# Changes in Mood States and Biomarkers During Peginterferon and Ribavirin Treatment of Chronic Hepatitis C

Robert J. Fontana, M.D.,[1] Ziad Kronfol, M.D.,[2] Karen L. Lindsay, M.D.,[3] Linas A. Bieliauskas, Ph.D.,[2,4] Latha Padmanabhan, M.S.,[5] Carla Back-Madruga, Ph.D.,[6] Anna S.F. Lok, M.D.,[1] Anne M. Stoddard, Sc.D.,[5] and the HALT-C Trial Group

[1]*Division of Gastroenterology and* [2]*Department of Psychiatry, University of Michigan, Ann Arbor, Michigan;* [3]*Division of Gastrointestinal and Liver Diseases and* [6]*Department of Psychiatry and the Behavioral Sciences, Keck School of Medicine, University of Southern California, Los Angeles, California;* [4]*Psychology Service, Veterans Affairs Health System, Ann Arbor, Michigan; and* [5]*New England Research Institutes, Watertown, Massachusetts*

**OBJECTIVE:** Depression is a frequent side effect of interferon therapy in patients with chronic hepatitis C (CHC). The aim of this study was to identify baseline and on-treatment predictors of depression in CHC patients receiving peginterferon and ribavirin.

**METHODS:** In total, 201 prior nonresponders with advanced fibrosis were treated with peginterferon alfa-2a and ribavirin for 24 wk in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis trial. Of these, 74 continued on antiviral therapy through week 48. Mood states were assessed with the Beck Depression Inventory II and the Composite International Diagnostic Interview. Plasma cortisol and whole blood serotonin levels were measured in 101 subjects at weeks 0, 4, 24, 48, and 72.

**RESULTS:** The incidence of interferon-induced depression was 23% and 42% at weeks 24 and 48, respectively. Although 22% of patients had baseline depression, the absence of a week 20 virological response was the only independent predictor of interferon-induced depression at week 24 ($P = 0.0009$). Plasma cortisol levels did not change during treatment nor correlate with depression. In contrast, whole blood serotonin/platelet levels significantly decreased during treatment, but did not correlate with interferon-induced depression through week 24 ($P = 0.35$), nor through week 48 ($P = 0.51$).

**CONCLUSION:** Depression during peginterferon and ribavirin therapy was associated with a lower antiviral response. The significant reduction in whole blood serotonin levels over time suggest that further studies of the serotonergic pathway are warranted to identify the mediators of interferon-induced depression.

(Am J Gastroenterol 2008;103:2766–2775)

## INTRODUCTION

Commonly reported psychiatric side effects of interferon therapy include depression, anxiety, and irritability, although mood changes associated with the underlying medical condition can be difficult to discern from the medication effects (1, 2). Peginterferon and ribavirin combination therapy is associated with a clinically significant depression in 30–50% of patients with chronic hepatitis C (CHC) treated in clinical trials as well as in clinical practice (3, 4). Using a battery of objective and validated neuropsychological tests, cognitive function did not worsen during peginterferon and ribavirin treatment of CHC patients with advanced fibrosis (5). However, many CHC patients receiving antiviral therapy complain

not only of impaired cognition but also of moderate-to-severe depression, anxiety, and fatigue, as measured by self-report and interviewer-administered questionnaires (5, 6). As psychiatric side effects are a leading cause of interferon dose reductions or premature discontinuation, further studies regarding the incidence and pathogenesis of interferon-induced mood alterations are warranted.

Selective serotonin reuptake inhibitors (SSRI) can effectively treat and potentially prevent the depression seen in patients receiving interferon (7–10). However, the studies reported to date have identified different, and sometimes, conflicting predictors of interferon-induced depression including baseline mood status (10–13). Furthermore, the mechanism(s) of interferon-induced depression have not been elucidated, although a few studies have suggested a possible role for changes in the hypothalamic–pituitary adrenal

(HPA) axis as well as the central catecholamine and serotonin systems (14–17). The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial is a prospective multicenter study designed to investigate the potential benefit of maintenance peginterferon in reducing the rate of disease progression in prior interferon nonresponders with advanced fibrosis (18, 19). During the lead-in phase, all patients received peginterferon alfa-2a and ribavirin for 24 wk, and subjects who remained viremic at week 20 were eligible for randomization to low-dose peginterferon or no treatment. The subjects who had undetectable HCV RNA by polymerase chain reaction (PCR) at week 20 continued in the responder arm of the study to complete 48 wk of antiviral treatment. In this article, the incidence, baseline predictors, and potential biomarkers associated with mood disturbances in 201 HALT-C patients are reported. It was hypothesized that depressive symptoms at baseline would predict depression during antiviral therapy, and that depression scores would positively correlate with plasma cortisol levels and negatively correlate with whole blood serotonin levels.

## METHODS

### Patient Population and Study Design
The 201 patients enrolled in this ancillary study were recruited from the University of Michigan (UM) and the University of Southern California (USC). The inclusion criteria for the HALT-C trial were detectable serum HCV RNA, a liver biopsy within 12 months of enrollment showing an Ishak fibrosis score of 3–6, and a lack of response to previous interferon treatment. The exclusion criteria included any other co-existent liver disorder, a history of hepatic decompensation, and intolerance to interferon (19). Additional exclusion criteria were an active use of illicit drugs, ongoing excessive alcohol consumption, a history of suicide attempt or hospitalization for depression within the past 5 yr, and a history of severe or incapacitating psychiatric condition within the past 6 months. A history of major depression that was not severe or incapacitating at enrollment was not considered as an exclusion criterion. All patients provided written informed consent to participate in the HALT-C trial as well as in this ancillary study at UM and USC.

All subjects were treated with peginterferon alfa-2a at a dose of 180 $\mu$g per week (Pegasys; Roche Laboratories, Nutley, NJ) and ribavirin in doses of 1.0–1.2 grams per day (Copegus; Roche Laboratories). Serum HCV RNA testing was done using the quantitative COBAS Amplicor HCV Monitor test (Roche Molecular Diagnostics, Branchburg, NJ) with a lower detection limit of 600 IU/mL and the qualitative COBAS Amplicor HCV test (Roche Molecular Diagnostics) with a lower detection limit of 100 IU/mL. Patients who tested HCV RNA negative at week 20 were considered virological responders and continued with combination antiviral therapy for 48 wk in the responder arm of the study. Patients without detectable HCV RNA at week 72 were considered to have a sustained virological response (SVR). Adherence was defined as taking at least 80% of the prescribed peginterferon and ribavirin dose through week 12 and week 20 (20).

