Hepatitis C Virus Quasi-Species Dynamics Predict Progression of Fibrosis after Liver Transplantation

Juan I. Arenas,1 Juan F. Gallegos-Orozco,1,5 Tomasz Laskus,1 Jeff Wilkinson,1 Amer Khatib,1 Carlos Fasola,2 Debra Adair,1 Marek Radkowski,1,4 Karen V. Kibler,1 Marek Nowicki,1 David Douglas,1 James Williams,1 George Netto,1 David Mulligan,1 Goran Klintmalm,2 Jorge Rakela,1 and Hugo E. Vargas1

1Division of Transplantation Medicine, Mayo Clinic Scottsdale, Scottsdale, Arizona; 2Transplantation Services, Baylor University Medical Center, Dallas, Texas; 3Maternal-Child Virology Research Laboratory, University of Southern California, Los Angeles; 4Warsaw Medical Academy, Warsaw, Poland; 5Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán,” Mexico City, Mexico

Background. The dynamics of hepatitis C virus (HCV) quasi species in the E2 region may correlate with the course of infection after orthotopic liver transplantation (OLT).

Methods. Thirty-four patients who underwent transplantation for HCV-related cirrhosis were studied. Serum and liver samples were available before OLT and at 1 week, 4 months, and 1 year after OLT. Patients were divided into group 1 (Knodell/Ishak fibrosis stage [FS] at 1 year, <2) and group 2 (FS at 1 year, ≥2). Complexity was estimated by the number of bands in a single-strand conformational polymorphism assay, whereas diversity was measured by Shannon entropy (SE) and median mobility shift (MMS) values derived from the heteroduplex mobility assay. Diversity dynamics were measured at transmission (before OLT vs. 1 week after OLT) and after OLT (1 week after OLT vs. 1 year after OLT).

Results. Complexity was higher in group 1 patients than in group 2 patients before OLT (P < .02) and at 1 week after OLT (P < .04). Diversity decreased in group 1 at transmission, as measured by either SE (P < .01) or MMS (P < .04). However, diversity increased in this group after OLT, as measured by SE (P < .03) or MMS (P < .02). FS at 1 year after OLT correlated with transmission changes, as measured by SE (r = 0.642, P < .0001) and MMS (r = 0.443, P < .04), and with post-OLT changes (for SE: r = −0.583, P < .01; for MMS: r = −0.536, P < .01).

Conclusions. HCV complexity and diversity in the E2 region correlated with the severity of recurrence of HCV infection after OLT. Increased diversity of quasi species at transmission correlated with a higher FS at 1 year. However, increased diversity of quasi species in the post-OLT period correlated with a lower FS at 1 year. The dynamics of HCV quasi species in patients who undergo transplantation are predictive of outcome.
and may affect patient and graft survival [7, 8]. In the setting of a chronic and worsening donor-organ shortage, the identification of recipients at greater risk for more-severe recurrence of HCV infection after transplantation has become a high priority. Several factors could influence the outcome of recurrent HCV disease, such as genotype [8–10], virus load [11], HLA mismatch [8], immunosuppressive therapy [12, 13], and cytomegalovirus infection [14–16]. The association between HCV quasi species and the severity of recurrent HCV infection after OLT has also been investigated [17–23]. However, the results of such investigations are inconclusive, likely because of the small number of patients studied, the use of different techniques for quasi-species analysis, patient selection, and different time points for analysis. A missing element in all previous reports is concurrent evaluation of early and late changes in HCV quasi species after OLT.

In the present study, we analyzed the dynamics of HCV quasi species at 4 different time points during the first year after OLT, in a group of 34 patients. We analyzed 2 different viral regions: HVR1/E2 and 5′UTR. Although the HVR1/E2 region quasi species is believed to be influenced and shaped by the host immune response [24], 5′UTR changes could be related to viral fitness associated with its capability to direct translation [25, 26].

