NOTES

Assessment of Spontaneous Fluctuations of Viral Load in Untreated Patients with Chronic Hepatitis C by Two Standardized Quantitation Methods: Branched DNA and Amplicor Monitor

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Quantitation of hepatitis C virus (HCV) RNA in serum has been used to predict and monitor the efficacy of interferon therapy in chronic HCV infection. We prospectively studied the fluctuation of viremia by a longitudinal follow-up of HCV RNA levels for 2 months in six untreated patients. Spontaneous fluctuations of HCV RNA ranged from 2.8- to 5.7-fold with branched DNA assay and from 2.9- to 5.6-fold with Monitor. These large spontaneous fluctuations (up to 0.75 log), observed daily, weekly, and monthly, raise doubt about the clinical value of a single assessment of pretherapeutic viremia.

The response to alpha interferon therapy in chronic hepatitis C is inversely correlated with the level of hepatitis C virus (HCV) RNA before treatment (2, 4, 9). HCV RNA quantitation therefore requires both accuracy and standardization. The two standardized assays of serum HCV RNA quantitation measure the viral load in clinical samples and are based either on a signal amplification method called branched DNA (bDNA) (Quantiplex HCV RNA assay; Chiron Diagnostics, Cergy-Pontoise, France) (14) or on reverse transcriptase PCR (Amplicor HCV Monitor; Roche Diagnostic Systems, Paris, France) (13). There is little data (10) on baseline fluctuation of HCV genotypes were determined by the line probe assay (InnoLiPA HCV; Innogenetics, Ghent, Belgium) (12). One patient had genotype 1a, three patients had genotype 1b, and two patients had genotype 2a/2c.

HCV RNA was quantitated in serum by the bDNA test (HCV version 1.0) according to the manufacturer’s instructions (assays in duplicate) and by the HCV Monitor method. For each patient, the samples were analyzed by the same operator, on two microwell plates on 2 days for the bDNA method and on one microwell plate on 1 day for the Monitor method. The overall data analysis necessitated two microwell plates on 2 days for the bDNA assay and six microwell plates during 6 days for the Monitor assay. To assess the reproducibility of the bDNA and Monitor assays, the same operator tested six serum samples from six other chronic hepatitis C patients (A to F); for the bDNA assay, each sample was tested eight times on two microwell plates during 2 days, and for the HCV Monitor assay, each sample was tested eight times on four microwell plates on 4 days. These six patients had been selected according to their HCV RNA level (range, 0.4 × 10^6 to 8 × 10^6 eq/ml, by bDNA).

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Correlation analysis with Pearson correlation coefficients was used to assess the relationship between the bDNA and Monitor methods and between each method and aspartate transaminase (AST) or alanine transaminase (ALT) values. The reproducibility of each method was assessed by a coefficient of variation (% CV). P values of less than 0.05 were considered significant.

The data in Fig. 1 shows fluctuations of HCV RNA quantified by the two methods. These fluctuations occurred either daily, weekly, or monthly and were not related to change in AST or ALT values. Fluctuations ranging between 2.8- and 5.7-fold were observed for HCV RNA quantified by bDNA, and fluctuations of between 2.9- and 5.6-fold were observed with Monitor (not illustrated). Although the values for the two methods differed, the magnitude of the fluctuation was the same. No significant relationships were found between fluctuation of HCV RNA serum level and sex, age, genotype, histological activity, or route of contamination. Figure 2 shows the reproducibility of the bDNA and Monitor assays performed eight times on each of the six other serum samples (A to F). The mean CV for replicate determinations was 9.1% (range, 6.1 to 12.1%) for bDNA and 21.0% (range, 15.6 to 27.1%) for Monitor. HCV RNA levels assessed by bDNA and Monitor methods were correlated ($r = 0.70$; $P < 0.0001$). Nevertheless, the fluctuations observed are significantly higher than the variability of HCV RNA measurement in intra- and interassay reproducibility. Two experiments were required because the maximum number of tests in the bDNA assay in the same microplate is 42, and the maximum number of tests in the Monitor assay in the same microplate is 12. The interassay reproducibility of the bDNA assay was established by Detmer et al. (3). With two lots of reagents, they tested replicates of specimen panels in 24 separate assay runs by two operators over the course of 6 days. The CVs ranged from 9 to 21%, with a mean of 14%. Izopet et al. (7) evaluated the reproducibility of the Monitor assay in a multicenter study involving three laboratories. When 10 samples were processed in the same run in the three laboratories, the interassay CVs ranged from 2 to 47% (mean, 22%). In our study, the interassay reproducibility agrees with these two studies: 6 to 12% (mean, 9%) for the bDNA assay and 16 to 27% (mean, 21%) for the Monitor assay. These technical variations are lower than the spontaneous fluctuations from 2.8- to 5.7-fold observed in the study. Thus, the fluctuations observed in this study cannot be explained by a methodological bias. Centrifugation of samples immediately after formation of the clot and freezing at $-80^\circ$C within 1 h guarantee that HCV RNA values are reproducible (5). Unlike for HIV, it has not yet been established for HCV what changes in virus RNA levels are significant. Nguyen et al. (10) considered changes greater than threefold to be significant and reported that no such changes were obtained in their study. In our study, large fluctuations from 0.5 to 0.75 log occurred in patients with either chronic active hepatitis, chronic persistent hepatitis, or minimal lesions. Moreover, relatively narrow fluctuations of HCV RNA are not related to serum transaminases and may occur in patients with normal ALT values. Although the reasons for such fluctuations are not known, the change of viremia in untreated patients could be due to the emergence of quasispecies under the pressure of immune response (15). Another explanation could be, as re-

FIG. 1. Fluctuation of AST, ALT, and HCV RNA measured by bDNA and Monitor methods in six patients with chronic hepatitis C. Eleven serum samples were collected for 2 months and tested for AST, ALT, and HCV RNA levels. Symbols: open bar, AST; solid bar, ALT; □, HCV RNA measured by bDNA assay (equivalents per milliliter of serum); ○, HCV RNA measured by Monitor assay (copies per milliliter of serum).
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REFERENCES


FIG. 2. Correlation and reproducibility of two methods of quantitation of HCV RNA in serum. Six serum samples were tested eight times by bDNA and Monitor assays. Symbols: ■, HCV RNA measured by bDNA assay (equivalents per milliliter of serum); □, HCV RNA measured by Monitor assay (copies per milliliter of serum).