### Psychiatric Assessment
The Composite International Diagnostic Interview (CIDI) is a standardized instrument for the assessment of mental health disorders according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria (21). In this study, the computerized lifetime (LT) version of the CIDI that included depression, anxiety, alcohol, and substance abuse modules was self-administered at baseline (week 0). During antiviral therapy, the 12-month version of the depression module alone was administered at weeks 24, 48, and 72.

The Beck Depression Inventory (BDI)-II, is a 21-item, self-administered questionnaire designed to screen for depressive symptoms over the previous 2 wk (22). The BDI was administered at weeks 0 (baseline), 4, 12, 24, 36, 48, 60, and 72. A score <10 was considered as no depression, 11–14 as minimal, 15–19 as mild, 20–28 as moderate, and >28 as severe depression. A lifetime alcohol consumption was estimated using a modification of the Skinner survey (23). Years of education were coded by the highest level completed, and occupation was categorized on a scale of 1–6 with 1 = unskilled/farm labor and 6 = professional/technical (24).

### Definition of Depression
Depression at baseline (week 0) was defined as either a BDI $\geq$11 or fulfilling the DSM-IV criteria for major depression during the previous 12 months on the CIDI. For subjects without baseline depression, interferon-induced depression was defined at each postbaseline time point as a BDI $\geq$11 or fulfilling DSM-IV criteria for depression via the CIDI. For subjects with baseline depression (i.e., baseline BDI-II $\geq$11 or CIDI criteria for depression), interferon-induced depression was defined at each postbaseline time point as a BDI score that had increased by 6 points or more compared with baseline. The rationale for this criterion includes prior studies that used a numerical increase in BDI-II scores or other self-rating scales to define interferon-induced depression in subjects with baseline depression (25, 26). The use of antidepressants such as SSRI's (e.g., fluoxetine) and non-SSRI's (e.g., nortriptyline) was recorded, but was not part of the definition of depression.

### Cortisol and Serotonin Assays
In subjects enrolled at UM, fasting morning blood samples were processed immediately and stored at –70°C. The samples were tested in batches to minimize interassay variability. Plasma cortisol was measured at the UM Hospital Chemistry Laboratory by a chemiluminescent immunoassay on an Advia Centaur analyzer (Bayer Diagnostics, Tarrytown, NY). The coefficient of variation for the cortisol assay varied from 4.3% to 8.0% over the reference range for morning cortisol levels of 7–22 $\mu$g/dL (25). Whole blood serotonin was measured using a high-pressure liquid chromatography method with fluorescence detection (Bioanalytical Systems, West Lafayette, IN)

(26). The dynamic range of the assay was 1–10,000 nM/L, with a coefficient of variation of 3.0%. Samples with low or undetectable whole blood serotonin levels were repeated to verify the results. As platelets are a rich source of serotonin in whole blood, and interferon therapy is known to reduce platelet counts, the whole blood serotonin/platelet ratio was analyzed as a biomarker of whole body serotonin stores.

### Data Analyses

A Kaplan-Meier product-moment analysis was used to estimate the cumulative incidence of depression at week 24 and week 48. A Cox-proportional hazard analysis was used to identify baseline predictors associated with depression in both week 24 and week 48 cohorts. In addition, adherence to antiviral medications as well as week 12 virological response (*i.e.*, decline in HCV RNA by $>2$ $\log_{10}$ or undetectable) and week 20 virological response (*i.e.*, HCV RNA undetectable) were tested as predictors of depression. The association of each variable with interferon-induced depression was tested alone, and a multivariable model was computed that included all variables with $P < 0.15$ and controlling for baseline depression. The association between the biological markers and baseline BDI-II scores in the UM patients was evaluated by the Pearson correlation coefficient. The change over time in the biological markers was evaluated using a repeated-measures analysis of variance (ANOVA). The association between the changes in biomarkers and development of interferon-induced depression through weeks 24 and 48 was evaluated using the Cox proportional hazard analysis. All analyses were carried out using the SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC).

## RESULTS

### Study Population

The baseline characteristics of the 102 subjects enrolled at UM and the 99 subjects enrolled at USC were comparable in all respects except for the proportion of Hispanics, number of HCV genotype 1 patients, and the duration of HCV infection. Therefore, the data from the 201 subjects were grouped together for analysis (Table 1). At week 24, 185 subjects completed the BDI-II, while the remaining 16 subjects had withdrawn from the HALT-C study (N = 13), the cognitive study (N = 2), or failed to complete the BDI-II (N = 1). Seventy-four of the 201 patients (37%) had undetectable HCV RNA at week 20 and continued in the responder arm of the trial through week 48. The remaining 127 subjects with detectable HCV RNA at week 20 had antiviral therapy discontinued at week 24 and were eligible for the randomized phase of HALT-C.

The baseline features of the 201 HALT-C patients demonstrated a mean age of 50 yr and 71% were male (Table 1). The mean baseline BDI-II score was 6.9, with 22% having a score greater than 11, 5.5% having a score $\geq 20$, and 7% meeting the DSM-IV criteria for depression in the previous 12 months. The 51 HALT-C patients (25%) with baseline depression were significantly more likely to be female, non-Caucasian, and have lower educational levels compared with the 150 nondepressed patients ($P < 0.05$). In addition, the depressed subjects were significantly more likely to be receiving an antidepressant or anxiolytic at week 0. Finally, the whole blood serotonin/platelet levels tended to be lower in the patients with baseline depression ($P = 0.10$).