### PATIENTS, MATERIALS, AND METHODS

#### Patient Population

The study group comprised 34 patients who underwent OLT for HCV-related cirrhosis either at Baylor University Medical Center (Dallas, TX; n = 10) or Mayo Clinic Scottsdale (Scottsdale, AZ; n = 24) from 1995 through 2001. Data were obtained in accordance with institutional review board requirements, and patients provided written, informed consent. All patients were positive for HCV RNA in serum before OLT, and all had recurrent HCV disease. “Recurrence” was defined by the presence of viremia detected by reverse-transcriptase polymerase chain reaction (RT-PCR) analysis after OLT. Patients with hepatitis B virus and other causes of liver disease were excluded from the study.

The mean age (±SD) of the OLT recipients was 49.6 ± 6 years. Twenty-nine patients were men, and 27 patients were infected with genotype 1 (12 patients were infected with genotype 1b, and 15 patients were infected with genotype 1a). Immunosuppressive therapy consisted of tacrolimus (for 27 patients) and cyclosporine (for 7 patients). All patients received oral prednisone for approximately 4 months. Twenty patients also received mycophenolate mofetil. The frequency of acute cellular rejection that required treatment was 57%, and treatment consisted of three 1-g intravenous doses of methylprednisolone. For 2 patients, anti-CD3 antibody preparation was used for steroid-resistant rejection.

None of the patients in the present study received antiviral therapy during follow-up, except for 2 patients who had liver failure secondary to fibrosing cholestatic hepatitis (FCH). These 2 patients died at 3 and 5 months after OLT, respectively.

Serum samples were obtained immediately before OLT and at 3–4 months and 1 year after OLT. An additional serum sample was obtained from 24 patients at 1 week after OLT. The samples were stored at −80°C until they were analyzed in the present study.

We were unable to amplify the HVR1/E2 region at any of the time points, for 3 patients. For 2 additional patients, we were unable to amplify the region in the pre-OLT and 1-week post-OLT samples. These 5 patients were excluded from the study, and 29 patients were fully examined. For 4 patients, we could not amplify the HVR1/E2 region in the sample obtained 1 week after OLT, most likely because of the low virus titers seen at that time.

All patients had a protocol liver biopsy performed at 1 year after OLT, and histologic findings for the specimens obtained

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Age, mean ± standard error, years</td>
<td>49 ± 2</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>14/3</td>
<td>8/4</td>
</tr>
<tr>
<td>Genotype 1, no. (%) of patients</td>
<td>14 (82)</td>
<td>11 (91)</td>
</tr>
<tr>
<td>RNA titer, mean ± SD, IU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before OLT</td>
<td>3.9 × 10^5 ± 1.0 × 10^6</td>
<td>4.3 × 10^5 ± 1.7 × 10^6</td>
</tr>
<tr>
<td>After OLT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 week</td>
<td>1.4 × 10^6 ± 4.7 × 10^4</td>
<td>4.4 × 10^4 ± 4.3 × 10^4</td>
</tr>
<tr>
<td>At 1 month</td>
<td>3.7 × 10^5 ± 0.9 × 10^5</td>
<td>6.0 × 10^5 ± 2.2 × 10^5</td>
</tr>
<tr>
<td>At 1 year</td>
<td>1.3 × 10^6 ± 4.6 × 10^5</td>
<td>2.2 × 10^6 ± 1.6 × 10^5</td>
</tr>
<tr>
<td>Rejection, % of patients</td>
<td>31</td>
<td>20</td>
</tr>
</tbody>
</table>

**NOTE.** OLT, orthotopic liver transplantation. For all comparisons of group 1 and group 2, the P value was not significant.
were graded according to the Knodell/Ishak hepatitis activity index [27]. All biopsy findings were interpreted by 1 of 2 pathologists who were unaware of the clinical and virological results. Patients were divided into 2 groups, according to the Knodell/Ishak fibrosis stage (FS; range, 0–6); group 1 consisted of 20 patients who had an FS <2 for the biopsy specimen obtained 1 year after OLT, and group 2 consisted of 14 patients who had an FS ≥2. This classification of patients was selected for two reasons. Given the mean follow-up of the patients, there were very few patients for whom we had very advanced FSs, thus making the analysis with higher fibrosis meaningless. Moreover, the aforementioned grouping is the cutoff used at our centers for initiation of antiviral therapy for patients, and, therefore, it has important clinical implications. There were no statistical differences between the 2 groups, with regard to age, sex, genotype, immunosuppressive regimen, virus titers, or incidence of rejection (table 1).

