

# Effect of Peginterferon Alfa-2a on Liver Histology in Chronic Hepatitis C: A Meta-analysis of Individual Patient Data

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Multicenter randomized trials have shown that once-weekly pegylated interferon (peginterferon) alfa-2a is more efficacious than conventional interferon alfa-2a (IFN) in patients with chronic hepatitis C. We performed a meta-analysis of 1,013 previously untreated patients (from 3 randomized trials) with pretreatment and post-treatment liver biopsies to assess the differences between peginterferon alfa-2a and IFN in terms of their effects on liver histology. Reported values were standardized mean differences (SMD) between patients receiving peginterferon alfa-2a and those receiving IFN (post-treatment value minus baseline value for each group). We used a random-effects model to quantify the average effect of peginterferon alfa-2a on liver histology. Peginterferon alfa-2a significantly reduced fibrosis compared with IFN (SMD,  $-0.14$ ; 95% CI:  $-0.27, -0.01$ ;  $P = .04$ ). A reduction in fibrosis was observed among sustained virologic responders (SMD,  $-0.59$ ; 95% CI:  $-0.89, -0.30$ ;  $P < .0001$ ) and patients with recurrent disease (SMD,  $-0.34$ ; 95% CI:  $-0.54, -0.14$ ;  $P = .0007$ ), whereas no significant reduction was observed among nonresponders (SMD,  $-0.13$ ; 95% CI:  $-0.32, 0.05$ ;  $P = .15$ ). Logistic regression analysis indicated that patients with sustained virologic responses (SVRs) had an odds ratio (OR) of 1.61 (95% CI: 1.14, 2.29) for reduction in fibrosis compared with patients without SVRs, whereas obese patients (body mass index [BMI]  $> 30 \text{ kg/m}^2$ ) had an OR of 0.56 (95% CI: 0.35, 0.90) compared with normal-weight (BMI  $< 25 \text{ kg/m}^2$ ) and overweight patients (BMI, 25–30  $\text{kg/m}^2$ ). In conclusion, in patients with chronic hepatitis C with or without cirrhosis, peginterferon alfa-2a (relative to IFN) significantly reduced fibrosis. The beneficial effects of peginterferon on liver histology are closely related to virologic response. (HEPATOLOGY 2004;39: 333–342.)

Patients with chronic hepatitis C typically undergo liver biopsy to determine the severity of disease and thereby assess the urgency of treatment.<sup>1</sup> The histologic findings on biopsy also enable the clinician to

evaluate the probability of a response to interferon alfa-2a (IFN).<sup>2</sup> Liver histology provides direct evidence of hepatic necroinflammatory activity, fibrosis, and progression to cirrhosis,<sup>3</sup> and is a surrogate endpoint of the long-term efficacy of IFN treatment.<sup>4</sup>

As a tool for evaluating response to treatment, histologic findings have several significant limitations and sources of bias.<sup>5,6</sup> For example, different scoring systems can be used to compare paired liver biopsies; in addition, biopsies may be performed at different times, and they may be evaluated by different pathologists in multicenter trials of antiviral therapy. Therefore, it is difficult to accurately and reliably assess the relatively small changes induced by treatment over short time periods.

The most frequently used systems in randomized controlled trials (RCTs) are the Histologic Activity Index (HAI)<sup>7</sup> and the METAVIR system.<sup>6</sup> The use of the combined HAI (Knodell score), in which four discontinuous scales (three for necroinflammation and one for fibrosis) are combined, represents a source of inconsistency in many clinical trials.<sup>8</sup> However, the HAI's discontinuous

Abbreviations: IFN, conventional interferon alfa-2a; RCT, randomized controlled trial; peginterferon, pegylated interferon; HAI, Histologic Activity Index; MIPD, meta-analysis of individual patient data; ALT, alanine aminotransferase; HCV, hepatitis C virus; SVR, sustained virologic response; SMD, standardized mean difference; BMI, body mass index; OR, odds ratio.

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fibrosis scale has an important advantage over the continuous METAVIR scale. In particular, the HAI score increases both intraobserver and interobserver reliability by clearly separating mild (+1) fibrosis from extensive (+3) fibrosis, which possesses the most relevant prognostic impact.<sup>3</sup>

In 1997, a meta-analysis of 17 RCTs ( $n = 1223$ ) demonstrated that conventional IFN significantly improved liver histology compared with no treatment.<sup>9</sup> Histologic improvement was clearly related to antiviral responses. Recently, pegylated interferon (peginterferon) was developed by attaching a polyethylene glycol moiety to IFN alfa. Subsequently, several large multicenter RCTs<sup>10–14</sup> have clearly demonstrated that peginterferon alfa-2a produces significantly greater virologic responses compared with conventional IFN. Nonetheless, the results of published studies on the effects of this new drug on liver histology remain inconsistent, and the overall effect of treatment is difficult to evaluate.<sup>11–14</sup>

To overcome some of the limitations associated with the use of histologic findings, to increase the relevance of statistical analysis, and to improve estimates of effect magnitudes, we performed a meta-analysis of individual patient data (MIPD). The aims of the current MIPD were 1) to assess the differences between peginterferon alfa-2a and conventional IFN with respect to changes in liver histology; 2) to identify predictors of histologic improvement; and 3) to evaluate the optimal treatment schedule for bringing about histologic improvement.

## Materials and Methods

**Patients.** The current MIPD was designed to pool data on a large number of individuals from tertiary referral specialty units in an attempt to define the efficacy of peginterferon alfa-2a with respect to liver histology. We analyzed data on individual patients from three RCTs of peginterferon alfa-2a.<sup>11,12,14</sup> These studies are the only three RCTs that compare peginterferon alfa-2a with conventional IFN and include previously untreated patients with pretreatment and post-treatment liver biopsies. Written consent was obtained from the principal investigator at each trial center. The study database consisted of patients who had the following baseline characteristics: age greater than 18 years, alanine aminotransferase (ALT) levels greater than the upper limit of normal on 2 occasions during the preceding 6 months, positive status for anti-hepatitis C virus (HCV) antibody, negative status for serum hepatitis B surface antigen, negative status for human immunodeficiency virus antibody, and self-reported complete abstinence from alcohol. Laboratory tests, including assessment of serum HCV RNA levels

(Cobas Amplicor HCV Monitor [Version 2.0]; Roche Diagnostics, Branchburg, NJ), were performed at a central laboratory. Sustained virologic response (SVR) was defined as the absence of detectable HCV RNA at the end of treatment and at 24 weeks after cessation of therapy. Recurrent disease was defined by HCV RNA levels that were undetectable at the end of treatment but were detectable again within 6 months of cessation of treatment. Lack of response was defined by detectable HCV RNA levels at 6 months after the start of therapy.