### Depression Through Week 24

The cumulative incidence of interferon-induced depression in the 201 subjects increased from 2% at week 4 to 6% at week 12 and 22% at week 24 (Fig. 1). The use of antidepressant/anxiolytic medications at baseline ($P = 0.054$) and the failure to achieve a week 12 virological response ($P = 0.018$) or week 20 virological response ($P = 0.0023$) were associated with interferon-induced depression (Table 2). As the HALT-C study had called for 24 wk of antiviral therapy, and there was a significant interaction between week 12 and week 20 virological response (kappa $= 0.60$, $P < 0.0001$), only week 20 virological response and other variables with $P < 0.15$ were used in the multivariate model. In the multivariable analysis, only the absence of a week 20 virological response remained significantly associated with interferon-induced depression ($P = 0.0009$).

Among the 150 subjects without baseline depression, the cumulative incidence of depression was 2% at week 4, 7% at week 12, and 21% at week 24 (Fig. 1). Similarly, in the 51 subjects who met the criteria for baseline depression, the cumulative incidence of interferon-induced depression was 4% at weeks 4 and 12, and 32% at week 24 (Fig. 1). Therefore, the incidence of interferon-induced depression was not significantly different in patients with and without baseline depression (hazard ratio 1.47, 95% confidence interval [CI] 0.79–2.73, $P = 0.22$).

### Depression Through Week 48

Among the 74 subjects who continued on the antiviral therapy through week 48, 64 (86%) had undetectable HCV RNA at week 48, and overall 38 (51%) achieved an SVR at week 72. The actuarial incidence of interferon-induced depression in these subjects increased from 4% at week 4 to 9% at week 24, and 42% at week 48 (Fig. 2). However, none of the baseline features including baseline BDI-II scores or adherence to peginterferon or ribavirin were associated with the development of depression through week 48 (data not shown). Among the 66 subjects with available BDI-II scores through week 72, the mean BDI-II scores at week 72 were significantly lower compared with week 24 ($4.7 \pm 0.7$ *vs* $7.8 \pm 0.7$, $P = 0.0009$) and week 48 ($4.7 \pm 0.7$ *vs* $7.4 \pm 0.7$, $P = 0.0006$), demonstrating that interferon-induced depression is reversible.

### Changes in Serum Biomarkers

At baseline, plasma cortisol levels ranged from 2.2 to 24.3 $\mu$g/dL, with only 2% of the patients having cortisol levels that

**Table 1.** Clinical Characteristics of Overall Study Population and Patients With and Without Baseline Depression

| | Total Population (N = 201) | Baseline Depression (N = 51) | No Depression (N = 150) | P Value |
|---|---|---|---|---|
| Age (yr) | 50.23 (7.8) | 50.49 (7.2) | 50.15 (8.0) | 0.79 |
| % female | 29 | 41 | 25 | 0.0318 |
| Ethnicity | | | | 0.16 |
|   % white | 71 | 59 | 75 | |
|   % black | 1 | 22 | 12 | |
|   % Hispanic | 10 | 14 | 9 | |
|   % other | 4 | 6 | 3 | |
| Educational level (yr) | 13.38 (2.3) | 12.78 (1.6) | 13.58 ( 2.5) | 0.0096 |
| Occupational codes* | 4.02 (1.6) | 3.71 (1.6) | 4.12 (1.6) | 0.10 |
| % married | 69 | 57 | 73 | 0.0356 |
| Parenteral risk factors | | | | |
|   Ever received a transfusion, % | 40 | 53 | 35 | 0.0265 |
|   Ever experienced needle stick, % | 15 | 20 | 14 | 0.34 |
|   Ever used needles for recreational drugs, % | 45 | 41 | 47 | 0.50 |
|   None of the above, % | 14 | 8 | 17 | 0.12 |
| Time since last IFN therapy (days) | 814.16 (740.42) | 857.92 (842.7) | 799.29 (704.8) | 0.63 |
| % Ishak 5/6 | 38 | 39 | 37 | 0.81 |
| Log HCV RNA (IU/mL) | 6.51 (0.5) | 6.58 (0.5) | 6.48 (0.5) | 0.22 |
| % HCV genotype 1 | 88 | 90 | 87 | 0.59 |
| Serum ALT (IU/mL) | 2.52 (1.95) | 2.28 (1.63) | 2.60 (2.1) | 0.31 |
| Platelets ($10^3$/mL) | 167.43 (61.0) | 166.06 (59.79) | 167.90 (61.6) | 0.85 |
| % diabetics | 25 | 31 | 23 | 0.25 |
| % prior IFN + ribavirin | 71 | 73 | 71 | 0.80 |
| Lifetime alcohol consumption (drinks/day) | 2.06 | 2.58 | 1.89 | 0.23 |
| BDI-II score | 6.86 (6.5) | 15.39 (6.3) | 3.95 (3.1) | <0.0001 |
| % BDI $\geq$11 | 22 | 86 | 0 | <0.0001 |
| CIDI depression (last 12 months) | 7% | 29% | 0% | <0.0001 |
| CIDI depression (lifetime) | 15% | 43% | 6% | <0.0001 |
| % on anxiolytic/antidepressants | 29 | 55 | 20 | <0.0001 |
| Plasma cortisol ($\mu$g/dL) | 12.41 (4.5) | 12.53 (4.8) | 12.36 (4.5) | 0.87 |
| Whole blood serotonin (nM/L) | 512.73 (371.1) | 463.96 (462.9) | 530.53 (333.3) | 0.50 |
| Whole blood serotonin/platelets (nM/L/ $10^3$ platelets) | 3.12 (1.9) | 2.60 (2.3) | 3.31 (1.8) | 0.10 |
| % week 12 virological response | 58 | 59 | 57 | 0.85 |
| % week 20 virological response | 37 | 35 | 37 | 0.79 |
| % sustained virological response | 19 | 16 | 20 | 0.50 |
| % took >80% peginteferon through week 12 | 76 | 75 | 76 | 0.83 |
| % took >80 % ribavirin through week 12 | 70 | 63 | 72 | 0.21 |
| % took >80% peginterferon through week 20 | 67 | 61 | 69 | 0.26 |
| % took >80% ribavirin through week 20 | 59 | 49 | 63 | 0.09 |

1 = unskilled labor (N = 3); 2 = semiskilled, operative, service (N = 53); 3 = not in work force >10 yr (N = 21); 4 = skilled labor/craftsman/foreman (N = 25); 5 = manager/clerical/sales work (N = 53); 6 = professional/technical (N = 43).
Data reported as mean (SD) or %.
IFN = interferon.

exceeded the normal range. There was no significant correlation between the baseline cortisol levels and BDI-II scores. As noted in Figure 3, plasma cortisol levels decreased slightly by week 4 and then remained stable at week 24, with no significant change over time ($P = 0.60$). Furthermore, there was no significant association between cortisol levels and the development of interferon-induced depression through week 24 ($P = 0.61$) or with BDI-II scores through week 24 (data not shown).