RT-PCR

RNA was extracted from 200 μL of serum by use of a commercially available kit (Trizol; Invitrogen Life Technologies), and it was dissolved in 25 μL of water. A total of 5 μL of this RNA solution was reverse transcribed with Moloney murine leukemia virus and was PCR amplified. The E2 region, including HVR1, and the 5′ UTR were amplified as described elsewhere [28, 29]. The sequences for the primers used in HVR1/E2 amplification are as follows: initial reactions, (upstream) 5′-CTG GTG CTC ACT GGG GAG TCC T-3′ (nt 1387–1409) and (downstream) 5′-CAT TGC AGT TCA GGG CAG T(C/G) C T(A/G)-3′ (nt 1633–1610). Nested reactions used the following primers: (upstream) 5′-TCC ATG GTG GGG AAC TGG GC-3′ (nt 1428–1448) and (downstream) 5′-TGC CAA CTG CCA TTG GTG TT-3′ (nt 1604–1584).

Single-Strand Conformational Polymorphism (SSCP) Analysis and Heteroduplex Mobility Assays (HMAs)

We decided to use SSCP analysis and the HMA to monitor changes in HCV quasi species, because these techniques allow for rapid analysis of a large number of samples. In addition, these techniques avoid artifactual errors common for analysis of cloned sequences [30, 31].

The SSCP assay was performed as described elsewhere [28, 29], with minor modifications. This assay is highly sensitive; we can routinely detect any minor variant representing ≥3% of the whole population. PCR products were purified using a DNA-binding resin system (Wizard PCR; Promega) and were resuspended in 50 μL of water. Next, 2–4 μL of the PCR product was diluted in 13 μL of loading dye (10% saccharose, 0.5% bromophenol blue, and 0.5% xylene cyanol), was heated for 3 min at 97°C, and was instantaneously cooled on ice. Between 8 and 10 μL of the diluted PCR product was loaded onto a 10% PAGE gel (acylamide:bis-acylamide ratio, 19:1) with 1× Tris-borate EDTA buffer and was electrophoresed at 400 V applied for 4–5 h at a constant temperature of 25°C. The bands were visualized with silver staining (Silver Stain; Promega).

HMA was performed as originally described by Delwart et al. [32], with minor modifications. The PCR products were heated for 3 minutes at 94°C in 1× buffer (1 mol/L NaCl, 100 mmol/L Tris HCl [pH 7.8], and 200 mmol/L EDTA [pH 8]) and were snap-cooled on ice. Heteroduplexes were separated on 10% PAGE gel (acylamide:bis-acylamide ratio, 37:1) and were run at 250 V for 3 h at a constant temperature of 25°C. The gels were stained with silver staining (Silver Stain; Promega), because this provided for higher sensitivity and better resolution of individual bands, compared with ethidium bromide staining. To avoid the potential influence of sample DNA

![Figure 1](#)  
**Figure 1.** Single-strand conformational polymorphism (SSCP) analysis of the hypervariable region 1 (HVR1)/envelope 2 (E2) region in 2 patients. Patient 1 had changes in the SSCP band pattern, for samples obtained after orthotopic liver transplantation (OLT), compared with a sample obtained before OLT. Patient 2 had a very stable SSCP pattern throughout follow-up.

