRODUCTION

- Qualitative hepatitis C virus (HCV) RNA testing is the currently recommended procedure to monitor virological response to HCV treatment.
- Cure of HCV infection is defined as undetectable HCV RNA in serum after 6 months of cessation of therapy (sustained virological response, SVR). At present, treatment of HCV infection in HIV-coinfectd patients is associated with a high response rate at the end of 48 weeks of therapy (EOT response), but a significant proportion of patients that achieve EOT response go on to relapse and do not reach SVR.
- One potential explanation for relapses of HCV is that treatment does not completely suppress virus replication and very low replication rates remain in the compartment of replication competent cells (mainly hepatocytes). Thus, a more sensitive assay could be able to detect patients with EOT response (according to standard virological methods) at risk of relapse.

OBJECTIVES

To evaluate if a Transcription-Mediated Amplification (TMA)-based assay, with an HCV RNA limit of detection of 5 IU/mL, could:

- Detect residual viremia in patients with EOT response and thus could be helpful in identifying relapsers
- Help identify patients who do not respond to therapy in early treatment periods, compared to a PCR based method

SUBJECTS

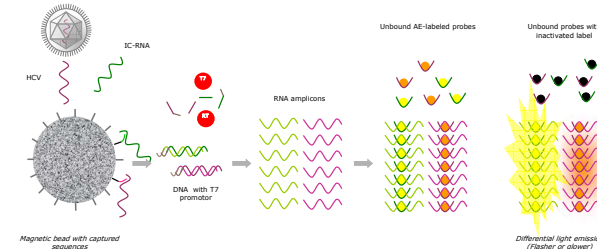
In order to be included in the study, subjects fulfilled the following criteria:

- HIV/HCV coinfection
- Pegylated Interferon plus ribavirin treatment
- PCR documented response/non response to HCV treatment
- Available archived PCR negative samples from HCV treatment follow-up

Patients were selected among the prospective HIV/HCV coinfection cohort followed at our hospital (N=366), by initial identification of the candidates in the clinic database.

METHODS

Sample availability (stored at -70°C) was confirmed for all the HCV PCR negative selected cases and the TMA assay was run in a blind fashion following the manufacturer instructions. Statistical analysis was done by means of SPSS 11.0.



Target capture
Target Capture Assay (TCA) releases nucleic acid from target organism. Captures both target RNA and Internal Control (IC) ARN on magnetic bead.

Target amplification
Transcription-mediated Amplification (TMA): add primers and enzymes (RT, T7 RNA polymerase) and generate billions of RNA amplicons via double stranded cDNA intermediates containing the T7 promoter sequence

Amplicon detection
Hybridization Protection Assay (HPA) and Dual Kinetic Assay (DKA): add acridinium-ester (AE) labeled probes and hybridization to IC and target amplicons. Inactivate unbound probes through chemical selection. Detect differential light emission. "Flasher" signal proves successful IC capture and amplification, whereas the "glower" signal indicates presence of target RNA in the original patient sample.



RESULTS

Table 1. Baseline characteristics

	Non sustained responders (n=27)	Sustained responders (n=63)
Sex		
Male	20	53
Female	7	10
Age, years	38.0 (36.5-41.0)	40.0 (38.0-43.0)
CD4 cell count, x10 ⁶ cells/l	544.0 (339.0-733.5)	538.0 (369.0-711.0)
HIV-1 RNA, Log ₁₀ copies/ml	1.7 (1.7-1.9)	1.7 (1.7-2.1)
HCV RNA, UI/mL	5x10 ⁵ (1x10 ⁵ -1x10 ⁶)	6x10 ⁵ (3x10 ⁵ -1x10 ⁶)
ALT, U/L	74.0 (54.0-165.0)	96.0 (54.0-149.5)
AST, U/L	83.0 (40.0-117.5)	67.0 (46.0-97.5)
GGT, U/L	126.0 (110.0-218.0)	98.0 (49.5-155.5)
Bilirubin, mg/dL	0.6 (0.4-0.8)	0.6 (0.4-0.8)

*Quantitative variables are expressed as median (Interquartile range, IQR)

Table 2. TMA results according to HCV genotype and SVR achievement

All genotypes (N=90)	W4 (N=24)		W12 (N=50)		W24 (N=50)		W48 (N=65)	
	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-
SVR	7	11	3	34	3	33	1	55
Non SVR	5	4	9	3	9	4	1	5
	p=0.154		p=0.064		p=0.030		p<0.001	
Genotype 2/3 (N=63)	W4 (N=17)		W12 (N=36)		W24 (N=34)		W48 (N=48)	
	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-
SVR	5	8	2	26	3	23	1	40
Non SVR	3	1	2	6	4	4	4	3
	p=0.294		p=0.207		p=0.037		p<0.001	
Genotype 1/4 (N=27)	W4 (N=7)		W12 (N=14)		W24 (N=16)		W48 (N=18)	
	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-	TMA+	TMA-
SVR	2	3	1	8	0	10	0	15
Non SVR	2	0	2	3	1	5	1	2
	p=0.428		p=0.505		p=0.375		p=0.166	

Table 3. TMA diagnostic value at different timepoints in HCV treatment monitoring

monitoring	PCR negative TMA diagnostic value			
	W4	W12	W24	W48
Sensitivity	83.3%	30.8%	35.7%	44.4%
Specificity	61.1%	91.1%	91.7%	98.2%
Positive predictive Value	41.7%	57.1%	62.5%	80.0%
Negative predictive value	91.7%	79.1%	78.5%	91.7%