Prior to antiviral therapy, whole blood serotonin levels ranged from 4 to 2,023 nM/L, while whole blood serotonin levels normalized to platelet count ranged from 0.02 to 8.80 nM/L/$10^3$ platelets. There were no significant correlations between baseline whole blood or normalized serotonin levels and BDI-II scores ($P = 0.59$ and $P = 0.23$, respectively).

The normalized serotonin levels significantly decreased from baseline through week 24 ($P < 0.0001$) (Fig. 4). However, neither the changes in normalized serotonin levels nor the whole blood serotonin levels correlated with interferon-induced depression through week 24 or week 48 ($P = 0.35$ and $P = 0.51$, respectively). In addition, there was no significant correlation of these biomarker levels with the BDI-II scores (data not shown).

## DISCUSSION

Previously published studies of mood states in CHC patients receiving an antiviral therapy have been limited by the small numbers of patients enrolled, the nonstandardized definitions
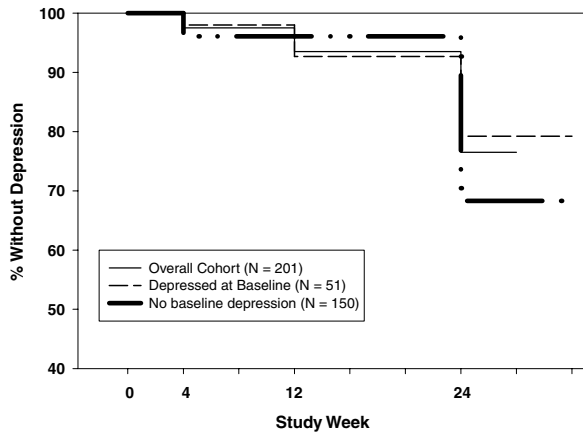
**Figure 1.** Interferon-induced depression through week 24 in 201 HALT-C patients. The incidence of interferon-induced depression increased to 23% by week 24. However, the incidence of interferon-induced depression was not significantly different in the 51 subjects with baseline depression compared with the 150 subjects without baseline depression (hazard ratio 1.47, 95% CI 0.79–2.73, $P = 0.22$).

of depression, and the heterogeneity of antiviral treatment regimens used (2, 27, 28). In this study, 201 CHC patients with advanced fibrosis entering a clinical trial of peginterferon and ribavirin were prospectively assessed for the changes in mood status over time using a series of validated instruments. The BDI-II was selected due to its brevity and established utility in detecting depression in patients with medical conditions (22, 29, 30). Furthermore, the BDI-II has been used in previous studies of CHC patients and correlated well with mental health professional assessment of mood disorders (31). The computerized CIDI depression module that is based upon the DSM-IV criteria was also used and previously shown to correlate with interviewer-administered questionnaires (21). As the CIDI is self-administered, this instrument may also be more likely to detect depression. Overall, 51 (25%) of the HALT-C patients met the criteria for depression before the antiviral therapy, although the depression was graded as mild in most. The high rate of baseline mood disorders may relate to the referral bias, the requirement for previous nonresponse to interferon and advanced fibrosis to enter the HALT-C study, and the prospective characterization of current and lifetime psychopathology. However, other prospective studies have also identified a 20–30% prevalence of mood disorders in CHC patients prior to antiviral therapy (13, 32)

The cumulative incidence of interferon-induced depression increased to 23% by week 24, a rate that is similar to what has been reported in other studies (Fig. 1) (4, 27, 32). In addition, 16 subjects (8%) had to prematurely discontinue therapy by week 24 due to various adverse events. Furthermore, 33% of the treated patients took less than 80% of the prescribed peginterferon dose through week 20 due to a variety of side effects including neuropsychiatric toxicity. Based upon the

**Table 2.** Bivariate and Multivariable Predictors of Interferon-Induced Depression Through Week 24 (N = 201 HALT-C Patients)

| Variable | Bivariate | | | Multivariable | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio | 95% CI | *P* Value | Hazard Ratio | 95% CI | *P* Value |
| Age | 0.97 | 0.94–1.01 | 0.11 | 0.97 | 0.94–1.01 | 0.22 |
| Female | 1.22 | 0.66–2.67 | 0.53 | | | |
| Ethnicity | | | 0.52 | | | |
| White | 1.60 | 0.22–11.63 | | | | |
| Black | 2.20 | 0.28–17.35 | | | | |
| Hispanic | 0.91 | 0.10–8.74 | | | | |
| Education level | 1.02 | 0.91–1.15 | 0.71 | | | |
| Occupation | 1.09 | 0.90–1.32 | 0.36 | | | |
| Married | 1.00 | 0.54–1.86 | 0.99 | | | |
| Baseline BDI-II score | 1.01 | 0.96–1.05 | 0.76 | | | |
| Parental risk factors | | | | | | |
| Received transfusion | 0.91 | 0.50–1.66 | 0.75 | | | |
| Needle stick | 1.39 | 0.67–2.88 | 0.38 | | | |
| Recreational drugs | 1.45 | 0.80–2.60 | 0.22 | | | |
| None of above | 0.72 | 0.29–1.83 | 0.49 | | | |
| Time since last IFN | 1.00 | 1.00–1.00 | 0.68 | | | |
| Cirrhosis | 0.86 | 0.46–1.60 | 0.64 | | | |
| Genotype 1 | 1.51 | 0.54–4.20 | 0.44 | | | |
| Antidepressant/anxiolytic at baseline | 1.79 | 0.99–3.23 | 0.054 | 1.45 | 0.73–2.88 | 0.29 |
| Depression at baseline | 1.47 | 0.79–2.73 | 0.22 | 1.01 | 0.48–2.14 | 0.98 |
| Diabetes | 0.55 | 0.27–1.33 | 0.15 | 0.51 | 0.21–1.22 | 0.13 |
| Prior combination therapy | 1.48 | 0.73–2.99 | 0.27 | | | |
| Total drinks per lifetime | 1.00 | 1.00–1.02 | 0.10 | 1.00 | 1.00–1.02 | 0.17 |
| Week 20 virologic response | 0.29 | 0.13–0.64 | 0.0023 | 0.21 | 0.08–0.52 | 0.0009 |
| Week 12 virologic response* | 0.79 | 0.27–0.88 | 0.018 | | | |
| Took >80% peginterferon through week 20 | 0.76 | 0.42–1.40 | 0.37 | | | |
| Took >80% ribavirin through week 20 | 1.24 | 0.67–2.31 | 0.49 | | | |
| Took >80% peginterferon through week 12 | 0.77 | 0.40–1.50 | 0.44 | | | |
| Took >80% ribavirin through week 12 | 1.12 | 0.58–2.16 | 0.75 | | | |

