Progression of Liver Fibrosis in Women Infected With Hepatitis C: Long-Term Benefit of Estrogen Exposure

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Female sex is a protective factor for the progression of fibrosis in patients with chronic hepatitis C virus (HCV) infection. Experimental data suggest that estrogens may have an antifibrotic effect. The objective of this study was to evaluate the influence of past pregnancies, oral contraceptives, menopause, and hormone replacement therapy (HRT) on liver fibrosis progression in HCV-infected women. Four hundred seventy-two HCV-infected women received a survey regarding prior pregnancies, menopause, and the use of oral contraceptives and HRT. The impact of these variables on liver fibrosis and its progression were evaluated using multivariate analyses considering all putative confounding factors. Two hundred one women completed the survey (43% response rate), 157 of whom had an estimated date of HCV infection (96 postmenopausal women, 96 women with previous pregnancies, and 105 women with past use of oral contraceptives). Through multivariate analyses, the estimated rate of fibrosis progression was higher in postmenopausal ($P < .05$) and nulliparous ($P = .02$) women and was associated with greater histological activity ($P < .001$). Prior use of oral contraceptives had no significant influence. Among postmenopausal women, the estimated rate of fibrosis progression (±SE) was lower in women who received HRT compared with untreated patients (0.099 ± 0.016 vs. 0.133 ± 0.006 METAVIR units/yr; $P = .02$) and was similar to that of premenopausal women (0.093 ± 0.012 METAVIR units/yr; P value not significant). In conclusion, menopause appears to be associated with accelerated liver fibrosis progression in HCV-infected women, an effect that may be prevented by HRT. Pregnancies may have a beneficial impact on the long-term progression of liver fibrosis. (HEPATOLOGY 2004;40:1426–1433.)

Despite a similar prevalence, chronic infection with hepatitis C virus (HCV) has been reported to be more severe in males than females. Progression to both cirrhosis[1,2] and hepatocellular carcinoma[3–5] is indeed more common in HCV-infected males. In a large crosssectional study modeling the rate of HCV-related liver fibrosis progression, male sex was an independent predictor of progression to cirrhosis, increasing the risk over 2.5-fold.[1] A low risk of cirrhosis in nondrinking women was confirmed by the true longitudinal observations of female cohorts infected by anti-D immunoglobulin.[6,7]

Several factors may account for the apparent beneficial influence of female sex on the outcome of chronic HCV infection, including a lower prevalence of alcohol and tobacco consumption[1,8] and a lower probability of iron overload and overweight, factors that negatively influence the course of chronic hepatitis C.[9,10] Recent data strongly suggest that estrogens and/or estrogen receptors have an impact on the course of liver disease. A decrease in estrogen receptors, marked after menopause, was reported to be associated with increased lipid peroxidation and impaired superoxide dismutase function, leading to increased susceptibility to hepatocellular carcinoma.[11] Moreover, experimental data in the dimethylnitrosamine rat model of liver fibrogenesis showing a protective effect of endogenous and exogenous estrogens on liver fibrosis provide consistent evidence of a direct beneficial effect of estrogens on fibrogenesis.[12]

Abbreviations: HCV, hepatitis C virus; HRT, hormone replacement therapy; BMI, body mass index; FPR, fibrosis progression rate.

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The effect of estrogens on HCV-related liver fibrosis in humans has rarely been examined. In a small case–control study, pregnancy was reported to increase the severity of necroinflammatory lesions,[13] but its long-term impact on liver fibrosis progression was not determined. Based on predominantly anecdotal reports, many physicians have been reluctant to prescribe oral contraceptives to HCV-infected women. Similarly, despite the recently reported risk of osteoporosis as an extrahepatic manifestation of chronic hepatitis C,[14] many avoid prescribing hormone replacement therapy (HRT) to postmenopausal women because of concerns regarding potential hepatotoxicity.

The objective of this study was to assess the long-term impact of estrogens on the progression of liver fibrosis in HCV-infected women. Specifically, past pregnancies, menopausal status, and the use of oral contraceptives and HRT were examined as determinants of estrogen exposure.