The current study was performed in accordance with the principles of good clinical practice, the principles of the Declaration of Helsinki and its appendices, and local and national laws. To maintain patients' privacy, patient names were replaced in the database by codes, dates of birth, and/or ages.

**Role of the Funding Source.** The current study was designed by the principal investigators. Hoffmann–La Roche (Basel, Switzerland) staff members collected data from designated clinical sites and assembled the database, and Hoffmann–La Roche supported the study with an investigational grant. The principal investigators analyzed and interpreted the data, wrote the current article, and submitted the article for publication.<sup>15</sup>

**Assessment of Histology.** Slides of liver biopsy specimens obtained before the study and at 24 weeks after discontinuation of treatment were coded and read by a single pathologist (Sugantha Govindarajan), who was unaware of patient identities, treatment regimens, and biopsy dates. A minimum length of 15 mm of liver biopsy specimen was required. Liver biopsy specimens were evaluated for changes in HAI score (maximum score, 22)<sup>7</sup> relative to the values recorded at the beginning of treatment (*i.e.*, at baseline). The following four categories were assessed and scored using the Knodell system: 1) piecemeal and bridging necrosis; 2) intralobular necrosis; 3) portal inflammation; and 4) fibrosis.

**Statistical Methods.** As a measure of the effect of treatment, we used the mean difference between the change in histologic score for patients who received peginterferon alfa-2a and the change for those who received IFN (final value minus baseline value for each group). This difference is referred to as the *net change*. The standardized mean difference (SMD) was calculated for each study by dividing the net change by the pooled, within-group sample standard deviation.<sup>16</sup> Therefore, the reported results are expressed in terms of standard deviation units rather than in terms of the original units of measurement.

Estimates of the average effect of treatment on liver histology, along with 95% CIs, were calculated using models based on both fixed-effects and random-effects

assumptions.<sup>17,18</sup> In addition to within-study variance, heterogeneity among studies also is considered by the random-effects model. Because of the diverse clinical settings and groups of patients analyzed, and because the tests for heterogeneity lack statistical power, we have presented the results obtained using random-effects models. Statistical analyses were performed using Metaview 4.0 (Oxford, England: The Cochrane Collaboration, 1999).

In the trials conducted by Heathcote et al.<sup>11</sup> and Pockros et al.,<sup>14</sup> two different peginterferon alfa-2a treatment arms (180  $\mu\text{g}$  vs. 90  $\mu\text{g}$  in the former and 180  $\mu\text{g}$  vs. 135  $\mu\text{g}$  in the latter) were compared, with the same conventional IFN arm as a control in both. To avoid stochastic dependencies involving effect magnitudes, we combined the results from the peginterferon alfa-2a arms in both trials. Therefore, the statistical analysis involved only independent estimators of effect magnitude.<sup>19</sup>

We defined a histologic response as a decrease of at least 1 point in the fibrosis score (staging) relative to the baseline biopsy score or a decrease of at least 2 points in the activity score (grading). This definition was based on recommendations appearing in the most recent consensus statements of the European Association for the Study of Liver Disease<sup>20</sup> and the National Institutes of Health Consensus Development Conference on Hepatitis C.<sup>21</sup>

To examine the extent to which differences in the observed treatment effects could be explained by differences in patient characteristics or in the therapeutic regimens administered, several independent variables were included in a multivariate model. Thus, two binary logistic regression models were developed to investigate potential correlations between histologic response probability and the explanatory variables. The dependent variable in the first model was the change in activity score (grading) from before treatment to after treatment, coded as 0, *worsened or stabilized* (any increase, no change, or a decrease of less than 2 points in activity score relative to baseline biopsy); or 1, *improved* (a decrease of at least 2 points in activity score relative to baseline biopsy). The dependent variable in the second model was the change in fibrosis score (staging) from before treatment to after treatment, coded as 0, *worsened or stabilized* (any increase or no change in fibrosis score relative to baseline biopsy); or 1, *improved* (a decrease of at least 1 point in fibrosis score relative to baseline biopsy). In the second model, patients with Stage 4 fibrosis (*i.e.*, cirrhosis) at baseline were excluded, because it was not possible for their fibrosis score to increase. Likewise, patients with Stage 0 fibrosis (no fibrosis) at baseline were excluded, because their score could not decrease. A subgroup analysis of patients with cirrhosis was performed to avoid the loss of information that would have been caused by eliminating this subgroup from the mul-

tivariate analysis. As candidate predictors of improvement in terms of fibrosis score and activity score, we selected age, sex, race, body mass index (BMI; obese [ $> 30 \text{ kg/m}^2$ ] vs. normal [ $< 25 \text{ kg/m}^2$ ] vs. overweight [ $25\text{--}30 \text{ kg/m}^2$ ]), source of infection, baseline ALT quotient (calculated as the average of the serum ALT levels before treatment divided by the upper limit of normal;  $> 3$  vs.  $\leq 3$ ), HCV RNA levels before treatment, treatment regimen, and virologic response (SVR vs. no SVR). Variables found to be significant on univariate analysis ( $P < .05$ ) were included in the multivariate logistic regression model. Statistical significance was tested using the Wald chi-squared test. Regression analysis was performed using PROC LOGISTIC software (SAS Institute, Cary, NC).