*Week 12 virological response defined as a decrease in HCV RNA by $> 2\log_{10}$ IU/mL compared with baseline or undetectable
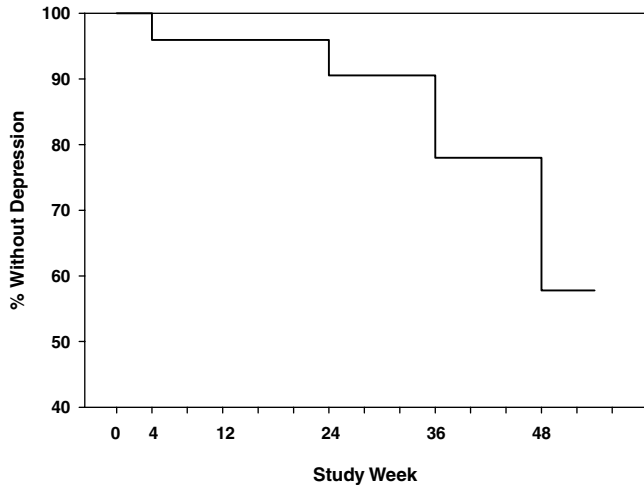
**Figure 2.** Interferon-induced depression through week 48 in 74 HALT-C patients. The incidence of interferon-induced depression continued to increase from 9% at week 24 to 42% at week 48.

studies of interferon therapy in melanoma and CHC patients, subjects with baseline depression were expected to be at an increased risk of interferon-induced depression (10, 13, 33). In support of this, the use of antidepressants/anxiolytics at baseline was associated with interferon-induced depression on the univariate analysis (Table 2). Although expected trends were noted for several other baseline variables (*i.e.*, baseline depression [$P = 0.22$], age [$P = 0.11$]), none remained significant in the multivariate analysis. The absence of a significant association of baseline depression with interferon-induced depression may have been due to the better tolerability of peginterferon compared with high-dose standard interferon used in prior studies and the selection of interferon-"tolerant" patients for the HALT-C study (34, 35). It is also possible that
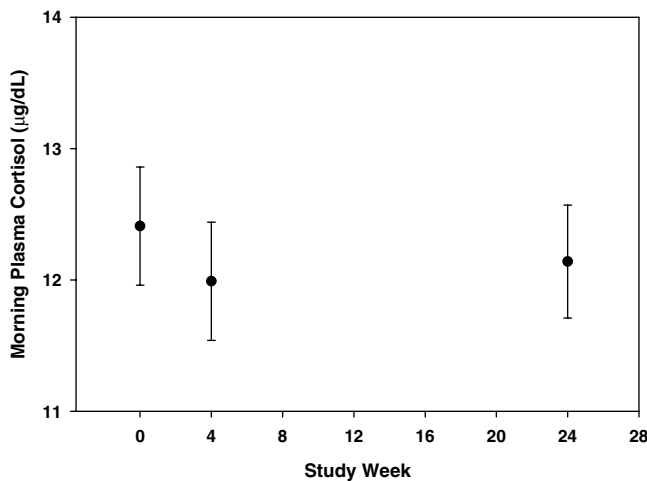


**Figure 3.** Morning plasma cortisol levels through week 24 in 101 HALT-C patients. There was no significant change in the mean plasma cortisol levels over time ($P = 0.60$). Values plotted as mean ± standard error.
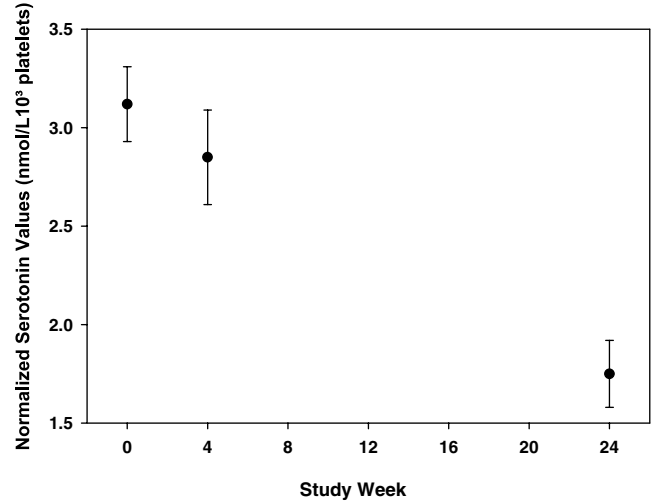


**Figure 4.** Normalized whole blood serotonin levels through week 24 in 101 HALT-C patients. The y-axis value was determined by dividing the whole blood serotonin level by the platelet count at each time point and is reported as the mean ± standard error. The mean whole blood serotonin/platelet level did significantly decline during antiviral therapy ($P < 0.0001$)

the definition of interferon-induced depression used in the current study differs from that used in prior studies. As mentioned, a validated depressive symptom rating scale (*i.e.*, the BDI-II) and the CIDI were used to define interferon-induced depression. To address potential ceiling effects with the BDI-II, an increase in the BDI-II score of >6 points was required during treatment for subjects with an elevated baseline score which would change the classification of depression from minimal to mild and mild to moderate. In addition, prior studies have suggested that an increase in the BDI-II score of 6 points is clinically significant (25, 26). An alternative approach would have been to use a threshold severity construct to define interferon-induced depression (*e.g.*, a BDI-II score >18). Finally, a frequent assessment of patients during antiviral therapy by a mental health professional would be considered the "gold standard" for verifying the presence of a mood disorder. However, this approach was not feasible due to logistical considerations.