![Figure 2](#)  
**Figure 2.** Mean no. of bands (i.e., complexity) at each time point, as measured by single-strand conformational polymorphism (SSCP) analysis. Patients in group 1 (fibrosis stage, <2) displayed higher complexity in the sample obtained before orthotopic liver transplantation (OLT) and in the sample obtained 1 week after OLT (by the Mann-Whitney U test). SE, standard error. Group 2, patients with a fibrosis stage ≥2.
quantification on Shannon entropy (SE) and median mobility shift (MMS) readings, we standardized DNA amounts for each sample analyzed throughout the whole study.

Data Analysis

Complexity. The genomic complexity of HCV in the HVR1/E2 region was defined by the number of bands on the SSCP gel. The images were recorded as tagged image file format files, with the use of a high-resolution scanner (Epson expression 1680; Epson America). To avoid the bias associated with manual counting, bands were counted using Quantity One software (Bio-Rad Laboratories). We used the same sensitivity level for every analysis.

Diversity. The genomic diversity of HCV in the HVR1/E2 region was assessed by HMA. By use of the high-resolution scanner, each lane was recorded from the bottom of the gel well to the position immediately below the homoduplex. To simplify calculations and to allow for unbiased comparison between various gels, each lane was standardized by dividing it into 449 equal partitions, and the signal intensity at each division along the lane was transferred to an Excel workbook (Microsoft) that contained formulas for data analysis.

SE analysis is a generalized mathematical tool used to estimate variability [33, 34]. It has been defined by the equation $H = -\sum p_i \log p_i$, where $p_i$ is the frequency of each variant in the HMA gel, and where $H$ is the entropy of the set of probabilities $p_i$. If all but 1 of the $p_i$ values is zero, the entropy value would be zero, and, if all of the $p_i$ values are equal, the entropy value would be the maximum (equivalent to $\log n$).

To facilitate data presentation, the entropy values were normalized as $H/\log n$, where $n$ is the total number of analyzed compartments that provide convenient values within the range of 0–1. Therefore, the minimum entropy value of zero would denote a situation in which all the signal is present in 1 compartment, whereas the maximum value would indicate that the signal is evenly distributed among all 449 compartments. SE calculations previously have been used to characterize the diversity of HIV quasi-species populations over time [32, 35].

MMS was measured as proposed by Delwart et al. [32]. The signal intensity along the lane was added, starting from the bottom, until it reached 50% of the total value. The MMS value was calculated by dividing the position of the compartment most closely matching 50% of total lane intensity by 449 (the standardized number of total compartments in each lane). An MMS value of 0.5 would denote that the signal is evenly distributed in the top half of the lane, whereas lower and higher values would denote a bottom-weighted signal and a top-weighted signal, respectively.

Figure 3. Single-strand conformational polymorphism (SSCP) analysis and heteroduplex mobility assay (HMA) of the hypervariable region 1 (HVR1)/envelope 2 (E2) region in 2 patients with fibrosing cholestatic hepatitis. The band patterns were stable throughout the study.

Figure 4. Mean values for Shannon entropy and median mobility shift (MMS) before orthotopic liver transplantation (OLT) and at 1 week, 3 months, and 1 year after OLT. No significant differences were observed between the 2 groups. SE, standard error.
Figure 5.  A. Phylogenetic reconstructions showing the evolutionary relationship of hepatitis C virus sequences in patients 2 and 3. Bootstrap proportions of >50 of 100 bootstrap replicates are shown at appropriate branch points. The taxa are labeled as “A–D,” indicating time points before orthotopic liver transplantation (OLT) and at 1 week, 4 months, and 1 year after OLT. For patient 3, the time point at 4 months was not analyzed. For patient 2, there was a substantial intermingling of viral sequences from different time points; however, for this patient, the viral population at 1 week is strikingly monophyletic. For patient 3, phylogenetic reconstruction shows sequential shifts in viral populations corresponding to changes observed in single-strand conformational polymorphism and heteroduplex mobility assay gels. Comparison between median mobility shift (MMS) and Shannon entropy (SE) values of patients 2 and 3 (B) and mean distances (C) within quasi species at the same time points (labeled as “1–4” and denoting time points before OLT and at 1 week, 4 months, and 1 year after OLT), calculated according to the Kimura 2-parameter model from cloned sequences [39].
Table 2. Differences between Shannon entropy (SE) and median mobility shift (MMS) values of patients in group 1 (fibrosis stage, <2) and patients in group 2 (fibrosis stage, ≥2).