Examination of the association between the change in a variable and its initial value is complicated by the presence of errors in measurement and by intrinsic within-subject variability.<sup>22</sup> Because of such variation, the actual levels, on average, will decrease even in the absence of any treatment. This artifactual decrease, an example of 'regression to the mean', will be greatest for patients with the highest initial recorded values and therefore will induce a spurious association between observed change and initial value. To assess whether the amount of improvement in grade after treatment depended on the baseline value, the average of the initial and final values was included in the multivariate model.<sup>22</sup>

## Results

**Baseline Patient Characteristics.** Of the 1,441 patients enrolled in the 3 RCTs, 1,013 (70.3%) had paired liver biopsies that were obtained before treatment and at 24 weeks after the end of treatment. The main baseline characteristics of these 1,013 patients did not differ significantly from the characteristics of the entire patient series. The proportion of patients who underwent a second liver biopsy was similar across the studies, ranging from 62.5% to 79.5%, regardless of virologic response. Patient characteristics were comparable in all trials (Table 1). Sixty-eight percent of patients were male, and 86.3% were Caucasian. The proportion of intravenous drug users was high (42%), and the majority of patients were overweight (mean BMI  $\pm$  standard deviation,  $26.7 \pm 4.8 \text{ kg/m}^2$ ). Inclusion criteria were uniform in all but one RCT, which included only patients with cirrhosis or bridging fibrosis.<sup>11</sup> Overall, 198 patients (19.5%) had cirrhosis at baseline. In all studies, the duration of treatment was 48 weeks. The peginterferon alfa-2a dose ranged from 90 to 180  $\mu\text{g}/\text{week}$ .

Baseline characteristics of patients treated with peginterferon alfa-2a and patients treated with conventional

**Table 1. Baseline Characteristics of Patients With Paired Biopsies in the Three Randomized Trials**

Characteristic	Heathcote et al. <sup>11</sup> (n = 271)			Pockros et al. <sup>12</sup> (n = 639)			Zeuzem et al. <sup>12</sup> (n = 531)		Total
	IFN 3 MU	PEG-IFN alfa-2a 90 µg	PEG-IFN alfa-2a 180 µg	IFN 3 MU	PEG-IFN alfa-2a 135 µg	PEG-IFN alfa-2a 180 µg	IFN 6/3 MU	PEG-IFN alfa-2a 180 µg	
All patients	88	96	87	214	215	210	264	267	1,441
Patients with paired biopsies (%)	55 (62.5)	61 (63.5)	68 (78.1)	147 (68.7)	171 (79.5)	160 (76.2)	167 (63.2)	184 (68.9)	1,013 (70.3)
Mean age ± SD (yrs)	47.8 ± 7.1	47.4 ± 8.3	46.5 ± 8.5	42.1 ± 8.3	41.8 ± 8.6	43.0 ± 7.8	41.5 ± 8.7	41.1 ± 10.6	43.9 ± 8.5
Male sex (%)	35 (69.6)	45 (73.7)	49 (72)	86 (58.5)	123 (72)	115 (72)	115 (69)	118 (64)	686 (68)
Race (%)									
White	48 (87.2)	58 (95)	60 (88.2)	127 (86.3)	150 (87.7)	137 (85.6)	137 (82)	157 (85.3)	874 (86.3)
Other	7 (12.8)	3 (5)	8 (11.8)	20 (13.7)	21 (12.3)	23 (14.4)	30 (18)	27 (14.7)	139 (13.7)
Source of infection (%)									
Intravenous drug use	27 (49)	36 (59)	32 (47)	57 (36.7)	72 (42.1)	74 (46.2)	61 (36.5)	66 (45.8)	425 (42)
Other	28 (51)	25 (41)	36 (53)	93 (63.3)	99 (57.9)	86 (53.8)	106 (63.5)	118 (54.2)	591 (58)
Mean weight ± SD (kg)	82.2 ± 15.3	84.9 ± 14.9	81.3 ± 17.2	80.3 ± 17.6	82 ± 19.4	81.6 ± 16.8	76 ± 14.7	74.9 ± 15.4	80.4 ± 16.4
Mean BMI ± SD (kg/m <sup>2</sup> )	28.2 ± 6.0	28.1 ± 4.5	27.1 ± 4.8	27.2 ± 4.8	26.9 ± 4.8	26.9 ± 4.7	25.9 ± 4.2	25.6 ± 5.1	26.7 ± 4.8
Mean ALT ± SD (> ULN)	3.3 ± 1.6	3.2 ± 1.8	4.2 ± 2.2	2.9 ± 1.8	3.0 ± 1.7	2.9 ± 1.8	3.1 ± 1.9	3.2 ± 2.4	3.2 ± 1.9
Histology at first biopsy									
Grade (%)									
Minimal	1 (1.8)	1 (1.6)	0	18 (12.2)	23 (13.4)	12 (7.5)	19 (11.3)	30 (16.3)	104 (10.3)
Mild	16 (29)	26 (42.6)	15 (22)	51 (34.6)	82 (47.9)	81 (50.6)	73 (43.7)	71 (38.6)	415 (40.9)
Moderate/severe	38 (69)	34 (55.8)	53 (78)	78 (53.0)	66 (38.6)	67 (41.8)	75 (45)	83 (45.1)	494 (48.8)
Stage (%)									
0	0 (0)	0 (0)	0 (0)	9 (6.2)	6 (3.5)	6 (3.7)	7 (4.1)	12 (6.5)	40 (3.9)
1	0 (0)	0 (0)	0 (0)	90 (61.2)	105 (61.4)	94 (58.8)	106 (63.4)	131 (71.3)	526 (51.9)
3	16 (29.1)	16 (26.3)	12 (17.6)	40 (27.2)	46 (26.9)	50 (31.2)	34 (20.3)	35 (19)	249 (24.6)
4	39 (70.9)	45 (73.7)	56 (82.4)	8 (5.4)	14 (8.2)	10 (6.3)	20 (12)	6 (3.2)	198 (19.6)
Genotype (%)									
1	30 (54)	37 (60)	38 (55)	84 (57)	99 (58)	93 (58)	105 (63)	112 (61)	598 (60.2)
2	8 (14)	4 (7)	11 (16)	15 (10)	15 (9)	16 (10)	15 (9)	24 (13)	108 (10.7)
3	16 (31)	17 (27)	15 (23)	47 (32)	55 (32)	51 (32)	42 (25)	44 (24)	287 (28.3)
Other	1 (2)	3 (5)	4 (5)	1 (1)	2 (1)	0 (0)	5 (3)	4 (2)	20 (2)
Serum HCV RNA (%)									
≥8 × 10 <sup>5</sup> copies/mL	26 (47.3)	26 (42.7)	34 (50)	72 (49)	74 (43.3)	70 (43.8)	70 (42)	89 (48.4)	461 (45.5)
<8 × 10 <sup>5</sup> copies/mL	29 (52.7)	35 (57.3)	34 (50)	75 (51)	97 (56.7)	90 (56.2)	97 (58)	95 (51.6)	552 (54.5)

Abbreviations: MU, megaunits; PEG-IFN, peginterferon; ULN, upper limit of normal.