A significant association between failure to clear HCV RNA by week 20 and developing depression during the antiviral therapy was observed in the current study. A previous study suggested that CHC patients with more severe psychiatric side effects during treatment were more likely to achieve a virological response (36). However, this association was not found in four other studies that demonstrated a negative relationship between fatigue and response to antiviral therapy (37–40). In the current study, there was no significant difference in the BDI-II score at week 4 or week 12 compared with baseline in the week 20 virologic responders *versus* nonresponders (data not shown). Therefore, the association noted in our study may have been due, in part, to the sharing of

HCV RNA test results with patients during the study and the expected negative impact that the knowledge of persistent viremia could have on a patient's mood. Future studies that blind patients to virological test results may better discern the biological effect of interferon on mood *versus* the knowledge of the treatment response.

The incidence of interferon-induced depression in the subjects who continued beyond 24 wk increased from 9% at week 24 to 42% by week 48 (Fig. 2). These data suggest that the neuropsychiatric side effects of peginterferon and ribavirin increase over time. Importantly, however, the mean BDI-II scores of subjects returned to pretreatment baseline levels by week 72, demonstrating the reversibility of interferon-induced depression. The lack of a significant association between any of the baseline variables and interferon-induced depression in this subgroup of 74 subjects may, in part, relate to the smaller sample size and the exclusion of nonresponders beyond week 24.

In addition to determining the frequency and severity of interferon-induced depression, the potential mechanism(s) of neuropsychiatric side effects was explored in the current study. Previous studies of short-term interferon therapy have demonstrated dramatic changes in the HPA axis in healthy individuals and CHC patients (14, 41, 42). Presumably, the induction of fever and systemic side effects by interferon can lead to a marked stress response, with characteristic increases in adrenocorticotropin-releasing hormone (ACTH) and plasma cortisol levels, as previously noted in subjects with endogenous depression (1). However, studies of prolonged interferon therapy demonstrate the development of a tolerance to this effect (14). As seen in Figure 3, no significant changes in plasma cortisol levels were noted over time. In addition, baseline plasma cortisol levels did not significantly correlate with baseline BDI-II scores, and the changes in cortisol levels over time did not track with the appearance of interferon-induced depression or serial BDI-II scores. These data suggest that alterations in the HPA axis as reflected in morning cortisol levels are not associated with interferon-induced depression in CHC patients. However, it remains plausible that more sensitive and specific measurements of subtle changes in the HPA axis such as the ACTH stimulation test or dexamethasone suppression test could have led to different results.

Previous studies have demonstrated that interferon can lead to downregulation of serotonin synthesis in the brain and depletion of serotonin precursors throughout the body (16, 43). In addition, depletion of whole body serotonin stores via interferon-mediated induction of indoleamine-2,3-dioxygenase activity has been postulated as a potential mechanism of interferon-induced depression (44). In the current study, whole blood serotonin levels were measured, which include contributions from platelet-derived serotonin. As interferon is known to cause thrombocytopenia via direct effects on the bone marrow, the changes in normalized whole blood serotonin to platelet levels were also investigated. At

baseline, a wide range of whole blood serotonin levels were noted, and subjects with baseline depression tended to have lower levels (Table 1). However, neither baseline whole blood nor normalized serotonin levels were associated with baseline BDI-II scores. Nonetheless, significant changes in normalized whole blood serotonin levels were seen during the antiviral therapy, although the degree of change did not correlate with the occurrence of depression (Fig. 4) nor with the serial changes in the BDI-II scores. The lack of an association between the changes in whole blood serotonin levels and interferon-induced depression may have been due to the discordance between whole blood and intracerebral serotonin levels. In addition, the nonspecific reduction of platelet count in treated patients from interferon effects on the bone marrow may have obscured a relationship. Finally, the use of SSRI's in 17% of the HALT-C patients during the lead-in phase and 15% during the responder arm may have confounded a relationship between these parameters. Therefore, investigation of more sensitive markers of serotonin metabolism such as serotonin gene polymorphisms, serotonin receptor expression in peripheral lymphocytes, and serum peptidases induced by interferon would be worthwhile (43–51).

The strengths of the current study include the enrollment of a large number of well-characterized CHC patients at two clinical sites with varying ethnicity, age, and gender. In addition, standardized and objective measures of mood states were used rather than the subjective clinical assessment of a hepatologist. Although a formal assessment of suspected mood disorders by a mental health professional would have been preferred, this was not feasible. Other strengths of the current study include the assessment of mood status within a clinical trial wherein all patients were seen at regular and frequent intervals and standardized rules for antiviral dose reductions were utilized. Nevertheless, the results of this study may not be generalizable to other patients with CHC due to the strict inclusion criteria of the HALT-C trial. In particular, the inclusion of patients who had previously tolerated a course of interferon therapy may have excluded patients at risk for severe neuropsychiatric toxicity. In addition, subjects with severe or recent psychopathology at baseline were excluded from enrollment into this study of long-term peginterferon therapy. Finally, as in any study that uses a self-administered questionnaire, some subjects could have overestimated psychiatric symptoms, while others may have underestimated their symptoms due to questionnaire fatigue. However, the cumulative incidence of interferon-induced depression at weeks 24 and 48 is similar to what has been reported in other studies of mood disorders in CHC patients (36–40).