<table>
<thead>
<tr>
<th>Value, changes</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>SE Early&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.035 ± 0.008</td>
<td>0.017 ± 0.007</td>
<td>.009</td>
</tr>
<tr>
<td>SE Late&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.023 ± 0.01</td>
<td>−0.014 ± 0.01</td>
<td>.045</td>
</tr>
<tr>
<td>MMS Early</td>
<td>−0.10 ± 0.05</td>
<td>0.08 ± 0.05</td>
<td>.047</td>
</tr>
<tr>
<td>MMS Late</td>
<td>0.08 ± 0.03</td>
<td>−0.10 ± 0.06</td>
<td>.040</td>
</tr>
</tbody>
</table>

NOTE. Data are the mean ± standard error, unless otherwise indicated.

<sup>a</sup> The Mann-Whitney U test was used for statistical analysis.

<sup>b</sup> Early changes (transmission) are defined as the difference between the MMS and SE values for the first sample obtained after orthotopic liver transplantation (OLT) and those for the pre-OLT sample.

<sup>c</sup> Late changes (occurring after OLT) are defined as the difference between the MMS and SE values for the sample obtained 1 year after OLT and those for the first post-OLT sample.

Sequence Analysis

For sequence analysis, RT-PCR products were cloned into the TA cloning vector (Invitrogen). Individual clones were then directly sequenced using an ABI 377 automatic sequencer (Perkin Elmer).

Multiple alignment of DNA sequences was performed with MegAlign (DNASTAR). Phylogenetic reconstruction was performed using the neighbor-joining method with the Kimura 2-parameter distance matrix [36]. Positions where gaps were inserted to maintain alignment were discounted. A bootstrap analysis that used 100 bootstrap replicates was performed to assess the reliability of tree construction. The mean distances within viral sequences, for each time point, were calculated to assess the stability of virus quantification before OLT and at 1 month after OLT. Quantification was performed using branched DNA assays (Quantiplex HCV-RNA 2.0; Roche).

HCV Genotyping and RNA Quantification

HCV genotypes were determined using a second-generation line probe assay (INNO-LiPA HCV II; Innogenetics [distributed by Bayer Diagnostics]). HCV RNA was quantified at 5 time points: before OLT and at 1 week, 1 month, 3 months, and 1 year after OLT. Quantification was performed using branched DNA assays (Quantiplex HCV-RNA 2.0; Roche).

Statistical Analysis

Continuous variables were described as the mean ± the standard error and were compared using the nonparametric Mann-Whitney U test. Spearman’s coefficient was used to calculate the correlations between the values for the early and late changes and the histologic scores. P ≤ .05 was considered to be statistically significant. Calculations were performed using SSPS (version 8.0; SPSS).

RESULTS

There were no statistical differences between group 1 and group 2 with respect to virus quantification before OLT and at 1 month after OLT. For patients in group 1 and patients in group 2, the mean HCV RNA levels (± SD) before OLT were 3.9 × 10<sup>5</sup> ± 1.0 × 10<sup>5</sup> IU and 4.3 × 10<sup>5</sup> ± 1.7 × 10<sup>5</sup> IU, respectively (not significant [NS]). For patients in group 1 and patients in group 2, the mean HCV RNA levels at 1 week after OLT were 1.4 × 10<sup>5</sup> ± 4.7 × 10<sup>4</sup> IU and 4.4 × 10<sup>4</sup> ± 4.3 × 10<sup>4</sup> IU, respectively (NS). The titers for 1 month, 3 months, and 1 year after OLT were measured but did not have statistical significance.