IFN are shown in Table 2. No significant differences were observed at baseline between the two treatment groups. The peginterferon alfa-2a and conventional IFN groups were comparable with respect to total HAI score and with respect to each of the four HAI components at baseline. The likelihood of SVR was significantly greater among patients treated with peginterferon alfa-2a (33.4%) compared with patients treated with conventional IFN (17.6%;  $P = .001$ ). Similar results were observed in terms of sustained biochemical response (Table 2). Nearly all patients who experienced SVR also had normal ALT values at the end of follow-up (269 of 280 [96%]). Of the 280 patients who achieved SVR (215 treated with peginterferon and 65 treated with IFN), 94 (33.5%) had abnormal ALT values at the end of treatment. Of these 94 patients, 78 (36.2% of the peginterferon SVR group) were treated with peginterferon and 16 (24.6% of the IFN SVR group) were treated with IFN ( $P = .08$ ). A sustained biochemical response eventually was observed in 83 of these 94 patients (88.3%), while ALT values remained abnormal in 11 of 94 patients (11.7%; 7 treated with IFN and 4 treated with peginterferon) 24 weeks after the end of treatment.

The overall rate of improvement in grading was 81.1% (151 of 186 patients) among patients with SVR and normal ALT levels at the end of treatment and 81.9% (77 of 94 patients) among those with SVR and abnormal ALT levels at the end of treatment ( $P = .80$ ). Staging improved at a comparable rate among patients with SVR and normal ALT levels at the end of treatment (64 of 186 patients [34.4%]) and patients with SVR and abnormal ALT levels at the end of treatment (28 of 94 patients [29.7%];  $P = .43$ ).

**Effect Magnitude for Continuous Data.** We analyzed the SMDs in grading and staging between the peginterferon alfa-2a and conventional IFN groups (Fig. 1). Peginterferon alfa-2a was more efficacious than was conventional IFN in improving both grading and staging in all comparisons. The SMDs in grading for the individual trials ranged from  $-0.30$  to  $-0.04$ , and the SMDs for staging ranged from  $-0.23$  to  $-0.03$ . The overall SMDs were  $-0.12$  (95% CI:  $-0.25, 0.01$ ;  $P = .06$ ) for grading and  $-0.14$  (95% CI:  $-0.27, -0.01$ ;  $P = .04$ ) for staging.

Data on the correlation between histologic improvement 6 months after the end of treatment and virologic

**Table 2. Baseline Clinical Characteristics and Virologic and Biochemical Response Data According to Type of Interferon Received**

	PEG-IFN alfa-2a (40 kd) (n = 644)	IFN (n = 369)	P Value
Mean age ± SD (yrs)	42.9 ± 9.2	42.7 ± 8.5	0.72
Male sex (%)	450 (69.8)	236 (63.9)	0.052
Race (%)			
White	562 (87.2)	312 (84.5)	0.22
Other	82 (12.8)	57 (15.5)	
Mean weight ± SD (kg)	80.1 ± 17.3	78.7 ± 16.1	0.19
Mean BMI ± SD (kg/m <sup>2</sup> )	26.7 ± 4.9	26.8 ± 4.8	0.73
Mean ALT ± SD (× ULN)	3.2 ± 2.0	3.1 ± 1.8	0.42
Source of infection (%)			
Intravenous drug use	280 (43.4)	142 (38.4)	0.12
Other	364 (56.6)	227 (61.6)	
Histology at first biopsy			
Grade (%)			
Minimal	66 (10.2)	38 (10.2)	0.30
Mild	275 (42.7)	140 (37.1)	
Moderate/severe	303 (47.1)	191 (51.7)	
Stage (%)			
0	24 (3.7)	16 (4.3)	
1	330 (51.2)	196 (53.1)	
3	159 (24.6)	90 (24.3)	0.8
4	131 (20.3)	67 (18.1)	
Mean total HAI score ± SD	9.7 ± 3.2	9.8 ± 3.1	0.88
Serum HCV RNA (%)			
≥8 × 10 <sup>5</sup> copies/mL	351 (54.5)	201 (54.4)	0.99
<8 × 10 <sup>5</sup> copies/mL	293 (45.5)	168 (45.6)	
Virologic response (%)			
Nonresponders	233 (36.1)	265 (71.2)	0.001
Patients with recurrent disease	196 (30.4)	39 (10.5)	
Patients with SVRs	215 (33.4)	65 (17.6)	
Biochemical response (%)			
Nonresponders	246 (38.2)	207 (56.1)	0.001
Patients with recurrent disease	142 (22.0)	68 (18.4)	
Patients with sustained responses	256 (39.7)	94 (25.4)	

Abbreviations: PEG-IFN, peginterferon; ULN, upper limit of normal.

response for patients treated with peginterferon alfa-2a are summarized in Fig. 2A (for grading) and Fig. 2B (for staging). In nonresponders, we observed negligible changes in necroinflammation (Fig. 2A) and no significant change in fibrosis (Fig. 2B); the overall SMDs were  $-0.04$  (95% CI:  $-0.22, 0.14$ ;  $P = .70$ ) for activity and  $-0.13$  (95% CI:  $-0.32, 0.05$ ;  $P = .15$ ) for fibrosis. In patients with recurrent disease, histologic improvement was statistically significant in terms of both necroinflammation (Fig. 2A) and fibrosis (Fig. 2B); the overall SMDs were  $-0.37$  (95% CI:  $-0.62, -0.12$ ;  $P = .004$ ) for activity and  $-0.34$  (95% CI:  $-0.54, -0.14$ ;  $P = .0007$ ) for fibrosis. Among patients with SVRs, there were substantial reductions in necroinflammation (Fig. 2A) and fibrosis (Fig. 2B); the overall SMDs were  $-1.77$  (95% CI:  $-2.02, -1.53$ ;  $P < .00001$ ) for activity and  $-0.59$  (95% CI:  $-0.89, -0.30$ ;  $P < .0001$ ) for fibrosis.

**Variables Associated With Histologic Improvement.** The activity grade improved in 48.4% of patients, it remained stable in 34.5% of patients, and it became worse in 16.9% of patients (Table 3). Improved grading was observed in 231 of 427 patients (54.1%) with early biochemical responses (defined by normal ALT levels by Week 12) and in 249 of 566 patients (43.9%) without early biochemical responses ( $P = .006$ ).