In summary, the cumulative incidence of interferon-induced depression in patients receiving peginterferon and ribavirin enrolled in the HALT-C trial was 23% at week 24 and 42% at week 48. Contrary to initial hypotheses, many pretreatment characteristics were not associated with interferon-induced depression including the baseline depression, liver

disease severity, and lifetime psychiatric and alcohol consumption history. However, as expected, receiving an antidepressant or anxiolytic medication at baseline was associated with depression, as was the absence of a virological response at week 12 and week 20. Morning plasma cortisol levels did not change significantly during therapy and were not associated with depression before or during antiviral therapy. In contrast, normalized serotonin levels decreased significantly during antiviral therapy, but were not associated with the development of interferon-induced depression. Additional studies of serotonergic pathways in the pathogenesis of mood disorders during antiviral therapy of CHC are warranted to improve our understanding of the biological basis of this common and dose-limiting side effect of the treatment.

## ACKNOWLEDGMENTS

### STUDY HIGHLIGHTS

**What Is Current Knowledge**

- Depression is a common and dose-limiting side effect of antiviral treatment in hepatitis C patients.
- The incidence, risk factors, and biological basis for interferon-induced depression are not well established.
- Prior studies have been limited by the small number of patients enrolled, nonstandardized assessment of depression, and heterogeneous antiviral treatment regimens.

### What Is New Here

- The incidence of depression was 23% at week 24 and 42% at week 48 in 201 patients who received pegylated interferon and ribavirin combination therapy in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial using validated instruments.
- Although 22% of patients had mild depression at baseline, the absence of virological clearance at week 20 was the only independent predictor of interferon-induced depression.
- The plasma cortisol levels did not change during treatment nor correlate with depression.
- Significant declines in whole blood serotonin levels were noted during treatment but did not correlate with depression. Additional studies of the seratonergic axis are indicated to further elucidate the mechanism of this common and dose-limiting side effect of the antiviral treatment in hepatitis C patients.

## REFERENCES

1. Kronfol Z. Immune dysregulation in major depression: A critical review of existing evidence. Int J Neuropsychopharmacol 2002;5:333–43.
2. Fontana RJ. Neuropsychiatric toxicity of antiviral treatment in chronic hepatitis C. Dig Dis 2000;18:107–16.
3. Lee SS, Peltekian K, Krajdens M, et al. Treating chronic hepatitis C with pegylated interferon alfa-2a (40kD) and ribavirin in clinical practice. Aliment Pharmacol Ther 2006;23:397–408.
4. Fried MW, Russo MA. Side effects of antiviral therapy for hepatitis C. Gastroenterology 2003;124:1711–9.
5. Fontana RJ, Bieliauskas LA, Lindsay KL, et al. Cognitive function does not worsen during peginterferon and ribavirin retreatment of chronic hepatitis C. Hepatology 2007;45:1154–63.
6. Hilsabeck RC, Hassanein TI, Carlson MD, et al. Cognitive functioning and psychiatric symptomatology in patients with chronic hepatitis C. JINS 2003;9:847–54.
7. Gleason OC, Yaters WR, Isbell MD, et al. An open-label trial of citalopram for major depression in patients with hepatitis C. J Clin Psychiatry 2002;63:194–8.
8. Hauser P, Khosla J, Aurora H, et al. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry 2002;7:942–7.
9. Raison CL, Woolwine BJ, Demetrashvili MF, et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. Aliment Pharmacol Ther 2007;25:1163–74.
10. Musselman DL, Lawson DH, Fumnick JF, et al. Paroxetine for the prevention of depression induced by high dose interferon-alfa. N Engl J Med 2001;344:961–6.
11. Constant A, Castera L, Dantzer R, et al. Mood alterations during interferon-alfa therapy in patients with chronic

hepatitis C: Evidence for an overlap between manic/hypomanic and depressive symptoms. J Clin Psychiatry 2005;66:1050–7.

12. Pariante CM, Landau S, Carpiniello B. Interferon alfa-induced adverse effects in patients with a psychiatric diagnosis. N Engl J Med 2002;347:148–9.

13. Raison CL, Borisov AS, Broadwell SD, et al. Depression during pegylated interferon-alpha plus ribavirin therapy: Prevalence and prediction. J Clin Psychiatry 2005;66:41–8.

14. Capuron L, Raison CL, Musselman DL, et al. Association of exaggerated HPA axis response to the initial injection of interferon alpha with the development of depression during interferon-alpha therapy. Am J Psychiatry 2003;160:1342–5.

15. Weissenborn K, Ennen JC, Bokemeyer M, et al. Monoaminergic neurotransmission is altered in hepatitis C virus infected patients with chronic fatigue and cognitive impairment. Gut 2006;55:1624–30.

16. Bonaccorso S, Marino V, Puzella A, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-a-based immunotherapy are related to interferon-a-induced changes in the serotonergic system. J Clin Psychopharmacol 2002;22:86–90.

17. Cai W, Khaoustove VI, Xie Q, et al. Interferon-a-induced modulation of glucocorticoid and serotonin receptors as a mechanism of depression. J Hepatol 2005;42:880–7.

18. Shifffman ML, Di Bisceglie AM, Lindsay KL, et al. Peginterferon Alfa-2a and ribavirin in patients with chronic hepatitis C who failed prior treatment. Gastroenterology 2004;126:1015–23.

19. Lee WM, Dienstag JL, Lindsay KL, et al. Evolution of the HALT-C trial: Pegylated interferon as a maintenance therapy for chronic hepatitis C in previous interferon nonresponders. Control Clin Trials 2004;25:472–92.

20. Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology 2007;132:103–12.

21. Wittchen HU. Reliability and validity studies of the WHO Composite International Diagnostic Interview: A critical review. J Psychiatr Res 1994;28:57–84.

22. Beck AT, Steer RA, Brown GK. BDI-II manual. The psychological corporation. San Antonio, TX: Harcourt Brace and Company, 1996.

23. Skinner HA, Sheu WJ. Reliability of alcohol use indices: The lifetime drinking history and the MAST. J Stud Alcohol 1982;43:1157–70.

24. Fontana RJ, Bieliauskas LA, Back-Madruga C, et al. Cognitive function in hepatitis C patients with advanced fibrosis enrolled in the HALT-C trial. J Hepatol 2005;43:614–22.

