# Clinical and Therapeutic Implications of Hepatitis C Virus Compartmentalization

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***Background & Aims:*** **Blood mononuclear cells (BMCs) frequently are infected by hepatitis C virus (HCV) variants that are not found in plasma. The influence of this compartmentalization on the natural and therapeutic outcome of hepatitis C is unknown.** ***Methods:*** **We studied 119 patients with previously untreated chronic HCV infection. Sixty-five of these patients started first-line treatment with pegylated interferon-alfa and ribavirin after enrollment in the study. The internal ribosomal entry site (IRES) of HCV RNA was amplified and compared between plasma and BMCs by means of single-strand conformational polymorphism (SSCP) analysis, line-probe assay, and cloning sequencing.** ***Results:*** **The IRES SSCP patterns differed between plasma and BMCs in 54 (48%) of 113 assessable patients. Twenty-seven (24%) of these patients were co-infected by 2 HCV types or subtypes, only 1 of which was detectable in BMCs (n = 25) or in plasma (n = 2). SSCP-defined compartmentalization was more frequent in former drug users than in others (35/56 [60%] vs 19/56 [34%];** $P < .01$**), and less frequent in patients with genotype 1 HCV in plasma (26/73 [24%] vs 28/40 [65%];** $P < .01$**). The only variables that were independently predictive of a sustained virologic response were SSCP-defined compartmentalization (25/31 vs 10/32;** $P = .0001$**) and genotype 2 or 3 infection of BMCs (22/31 vs 8/34;** $P = .002$**).** ***Conclusions:*** **A significant proportion of patients with hepatitis C are co-infected by 2 or more HCV variants with distinct IRES sequences and distinct cellular tropism. This compartmentalization is a strong independent predictor of treatment efficacy.**

Hepatitis C virus (HCV) is a positively stranded RNA virus that infects more than 1% of the world's population and is a major cause of cirrhosis and hepatocellular carcinoma. Phylogenetic analysis of HCV strains collected worldwide has identified at least 6 types and multiple subtypes.[1] Combination therapy with pegylated interferon-alfa and ribavirin yields a sustained virologic response (SVR) after 24 weeks in more than 80% of patients with genotype 2 or 3 HCV infection (based on plasma sampling), but in less than 50% of patients with genotype 1 or 4 infection treated for 48 weeks.

Many studies have shown the presence of positively and negatively stranded HCV RNA in blood mononuclear cells (BMCs) of chronically infected individuals.[2–7] Positive and negative HCV-RNA strands also were detected in BMCs harvested from long-term responders to interferon–ribavirin combination therapy[8,9] and from treatment-naive HCV-seropositive patients with undetectable viremia.[10] Finally, a recent report[11] showed the presence of HCV proteins and positive and negative HCV-RNA strands in lymph nodes. Infection of immune cells could be a mechanism by which HCV evades the host response, but no relation has been reported between BMC infection and the natural or therapeutic outcome of HCV infection.

HCV-RNA polymerase lacks proofreading ability, and this leads to strong genomic variability. In infected patients, HCV circulates as a population of closely related variants that are referred to collectively as a *quasispecies*. This quasispecies distribution may be involved in viral persistence in the early stages of infection.[12] HCV compartmentalization is a concept in which viral variants are distributed nonrandomly among the different sites of replication.[13] Studies of the hypervariable region of the HCV envelope E2 show that specific variants are frequent particularly in B lymphocytes and monocytes.[14] HCV compartmentalization also can be detected by analyzing the 5′ untranslated part of HCV RNA (the most strongly conserved region), corresponding to the internal ribo-

*Abbreviations used in this paper:* BMC, blood mononuclear cells; HVR, hypervariable region; IRES, internal ribosomal entry site; PCR, polymerase chain reaction; SSCP, single-strand conformational polymorphism; SVR, sustained virologic response; UTR, untranslated region.


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somal entry site (IRES) that regulates viral polyprotein translation. Some patients have been found to have different HCV genotypes in their brain and plasma.[15,16] In a recent study of 65 immunocompetent patients and 44 liver transplant recipients,[17] we found that the plasma and BMC IRES sequences differed in 39% of patients, and that the BMCs of 9% of patients harbored a genotype that could not be detected in their plasma. Infection through drug use or liver transplantation was associated with the presence of divergent BMC variants. The impact of such mixed infections on the natural and therapeutic outcome of HCV infection is not known.

Here we examined possible relations between IRES-defined HCV compartmentalization and the main clinical, histologic, and virologic characteristics of HCV infection, focusing on the SVR status more than 1 year after the end of interferon–ribavirin combination therapy.