Model I, which considered baseline ALT levels, pre-treatment serum HCV RNA levels, mean of the initial and final activity scores, virologic response, and treatment regimen, indicated that both SVR (odds ratio [OR], 6.20; 95% CI: 4.28, 8.98) and baseline ALT quotient  $> 3$  (OR, 1.35; 95% CI: 1.11, 1.63) were independent and significant predictors of improvement in grading (Table 4). Similar results were obtained when SVR was replaced by sustained biochemical response (OR, 4.90; 95% CI: 3.57, 6.73).

We performed subgroup analyses to assess the difference in the likelihood of activity score improvement between the standard (180  $\mu\text{g}$ ) and low-dose (90–135  $\mu\text{g}$ ) peginterferon alfa-2a treatment groups. Improvements in grading were significantly more common in the standard-dose group (232 of 412 patients [56.3%]) than in the low-dose group (101 of 232 patients [43.5%];  $P = .0002$ ).

Fibrosis stage improved in 25.7% of patients, it remained stable in 63.6% of patients, and it became worse in 10.5% of patients (Table 3). Among the 775 patients with Stage 1 or 3 fibrosis at baseline, fibrosis stage improved in 25% ( $n = 194$ ), remained stable in 65% ( $n = 503$ ), and became worse in 10% ( $n = 78$ ).

Logistic regression analysis showed that patients with SVRs had an OR of 1.61 (95% CI: 1.14, 2.29) for improvement in fibrosis relative to patients without SVRs and that obese patients had an OR of 0.56 (95% CI: 0.35, 0.90) relative to normal-weight and overweight patients. Similar results were obtained when SVR was replaced by sustained biochemical response (OR, 1.42; 95% CI: 1.10, 1.77). Among the 498 patients without virologic responses, the mean baseline BMI was  $26.7 \pm 3.9$  kg/m<sup>2</sup> for the 107 patients with reduced fibrosis,  $27.6 \pm 5.8$  kg/m<sup>2</sup> for the 313 patients with unchanged fibrosis, and  $28.2 \pm 4.9$  kg/m<sup>2</sup> for the 78 patients with increased fibrosis ( $P = .03$ ). Subgroup analysis indicated that there was no significant difference in terms of reduced fibrosis between the standard (117 of 412 patients [28.4%]) and low-dose (54 of 232 patients [23.3%]) peginterferon alfa-2a groups ( $P = .27$ ).

**Predictive Value of Early Virologic Response.** Nine hundred ninety-three patients were eligible for the analysis of early virologic response to treatment, whereas quan-

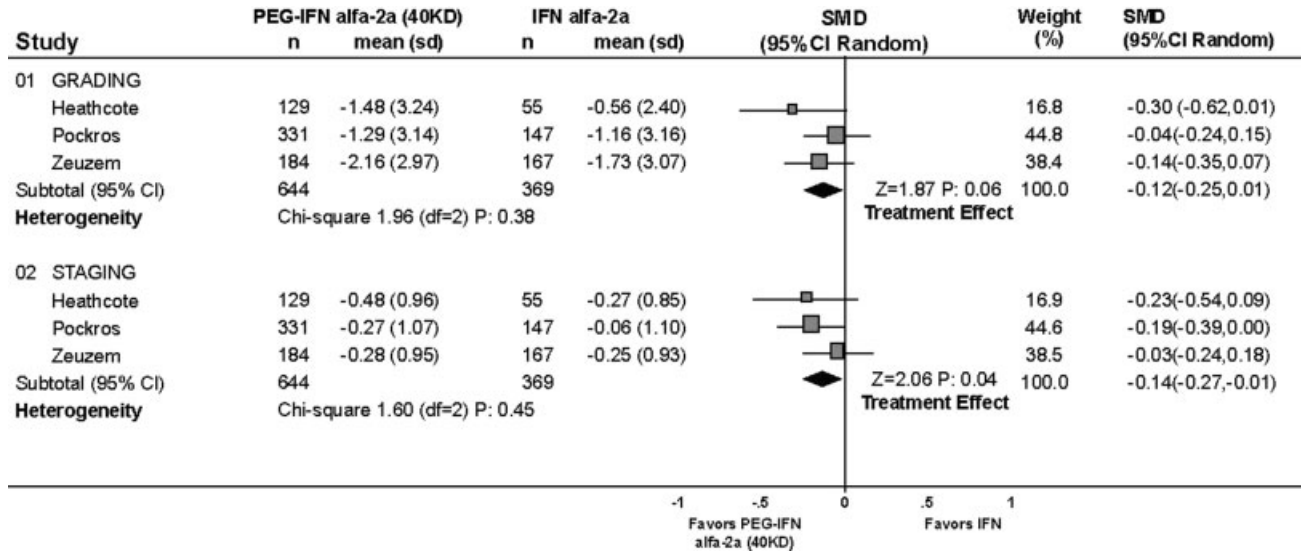


Fig. 1. Meta-analysis (using a random-effects model) of 3 RCTs comparing peginterferon (PEG-IFN) alfa-2a with conventional IFN for chronic hepatitis C. SMDs and 95% CIs for activity (*Grading*) and fibrosis (*Staging*) for patients receiving peginterferon alfa-2a compared with those receiving IFN are shown. *df*, degrees of freedom.