25. Malaguarnera M, DiFazio I, Restuccia S, et al. Interferon-alpha-induced depression in chronic hepatitis C patients: Comparison between different types of interferon alpha. Neuropsychobiology 1998;37:93–7.

26. Mistler LA, Brunette MF, Marsh BJ, et al. Hepatitis C treatment for people with severe mental illness. Psychosomatics 2006;47:93–107.

27. Whitley RJ, Meikle AW, Watts NB. Endocrinology, Part VI: Adrenocortical steroids. In:Burtis CA, Ashwood ER, eds. Textbook of clinical chemistry, 2nd Ed. Philadelphia, PA: WB Saunders, 1994:1822–5.

28. Xiao R, Beck O, Hjerndahl P. On the accurate measurement of serotonin in whole blood. Scand J Clin Lab Invest 1998;58:505–10.

29. Schaefer M, Schwaiger M, Garkisch AS, et al. Prevention of interferon-alpha associated depression in psychiatric risk patients with hepatitis C. J Hepatol 2005;42:793–8.

30. Dan AA, Martin LM, Crone C, et al. Depression, anemia, and health-related quality of life in chronic hepatitis C. J Hepatol 2006;44:498.

31. Rutledge T, Reis SE, Olson MB, et al. Depression severity and reported treatment history in the prediction of cardiac risk in women with suspected myocardial ischemia. Arch Gen Psychiatry 2006;63:874–80.

32. Grothe KB, Dutton GR, Jones GN, et al. Validation of the Beck Depression Inventory-II in a low-income African American sample of medical outpatients. Psychol Assess 2005;17: 110–4.

33. Dieperink E, Ho SB, Thuras P, et al. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. Psychosomatics 2003;44:104–12.

34. Golden J, O'Dwyer AM, Conroy RM. Depression and anxiety in patients with hepatitis C: Prevalence, detection, and risk factors. Gen Hosp Psychiatry 2005;27:431–8.

35. Capuron L, Raynaud A. Prediction of the depressive effects of interferon-alfa therapy by the patient's initial affective state. N Engl J Med 1999;340:1370

36. Rasenack J, Zeuzem S, Feinman SV, et al. Peginterferon alpha-2a (40 kD) (Pegasys) improves HR-QOL outcomes compared with unmodified interferon-2a in patients with chronic hepatitis C. Pharmacoeconomics 2003;21:341–9.

37. Kraus MR, Schafer A, Csef H, et al. Compliance with therapy in patients with chronic hepatitis C. Dig Dis Sci 2001;46:260–5.

38. Loftis JM, Socherman RE, Howell CD, et al. Association of interferon-alpha induced depression and improved treatment response in patients with hepatitis C. Neurosci Lett 2004;365:87–91.

39. Raison CL, Pariante CM, Capuron L, et al. The association of fatigue with poor virologic response in patients receiving interferon-alpha plus ribavirin for the treatment of hepatitis C [abstract]. Hepatology 2005;44:1216.

40. Raison CL, Broadwell SD, Barisov AS, et al. Depressive symptoms and viral clearance in patients receiving interferon alpha and ribavirin for hepatitis C. Brain Behav Immun 2005;19:23–7.

41. Maddock C, Landau S, Barry K, et al. Psychopathological symptoms during interferon-alpha and ribavirin treatment: Effects on virologic response. Mol Psychiatry 2005;10:332–3.

42. Castera L, Constant A, Henry C, et al. Impact of adherence and sustained virologic response of psychiatric side effects during peginterferon and ribavirin therapy for chronic hepatitis C. Aliment Pharmacol Ther 2006;24:1223–30.

43. Muller H, Hiemke C, Hammes E, et al. Sub-acute effects of interferon-alpha 2 on ACTH, cortisol, growth hormone and prolactin in humans. Psychoneuroendocrinology 1992;17:459–65.

44. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. Biol Psychiatry 2001;49:394–404.

45. Lopez JF, Chalmers DT, Little KY, et al. Regulation of serotonin 1A, glucocorticoid, and mineralocorticoid reception in rat and human hippocampus: Implications for the neurobiology of depression. Biol Psychiatry 1998;43:547–73.

46. Maes M, Bonaccorso S. Lower activities of serum peptidases predict higher depressive and anxiety levels following interferon-alpha-based immunotherapy in patients with hepatitis C. Acta Psychiatr Scand 2004;109:126–31.

47. Schlaak JF, Trippler M, Erim Y, et al. Identification of candidate genes that mediate depressive side effects of pegylated IFN-alpha 2a in patients with chronic hepatitis C [abstract]. Hepatology 2006;44:331.
48. Gladkevich A, Kauffman HF, Korf J. Lymphocytes as a neural probe: Potential for studying psychiatric disorders. Prog Neuropsychpharmacol Biol Psychiatry 2004;28:559–76.
49. Yoshida K, Alagbe O, Wang X, et al. Promoter polymorphisms of the interferon-alpha receptor gene and development of interferon-induced depressive symptoms in patients with chronic hepatitis C: Preliminary findings. Neuropsychobiology 2005;52:55–61.
50. Wichers MC, Koek GH, Robaeys G, et al. IDO and interferon-a-induced depressive symptoms: A shift in hypothesis from tryptophan depletion to neurotoxicity. Mol Psychiatry 2005;10:538–44.
51. Kraus MR, Al-Taie O, Schefer A, et al. Serotonin-1A receptor gene (*HTR1A*) variation predicts interferon-induced depression in chronic hepatitis C. Gastroenterology 2007;132:1279–86.

## CONFLICT OF INTEREST

**Guarantor of the article:** Robert J. Fontana.
**Specific author contributions:** All of the authors had meaningful input into the design, conduct, analysis, and writing of the manuscript including final drafts and figures and tables.

**Potential competing interests:** Robert J. Fontana is on the speaker's bureau of Hoffman-La Roche, Inc.; Karen L. Lindsay is a consultant to Hoffman-La Roche, Inc., and receives research support; and Anna S.F. Lok is a consultant to Hoffman-La Roche, Inc., and receives grant support. Z. Kronfol, L.A. Bieliauskas, C. Back-Madruga, L. Padmanabhan, and A.M. Stoddard have no potential competing interests.