Quasi-species analysis of the 5′UTR by use of SSCP analysis. Four patients (11%) had minor changes in the 5′UTR between the time that the pre-OLT sample was obtained and the time that the first post-OLT sample was obtained. These changes were either the emergence of new bands or the loss of bands present in the pre-OLT sample. The 5′UTR changes had no correlation with the clinical outcome.

Quasi-species analysis of the HVR1/E2 region by use of SSCP analysis. For the HVR1/E2 region, the pattern of major quasi-species variants during transmission (the pre-OLT sample vs. the first post-OLT sample) was stable in 75% of patients in group 2 and in 41% of patients in group 1 (NS).

During follow-up, 26% of the patients in group 1 had no changes in their quasi-species pattern, compared with 33% of patients in group 2 (NS). Examples of one patient with HVR1/E2 changes and another patient with a stable pattern are shown in figure 1.

However, complexity (i.e., the number of bands) was significantly higher in patients in group 1 than in patients in group 2, when the pre-OLT sample was compared with the sample obtained 1 week after OLT (figure 2). These differences were not present in the samples obtained at 3 months and 1 year after OLT. The 2 patients with FCH had a very stable quasi-species pattern throughout follow-up, with no changes noted at any of the time points (figure 3).

Quasi-species analysis of the HVR1/E2 region by use of HMA. To obtain information about the diversity (i.e., the genetic distances) within the HVR1/E2 quasi-species population, RT-PCR products were analyzed by HMA. The mean values for SE and MMS, for both groups, are shown in figure 4. No statistically significant differences between the 2 groups were seen at any of the time points. Next, we analyzed changes in SE and MMS over time. “Early changes” (i.e., transmission
changes) were defined as the difference between the MMS and SE values for the first post-OLT sample and those for the pre-OLT sample. “Late changes” (i.e., changes that occurred after OLT) were assessed, in similar fashion, by subtracting the MMS and SE values for the first post-OLT sample from those for the sample obtained 1 year after OLT.

To correlate the diversity calculated from HMA gels, for patients 2 (group 1) and 3 (group 2), RT-PCR products from different time points were cloned and sequenced. Fifty-seven clones from 4 time points were analyzed for patient 2, and 40 clones from 3 time points were analyzed for patient 3. As seen in figure 5, there was a good overall correlation between phylogenetic reconstructions and intra–quasi-species diversity, as estimated by the mean evolutionary distance and by MMS and entropy values calculated from HMA gels. For patient 3, the mean ratio of nonsynonymous to synonymous substitutions per site was higher than that for patient 2 (0.899 vs. 0.542); this finding suggests that the changes in quasi-species composition that were evident on SSCP analysis and HMA performed for patient 3 were driven, at least in part, by positive immune selection against the envelope epitopes.

The values for early changes and late changes, as measured by SE and MMS, are shown in table 2. The mean SE and MMS values decreased in group 1 and increased in group 2 during transmission, whereas the trend was reversed after OLT. All of these differences were statistically significant. Figure 6 shows
Figure 7. **A**, Fibrosis stage at 1 year after orthotopic liver transplantation (OLT) correlated with the changes in Shannon entropy (SE) and median mobility shift (MMS) during transmission. **B**, Fibrosis stage at 1 year after OLT was inversely correlated with the SE and MMS changes during post-OLT follow-up.

an example of SSCP analysis and HMA performed for 4 patients, 2 of whom had an FS <2 at 1 year and 2 of whom had an FS ≥2 at 1 year.

There was also a direct correlation between the FS at 1 year and MMS changes (r = 0.642, P < .0001), as well as SE changes (r = 0.642, P < .05), at the time of transmission (figure 7A). FS at 1 year was inversely correlated with post-OLT changes measured by MMS (r = −0.536, P < .01) and SE (r = −0.583, P < .01) (figure 7B).