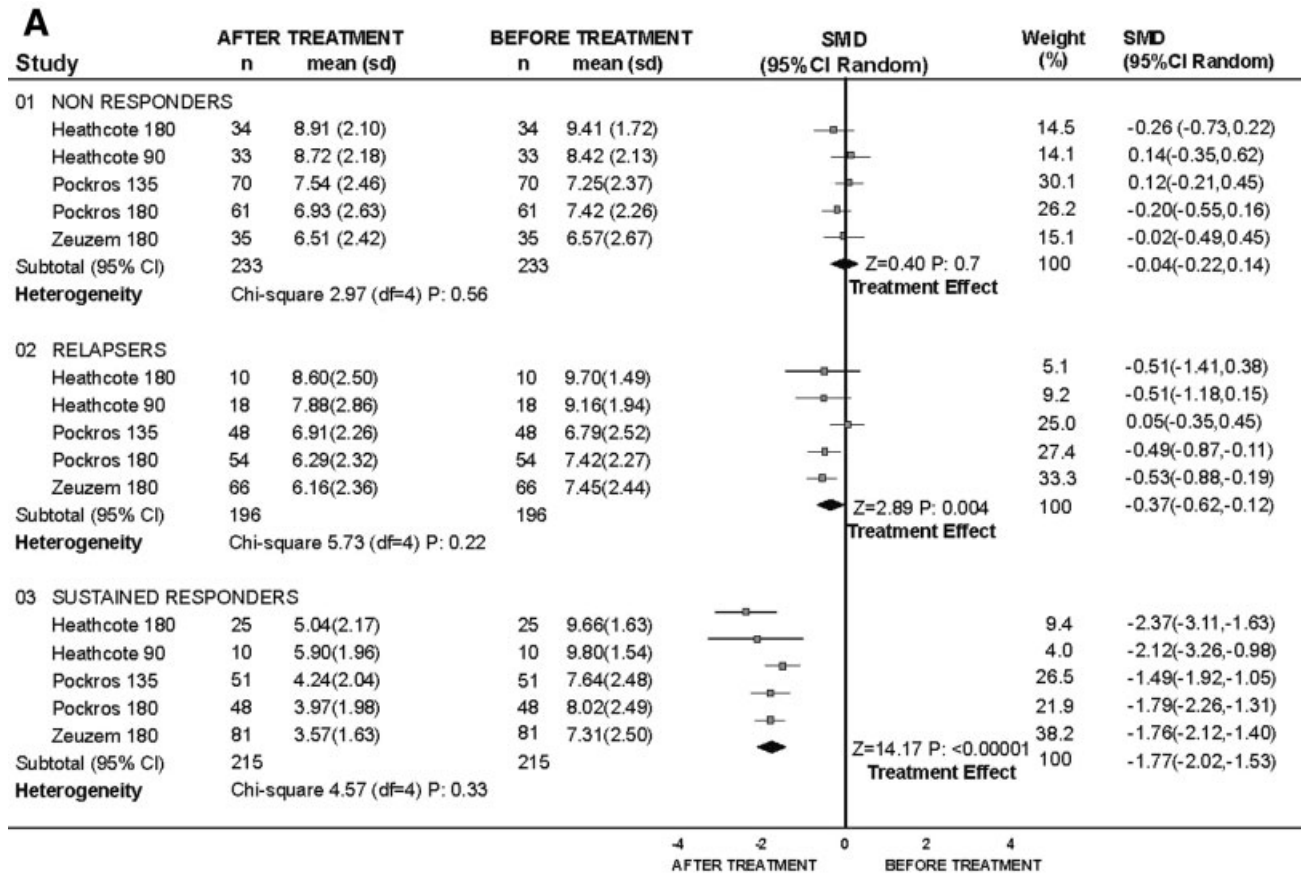


Fig. 2. Meta-analysis (using a random-effects model) of 3 RCTs comparing histologic score before and after treatment in patients with chronic hepatitis C treated with peginterferon alfa-2a. SMDs and 95% CIs for (A) activity and (B) fibrosis, according to virologic response to peginterferon alfa-2a, for nonresponders, patients with recurrent disease, and sustained responders are shown. *df*, degrees of freedom.

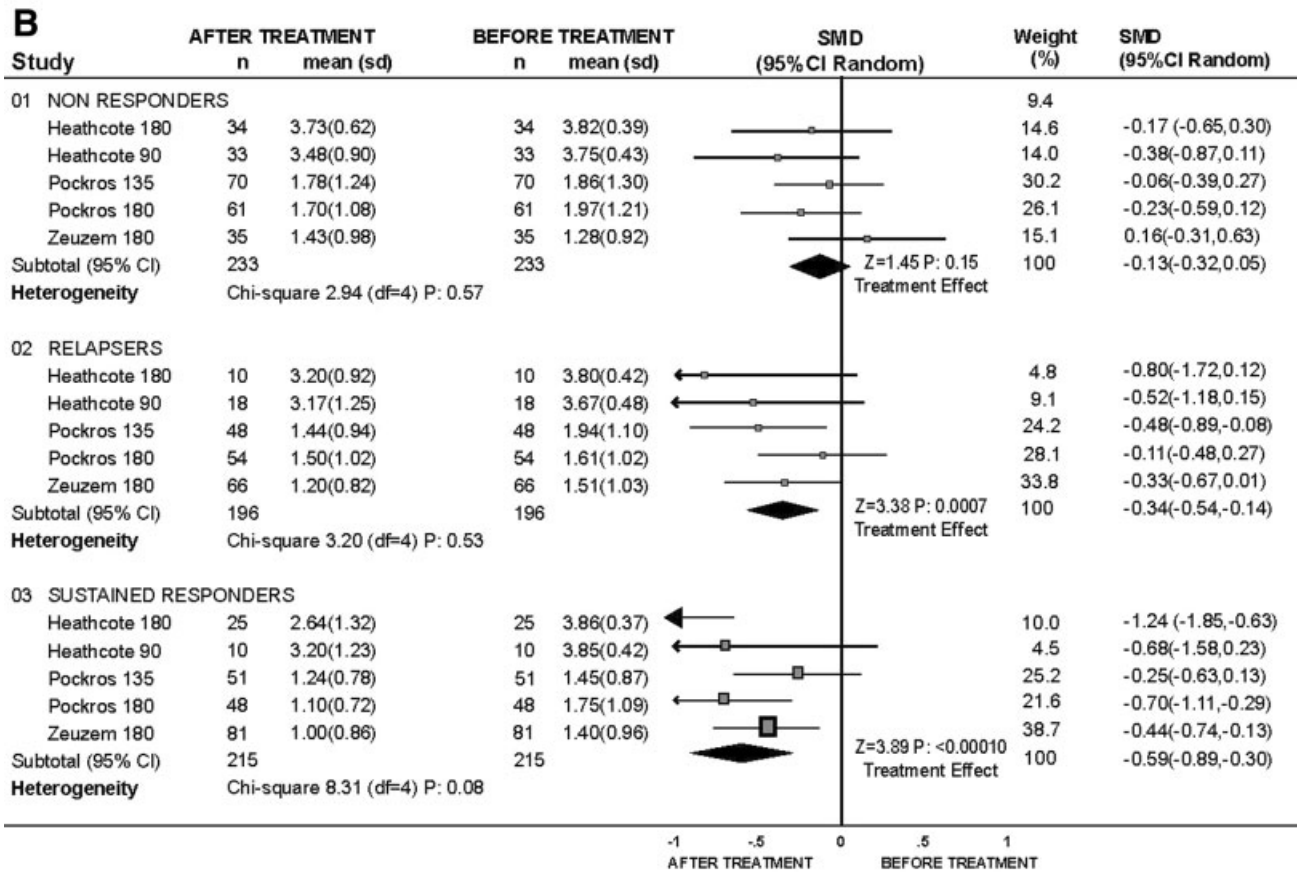


Fig. 2 (Cont'd.)

