Reassessment of Genotype 1 Hepatitis C Virus Subtype Misclassification by LiPA 2.0: Implications for Direct-Acting Antiviral Treatment

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The accuracy of LiPA 2.0 for hepatitis C virus 1 (HCV-1) subtype classification was analyzed. LiPA 2.0 genotype results from 101 HCV-1-infected patients were compared to genotype findings determined by direct core sequencing. Eleven (11%) samples were misclassified. Given the influence of the HCV-1-subtype in the anti-HCV therapy response, an alternative classification method is warranted.

The effect of the hepatitis C virus (HCV) genotype 1 subtype on the response to treatment can be dramatic for some direct antiviral agent (DAA)-containing regimens (1–9). This is the case for simeprevir, a DAA recently approved for clinical use, that in combination with pegylated interferon and ribavirin (peg-IFN/RBV) has a lower sustained virological response (SVR) rate for subtype 1a than for 1b (2). Simeprevir plus peg-IFN/RBV treatment achieves rates of SVR similar to those for peg-IFN/RBV treatment in HCV-1a-infected patients who present the viral variation Q80K. This variation is frequent in the HCV-1a subtype, but it is not found in HCV-1b (2, 5). Similarly, daclatasvir plus peg-IFN/RBV treatment also achieves higher SVR rates among HCV-1b-infected patients than among HCV-1a-infected individuals (4). For interferon-free regimens, DAA combinations such as asunaprevir plus daclatasvir may only be indicated for subtype 1b, because of high relapse rates observed among patients with subtype 1a (3, 4). Because of these, accurate subtyping of HCV genotype 1-infected patients is needed for those who are candidates for DAAs.

Sequencing of specific HCV genome regions with subsequent phylogenetic analysis is considered the gold standard of HCV genotyping (10). However, it includes several complex techniques and is time consuming. For these reasons, LiPA 2.0 is currently one of the most widely used genotyping assays. However, several studies have reported HCV genotyping errors using this commercial method (11–13). Those studies were not designed to evaluate the frequency of HCV-1 subtyping misclassifications, and they included few HCV-1-infected patients. Therefore, due to the high prevalence of HCV-1 infections in western countries and the importance of correct subtyping in clinical decisions, the accuracy of this method for HCV-1 subtyping needs to be evaluated in larger samples. For these reasons, we aimed to evaluate the accuracy of LiPA 2.0 for classifying HCV-1 by subtypes.

This study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki, and it was approved by the ethics committee of the Hospital Universitario de Valme (Seville, Spain).

The first 110 consecutive patients starting from October 2001, who received a course of peg-IFN/RBV treatment in our hospital and who were infected with HCV-1, according to the LiPA 2.0 determination, were selected. All of them were Caucasian with a median age (quartile 1 to quartile 3) of 42 years (range, 39 to 46 years). Among the patients, 82 (81%) were male, and 48 (47.5%) were infected with HIV.

To determine the HCV-1 subtype, we analyzed the entire core genetic sequence. This region is more conserved than those traditionally used for HCV genotyping (NS5B or core/E1) and allows higher amplification rates (13, 14). The core region was amplified by nested PCRs. The first PCR was performed with 5 µl of cDNA using primers 1 and 2 (Table 1). For the second PCR, 4 µl of the first PCR product and primers 3 and 4 (Table 1) were used. For those samples that were not amplified with this protocol, primers 1 and 5 for the LiPA 2.0-identified subtype 1a or primers 1 and 4 for subtype 1b were used to perform the external PCR and then primers 6 and 7 (1a) or 8 (1b) were used in the internal PCR (Table 1). The nested PCR products were bidirectionally sequenced using standard capillary electrophoresis techniques. ClustalW2 software (www.ebi.ac.uk/Tools/msa/clustalw2/) was used for alignment of the forward and reverse sequenced strains to obtain consensus sequences.