## Patients and Methods

### Patients

A total of 119 patients managed by 2 of the authors (C.F. and D.S.) at Paul Brousse Hospital, France, were enrolled in this study with their written consent. The inclusion criteria were anti-HCV and plasma HCV-RNA positivity, hepatitis B surface antigen and anti–human immunodeficiency virus negativity, no previous treatment with interferon or ribavirin, no anticancer chemotherapy or therapeutic immunosuppression, no intranasal or intravenous illicit drug use in the past 3 years, current alcohol intake less than 20 g/day, and normal liver function (prothrombin time >70%). When transfusion or intravenous/intranasal drug use was clearly the route of infection, 2 distinct durations of HCV infection and 2 distinct ages at infection were estimated, from the years of first and last transfusion (before 1991) and the years of first and last drug use. Among patients transfused before 1991, those who also used drugs formed a separate subgroup. Patients with no history of drug use or transfusion were classified as having an unknown route of infection, even in cases of previous tattooing, piercing, invasive medical investigations, or surgery. A liver biopsy specimen obtained the day of blood sampling was available for 111 of 119 patients. Hepatic fibrosis and inflammatory activity were graded with the METAVIR system.[18] Forty of the 119 patients were included in our previous study of IRES-defined BMC compartmentalization.[17] Finally, anti–HBc was positive in 16 of 55 patients who were former drug users and in 8 of 64 other patients ($P = .04$).

Sixty-five patients (52%) started treatment between January 2001 and January 2003, based on weight-dosed pegylated interferon-alfa 2b (Virapeg; Schering-Plough, Raritan, NJ) 1–1.5 μg/kg/wk (depending on predicted tolerability) and ribavirin 10–15 mg/kg (according to renal function and the red cell count). Therapy was stopped if viremia persisted at the 12th week. Otherwise, it was continued for another 12 weeks in patients with genotype 2 or 3 infection and for 36 weeks in other patients. Virologic responses and SVRs, respectively, were defined by undetectable HCV viremia (50 copies/mL) in the Amplicor Cobas assay (Roche Diagnostic, Meylan, France) at the 12th week after treatment initiation and the 12th month after treatment completion. The main characteristics of the treated and untreated patients are shown in Table 1.

**Table 1.** Characteristics of Treated and Untreated Patients

| Variables | Not treated (n = 54) | Treated (n = 65) | *P* value |
|---|---|---|---|
| Men | 29 (51%) | 32 (52%) | NS |
| Age at inclusion | 46 ± 12 | 43 ± 11 | NS |
| Age at infection | 26 ± 9 | 24 ± 7 | NS |
| Duration of infection | 18 ± 9 | 17 ± 7 | NS |
| HCV RNA (log copies/mL) | 5.7 ± 5.1 | 5.9 ± 6.2 | NS |
| Route of infection | | | |
| IVDU | 25 (45%) | 30 (46%) | NS |
| Transfusion | 15 (28%) | 15 (23%) | NS |
| IVDU and transfusion | 2 (4%) | 3 (5%) | NS |
| Unknown | 12 (22%) | 17 (26%) | NS |
| Plasma HCV genotypes | | | <.001 |
| 1 | 40 (74%) | 38 (58%) | |
| 2 | 1 (2%) | 12 (18%) | |
| 3 | 3 (6%) | 13 (20%) | |
| 4 | 10 (19%) | 2 (3%) | |
| Biopsy examination | 48 (89%) | 63 (97%) | NS |
| Fibrosis score | | | NS |
| 0 | 7 (15%) | 5 (8%) | |
| 1 | 23 (48%) | 28 (44%) | |
| 2 | 4 (8%) | 15 (24%) | |
| 3 | 1 (2%) | 8 (13%) | |
| 4 (cirrhosis) | 13 (28%) | 7 (11%) | |

NS, not significant; IVDU, intravenous drug use.

### Sampling and HCV-RNA Amplification

Blood was collected on the day of liver biopsy examination. BMCs were isolated from 10 mL of blood with the Vacutainer CPT (Becton Dickinson, Le Pont-De-Claix, France). RNA was extracted from 140 μL of plasma with the QIAmp Viral RNA kit (Qiagen GmBH, Germany), and from BMCs with the RNeasy minikit (Qiagen, Courtaboeuf, France).

To detect the HCV genome, one fifth of the plasma extract or 1 μg of cellular RNA extract was subjected to reverse-transcription polymerase chain reaction with Ready-To-Go reverse-transcription polymerase chain reaction beads (Pharmacia Biotech, Uppsala, Sweden). Three microliters of the first polymerase chain reaction (PCR) product was subjected to a second round of PCR using Ready-To-Go PCR beads, according to the manufacturer's instructions. A 250–base pair (bp) fragment (nucleotides 100–350) of the 5′untranslated region (5′UTR) was amplified with a nested reverse-transcription polymerase chain reaction protocol using previously published primers.[17] A 1500-bp fragment (nucleotides 100–1629) span-