tative data on HCV RNA were not available for 20 patients. By Week 12, 64.2% of patients (638 of 993) had experienced an early virologic response, defined as a 2-log decrease in HCV RNA levels relative to baseline (245 of 638 patients [38.4%]) or the absence of detectable serum HCV RNA (393 of 638 patients [61.6%]). Fifty-seven percent of patients with early virologic responses (364 of 638) subsequently experienced an improvement in grading, and 28% (179 of 638 patients) experienced an improvement in staging after treatment. Of the 355 patients who did not have early virologic responses, 239 (67%) did not experience an improvement in grading after treatment, and 278 (78.3%) did not experience an improvement in staging.

Among nonresponders, we did not observe a correlation between the 2-log HCV RNA reduction at 12 weeks and histologic response. The mean log change in HCV RNA levels from baseline to Week 12 of treatment was  $-1.63 \pm 1.77$  for the 106 patients who experienced an improvement in fibrosis and  $-1.49 \pm 1.60$  for the 383 patients who did not experience an improvement in fibrosis ( $P = .42$ ).

**Subgroup Analysis of Patients With Cirrhosis.** The database contained 198 patients with cirrhosis. At second biopsy, a reduction in fibrosis stage was observed in 67 of these patients (33.8%; to Stage 3 in 48 patients [24.2%] and

to Stage 1 in 19 patients [9.6%]). Complete resolution of cirrhosis (from Stage 4 to Stage 0) was not observed.

A 2-point decrease in grade was observed in 86 of the 198 patients with cirrhosis (43.4%). Among patients with cirrhosis who received peginterferon alfa-2a, the overall rate of improvement in terms of grading was 56.9% (41 of 72 patients) in the 180  $\mu\text{g}/\text{week}$  group and 37.3% (22 of 59 patients) in the 90–135  $\mu\text{g}/\text{week}$  group ( $P = .025$ ). In contrast, no significant difference in improved staging between the 180  $\mu\text{g}/\text{week}$  group (28 of 72 patients [38.9%]) and the 90–135  $\mu\text{g}/\text{week}$  group (16 of 59 patients [27.1%]) was observed ( $P = .15$ ). Virologic response was the only significant predictor for improvement in terms of both grading (OR, 23.7; 95% CI: 6.7, 80.9) and staging (OR, 2.16; 95% CI: 1.04, 4.47) among patients with cirrhosis.

## Discussion

In the current study, we found that peginterferon alfa-2a significantly reduces fibrosis compared with conventional IFN. Our MIPD has demonstrated conclusively that impressive improvements in terms of fibrosis can be achieved in patients with SVRs and, to a lesser

**Table 3. Changes in Staging and Grading Between Pretreatment and Post-treatment Biopsies**

	No. of Patients	Improved (%)	Stabilized (%)	Worsened (%)
<b>Staging</b>				
Heathcote et al. <sup>11</sup>				
IFN 3 MU	55	15 (27.2)	34 (61.8)	6 (10.9)
PEG-IFN alfa-2a 90 µg	61	15 (24.5)	43 (70.4)	3 (4.9)
PEG-IFN alfa-2a 180 µg	68	24 (35.3)	41 (60.3)	3 (4.4)
Pockros et al. <sup>14</sup>				
IFN 3 MU	147	29 (19.7)	95 (64.6)	23 (15.6)
PEG-IFN alfa-2a 135 µg	171	39 (22.8)	113 (66.0)	19 (11.1)
PEG-IFN alfa-2a 180 µg	160	46 (28.7)	97 (60.6)	17 (10.6)
Zeuzem et al. <sup>12</sup>				
IFN 6/3 MU	167	46 (27.5)	105 (62.8)	16 (9.5)
PEG-IFN alfa-2a 180 µg	184	47 (25.5)	117 (63.5)	20 (10.8)
All patients	1013	261 (25.7)	645 (63.6)	107 (10.5)
<b>Grading</b>				
Heathcote et al. <sup>11</sup>				
IFN 3 MU	55	13 (23.6)	33 (60.0)	9 (16.3)
PEG-IFN alfa-2a 90 µg	61	27 (44.2)	20 (32.7)	14 (22.9)
PEG-IFN alfa-2a 180 µg	68	35 (51.4)	19 (27.9)	14 (20.5)
Pockros et al. <sup>14</sup>				
IFN 3 MU	147	58 (39.4)	64 (43.5)	25 (17.0)
PEG-IFN alfa-2a 135 µg	171	74 (43.2)	55 (32.1)	42 (24.5)
PEG-IFN alfa-2a 180 µg	160	86 (53.7)	53 (33.1)	21 (13.1)
Zeuzem et al. <sup>12</sup>				
IFN 6/3 MU	167	87 (52.1)	53 (31.7)	27 (16.1)
PEG-IFN alfa-2a 180 µg	184	111 (60.3)	53 (28.8)	20 (10.8)
All patients	1013	491 (48.4)	350 (34.5)	172 (16.9)

Abbreviations: PEG-IFN, peginterferon; MU, megaunits.

degree, in patients with recurrent disease. No significant changes were observed in nonresponders.

Our data indicate that patients with BMI  $\leq 30$  kg/m<sup>2</sup> have the greatest likelihood of experiencing a reduction in fibrosis. The observation that obesity (BMI  $> 30$  kg/m<sup>2</sup>) is a risk factor for progression of fibrosis is consistent with the results of 3 recently published studies.<sup>23-25</sup> Although

the mechanisms responsible for the effect of BMI on liver histology are unknown,<sup>25</sup> a practical recommendation to reduce body weight before starting therapy may be beneficial. This statement is strengthened by our observation of reduced fibrosis in the small proportion of nonresponders with the lowest BMI values.