As the genotyping method, we analyzed the evolutionary history, which was inferred by using the maximum likelihood method based on the general time reversible model. Evolutionary analyses were conducted in MEGA5 software (15). The initial tree(s) for the heuristic search was obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach (15). A discrete gamma distribution was used to model the evolutionary rate differences among sites (4 categories [$+G$, parameter $= 0.3742$]). The analysis involved 110 sequences, including the sequences from patient samples and those selected as references. In the final data set, there were a total of 573 base pair positions corresponding to the complete core region for each sample.

TABLE 1 Primers used for amplification of the HCV core gene

Primer no.	Designation	Sequence (5′ to 3′)	Location in genome[a]
1	FAD-ES-Fw	CGAAAGGCCTTGTGGTACTG	272 → 291
2	FAD-EA-Rv	CTCCCGAACGCAGGGCAC	1020 ← 1037[b]
3	FAD-IS-Fw	TTGTGGTACTGCCTGATAGGGT	281 → 302
4	FAD-IA-Rv	ATGCTTGAGTTGGAGCAGTCG	956 ← 976[b]
5	JLM-Ea-Rv	CGCCTCGTACACAATACTCG	970 ← 989[b]
6	JLM-Iab-Fw	TGTGGTACTGCCTGATAGGG	282 → 301
7	JLM-Ia-Rv	GGGCAATCATTGGTGACATGG	944 ← 964[b]
8	JLM-Ib-Rv	TTGGAGCAGTCGTTCGTGAC	948 ← 967[b]

[a] Nucleotide position according to reference sequence H77 (GenBank accession number NC_004102).

[b] Reverse complement sequence with respect to that specified in the reference sequence.

Successful sequencing and genotyping were obtained in 101 (91.8%) patients. The 9 (8.2%) samples that were not amplified had a viral load of <3 log IU/ml.

According to LiPA 2.0, 58 (57.4%) samples were identified as HCV-1a, and the remaining 43 (42.6%) were identified as HCV-1b. By the sequence-based method, 67 (66.3%) samples were classified as genotype 1a, 33 (32.7%) samples were classified as 1b, and 1 (1%) was classified as 4d (Table 2). The overall Cohen kappa index obtained was 0.773 (95% confidence interval, 0.647 to 0.900). The proportion of patients with misclassified HCV-1 subtypes was 11%. All HCV-1 subtype misclassifications were observed among individuals identified by LiPA 2.0 as the HCV-1b subtype. Thus, 10 (23%) patients classified as HCV-1b by LiPA 2.0 were identified as HCV-1a by sequencing methods (Table 2). The sensitivity, specificity, positive predictive value, and negative predictive value for HCV-1b subtyping by LiPA 2.0 were 1, 0.85, 0.77, and 1, respectively. The corresponding numbers for HCV-1a subtyping were 0.85, 0.97, 0.98, and 0.77, respectively.

This is the largest study, to our knowledge, that has analyzed the accuracy of HCV-1 subtype identification by LiPA 2.0. The study of Avó et al. (11) included a total of 31 HCV-1 samples (of them, only 7 were 1b), whereas in the study of Bouchardeau et al. (12), there were a total of 62 HCV-1 samples and only 21 of them were 1b. In a similar study, HCV-1a-infected patients were not included because of the HCV genotype distribution (13).

In conclusion, our results show that the LiPA 2.0 assay is not an adequate tool for subtyping HCV genotype 1. This genotyping method has subtyping failures, and it is prone to errors with HCV subtype 1b. Because the HCV subtype 1 misclassification might lead to inadequate treatment associated with a high likelihood of nonresponse, more accurate genotyping methods than LiPA 2.0 should be used.

Nucleotide sequence accession numbers. The sequences obtained in this study were submitted to GenBank under accession numbers KF060663 to KF060714 and KJ739737 to KJ739786.

TABLE 2 HCV genotyping results obtained with sequence-based methods and the LiPA 2.0

HCV genotype by LiPA 2.0	HCV genotype by core sequence:			
	1a	1b	4d	Total
1a	57		1	58
1b	10	33		43
Total	67	33	1	101

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