Therefore, early changes in quasi-species parameters were predictive of the outcome: 83% of the patients with increased or stable SE values during transmission belonged to group 2, and 88% of the patients with decreased SE values during transmission belonged to group 1. For a patient with a decrease in SE values during transmission, the odds ratio for having an FS <2 when biopsy was performed at 1 year after OLT was 37.5 (95% confidence interval [CI], 4.5–311.5) (P < .0001). In the post-OLT period, for a patient with an increase in SE values during the first year after OLT, the odds ratio for having an FS <2 was 14.7 (95% CI, 1.5–146.9) (P < .01). MMS did not have predictive value.

**DISCUSSION**

Using HMA and SSCP analysis to measure quasi-species diversity and complexity, we obtained an overall picture of the patterns of transmission and evolution of HCV quasi species after OLT. This approach, compared with that of previous reports [18, 20, 22, 23], includes several new observations. Changes in HCV quasi species during the first week after transplantation have not previously been reported. We found that patients with a lower FS at 1 year (group 1 patients) had a decrease in HVR1/E2 quasi-species diversity, as measured by SE and MMS, during the first week after OLT. In contrast, patients with a higher FS at 1 year (group 2 patients) had either a slight increase or stability in quasi-species diversity during the first week after OLT.

Of importance, we were able to predict the clinical outcome at 1 year by analyzing very early changes in quasi-species diversity. Patients who had decreased quasi-species diversity during the first week after OLT, as measured by SE, were 37 times more likely to have an FS <2 at 1 year than were patients who had an increase in SE values. This measurement allows for early identification of patients who are most at risk.

Likewise, for patients with a higher FS at 1 year (group 2 patients), quasi-species diversity remained relatively unchanged during the first year. In contrast, patients with a lower FS at 1 year (group 1 patients) had increased quasi-species diversity during the first week after OLT and during the first year after OLT (table 2).

To our knowledge, there have been no reports of a clear association between the number of viral variants (i.e., complexity) and the outcome after transplantation. In the present study, we found that patients who had a lower FS when a protocol biopsy was performed at 1 year after OLT had higher quasi-species complexity in samples obtained before OLT and at 1 week after OLT. In a recently published study, Lyra et al. [23] studied HCV quasi species in 11 patients who were undergoing OLT, by cloning and sequencing pre-OLT and post-OLT serum samples. By studying 10 clones/patient, they found an inverse correlation of amino acid complexity and FS.

In the present study, 2 patients developed FCH and displayed a very stable quasi-species pattern with low complexity. These findings are in accord with those of Doughty et al. [20], who reported that patients with cholestatic hepatitis displayed a significantly lower number of bands on SSCP analysis and had a more stable band pattern than did patients with noncholestatic hepatitis.

The decreased diversity and complexity of quasi species within the HVR1/E2 region of immunosuppressed patients, including patients undergoing bone marrow transplantation [40], hypogammaglobulinemic patients [41], and HIV-HCV–coinfected individuals who had low CD4 T cell counts [42], suggest that the immune response acts as a major selective force on the HVR1/E2 region during HCV infection. Thus, patients with a better immune response before transplantation could induce the development of escape mutants, resulting in a higher quasi-species complexity. In the present study, patients with a
greater number of variants (i.e., complexity) had a better clinical outcome at 1 year after OLT. One potential reason for this observation is the possibility of a more robust host immune response to the virus.

A similar mechanism could drive quasi-species diversity. Thus, in patients with worse recurrence, stable quasi-species diversity could reflect the lack of immune pressure in these patients. We could also speculate that a weak immune response could favor the selection and fixation of more-virulent viral variants. This would be in accordance with what has been reported for HIV—1—that is, slower evolution of quasi species was observed among patients for whom progression to AIDS was rapid [32, 35].

In summary, we have shown that the clinical outcome for HCV-positive patients who undergo transplantation is closely correlated with virus variability in the HVR1/E2 region, which is most likely a reflection of the immune system response of the host. HCV quasi-species analysis may predict, within the first week after OLT, the outcome at 1 year with regard to liver fibrosis. This observation may help to determine the amount of immunosuppression and need for antiviral therapy.

References