According to recent reports, steatosis is a cofactor of fibrosis progression<sup>23,26</sup> as well as response to therapy in patients with chronic hepatitis C.<sup>27</sup> Unfortunately, we did not collect data on hepatic steatosis. Nonetheless, it recently was demonstrated that in patients with chronic hepatitis C, BMI is closely correlated with the degree of hepatic steatosis.<sup>23,26,28</sup> Therefore, we are confident in our assumption that BMI is a surrogate marker for steatosis in the current analysis. Finally, Bressler et al.<sup>28</sup> recently reported that high BMI, but not steatosis, was an independent risk factor for nonresponse to antiviral treatment.

Our data suggest that patients with high baseline ALT levels experience the largest improvements in grading. This finding may be linked to the higher degree of clearance of virally infected cells during the second phase of response to treatment.<sup>29</sup> Furthermore, the magnitude of the effect of treatment (*i.e.*, the reduction of inflammation under treatment) is more easily measurable when the initial grade of activity is high.

Poynard et al.<sup>30</sup> recently suggested that peginterferon alfa-2b combined with ribavirin slows the natural progression of fibrosis in virologic nonresponders, as well as in responders and patients with recurrent disease. The discrepancy between this finding and our conclusion probably stems from the fact that Poynard et al. based their analysis on the comparison of fibrosis progression rate per year before and after treatment. There are flaws

**Table 4. Logistic Regression Models for Predicting Improvement in Grading (Model I) and Staging (Model II) Between Pretreatment and Post-treatment Biopsies**

Variable	$\beta$	SE	P Value	OR (95% CI)
<b>Grading (Model I)</b>				
Virologic response				
0: No SVR	1.82	0.189	.0001	6.20 (4.28-8.98)
1: SVR				
Baseline ALT level	0.30	0.099	.0024	1.35 (1.11-1.63)
0: $\leq 3 \times$ ULN				
1: $> 3 \times$ ULN				
<b>Staging (Model II)</b>				
Virologic response				
0: No SVR	0.48	0.17	.006	1.61 (1.14-2.29)
1: SVR				
BMI				
0: Not obese (BMI $\leq 30.0$ kg/m <sup>2</sup> )	-0.56	0.23	.01	0.56 (0.35-0.9)
1: Obese (BMI $> 30$ kg/m <sup>2</sup> )				

NOTE. Model I:  $n = 1,013$ ;  $\chi^2 = 177$ , with 5 degrees of freedom;  $P = .0001$ ; adjustments were made for baseline serum HCV RNA, treatment regimen, and mean of initial and final activity scores. Model II:  $n = 775$ ;  $\chi^2 = 14.26$ , with 3 degrees of freedom;  $P = .0026$ ; adjustment was made for treatment regimen.

Abbreviation: ULN, upper limit of normal.

inherent in the use of fibrosis progression rate as a basis for modeling the indication for treatment, due to inaccurate estimation of the duration of infection and the nonlinearity of fibrosis progression over time. In fact, two studies of the validity of fibrosis progression rate demonstrated that this rate is not a reliable and accurate tool for predicting histologic improvement.<sup>31,32</sup> The discord between the findings of Poynard et al. and our findings also may result from the fact that many patients in the study by Poynard et al. received combination therapy, whereas patients in the current study received peginterferon monotherapy.

Poynard et al.<sup>30</sup> found six indicators that were useful in identifying patients with a high probability of reduction in fibrosis; among these indicators were low baseline staging and grading scores. The results reported by Poynard et al. appear to rely on the definition of the endpoint, described as the absence of significant fibrosis (*i.e.*, the absence of METAVIR Stage F2/F3/F4 fibrosis) on second biopsy. This definition is flawed, because the majority of patients (75%) included in the PEG-Fibrosis Project Group database already were free of significant fibrosis (*i.e.*, they had METAVIR Stage F0/F1 fibrosis) at baseline. Thus, the cross-sectional evaluation of staging on second biopsy only, regardless of the fibrosis score on first biopsy, practically hinders the evaluation of changes between paired biopsies.

Unlike Poynard et al.,<sup>30</sup> we did not observe complete resolution of cirrhosis (from Stage 4 to Stage 0) after treatment in any of nearly 200 patients with cirrhosis. A decrease in fibrosis score after treatment was observed in only 33% of patients with cirrhosis. The large size of the cohort with cirrhosis or bridging fibrosis (447 of 1013 patients [44%]) in the current study enhances the statistical relevance of our conclusions.

The main limitation of the current study, as well as other studies, is the very short time between paired biopsies. In RCTs, the post-treatment biopsy generally is performed only 24 weeks after the end of treatment. The current analysis indicated that approximately two-thirds of all patients did not experience a change in fibrosis stage between paired biopsies. Because histologic improvement is a slow process, particularly for fibrosis,<sup>4</sup> it may be advisable to repeat liver biopsy 2 or 3 years after the end of treatment to assess the treatment benefit with respect to fibrosis. Due to the large number of patients analyzed in the current MIPD, we believe that a 'moderate' but clinically relevant treatment benefit with respect to liver fibrosis was not overlooked. Because of the different clinical settings and groups of patients analyzed, and because the small number of studies included in the current meta-analysis reduces the statistical power to test for heterogeneity, we have presented the findings of random-effects models.

Many studies have attempted to identify the peginterferon dose that maximizes benefit with respect to liver histology. Poynard et al.<sup>30</sup> compared 10 different regimens of peginterferon alfa-2b and conventional IFN with and without ribavirin with a regimen of 3 megaunits of conventional IFN 3 times weekly for 24 weeks. We compared only 3 different regimens of peginterferon alfa-2a monotherapy with a control regimen of conventional IFN at a dose of at least 3 megaunits 3 times weekly for 48 weeks. The current MIPD provides evidence that of the three peginterferon doses assessed, a standard dose (180  $\mu\text{g}/\text{week}$ ) is the best option with respect to grading improvement in patients with or without cirrhosis.

The available evidence was sufficient to conclude that 1) peginterferon alfa-2a was more efficacious than conventional IFN alfa-2a in improving liver histology; 2) peginterferon alfa-2a induced a marked reduction in both stage and grade in patients with SVRs and, to a lesser degree, in patients with recurrent disease, but provided no benefit for nonresponders after 24–48 weeks of treatment; patients with BMI < 30 kg/m<sup>2</sup> and high pretreatment ALT levels had the greatest probability of experiencing histologic improvement; and 3) peginterferon alfa-2a at 180  $\mu\text{g}/\text{week}$  for 48 weeks was the best dosing option in terms of grading improvement.

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