Patients and Methods

Patients and Study Design. This was a bicenter retrospective cohort study conducted at the Hôpital Bicêtre and Pitié-Salpêtrière in France. All women with chronic hepatitis C evaluated between 1993 and 2001 were eligible for the study. Chronic hepatitis C was defined via a positive serological test for HCV using at least a second-generation enzyme-linked immunosorbent assay and positive HCV RNA using polymerase chain reaction assay. We excluded women with human immunodeficiency virus, hepatitis B virus, or hepatitis delta virus coinfection; women with other causes of chronic liver disease; women who did not undergo a liver biopsy; and women who died or were lost to follow-up. All eligible women received a survey by mail or at a medical visit. We considered women who responded to the survey and provided informed, written consent as eligible.

Survey. Between January 2000 and June 2001, a survey was administered to each eligible subject either by mail or on-site. On-site completion was independent and was performed mainly in the waiting room. Demographic data and information regarding chronic hepatitis C, comorbid conditions, and events of the reproductive life were recorded anonymously. HCV-related data included the estimated date and route of HCV infection, the date of the first liver biopsy, and prior antiviral therapy. The date of HCV infection was estimated as the date of transfusion or initial exposure to other parenteral sources. Data regarding comorbidity included factors known to influence the natural history of chronic hepatitis C, including self-reported alcohol consumption (in glasses per week, subsequently converted to grams per day; age at onset and cessation), self-reported tobacco consumption (number of cigarettes per day; age at onset and cessation), and history of overweight, diabetes, or dyslipidemia (with age at diagnosis, if any). Data concerning the estrogen-associated events included age at menarche and menopause, etiology of menopause (natural, ovariectomy, pelvic radiotherapy, chemotherapy), and therapy with oral contraceptives or HRT (via an exhaustive alphabetized listing of drugs appended to the survey). Information regarding previous pregnancies included maternal age at each pregnancy and duration according to outcome (live birth or spontaneous or therapeutic abortion). Data concerning estrogen-associated events were considered only if they occurred between the estimated date of HCV infection and the first liver biopsy. When the date of HCV infection could not be estimated, all events prior to the liver biopsy were reported in the population characteristics but were not considered for further analyses.

Liver Histology. Liver biopsy specimens more than 10-mm long were fixed in 10% formalin buffer, stained with hematoxylin-eosin, and read by a single pathologist unaware of the clinical and biological data. Liver inflammation and fibrosis were assessed according to the METAVIR scoring system.[15] The grading of activity, which evaluates the intensity of necroinflammatory lesions, was indicated as follows: A0, no histological activity; A1, mild activity; A2, moderate activity; and A3, severe activity. Liver fibrosis was staged on a scale of F0 to F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis).

Determination of the Rate of Liver Fibrosis Progression. The rate of liver fibrosis progression was determined as described previously.[1,16–18] Briefly, the fibrosis progression rate per year was calculated as the ratio between the stage of fibrosis (in METAVIR units) and the estimated duration of infection (in years). For example, for a patient with a fibrosis stage of F2 and an 8-year duration of infection, the estimated rate of fibrosis progression is $2/8 = 250 \times 10^{-3}$ METAVIR units per year.

Statistical Analysis. Quantitative variables were expressed as the mean $\pm$ SE or as the median (95% CI) in case of abnormal distribution. The relationship between histological features and the estimated rate of fibrosis progression, age, alcohol and tobacco consumption, body mass index (BMI), past pregnancies, menopause, years of reproductive life, and oral contraceptive or HRT were assessed using a chi-square test, a Student t test, linear regression, and log-rank tests for univariate analyses. For multivariate analyses, multiple linear regression analysis—validated using the jack-knife procedure—was performed to assess variables correlated with the estimated

rate of fibrosis progression. Parameters analyzed in the multivariate analyses were selected according to the results of univariate analysis and the absence of colinearity. Factors associated with the presence of septal fibrosis (F2 or more) were also assessed using logistic regression. For all these analyses, only women with a known date of HCV infection were considered. Hence, the events associated with estrogen exposure (past pregnancies, menopause, years of reproductive life, and oral contraceptive or HRT) considered for the analyses were only those that occurred between HCV infection and liver biopsy.

Results

Study Population. Out of 1,506 HCV-infected women evaluated at the Hôpital Pitié-Salpêtrière and Hôpital Bicêtre between 1993 and 2001, 840 women were eligible for the study. Four hundred eighty women were excluded because of human immunodeficiency virus coinfection (n = 84), hepatitis B surface antigen positivity (n = 19), or absence of a liver biopsy (n = 377). A total of 186 women were also excluded because they were lost to follow-up (n = 143) or had died (n = 43) by the initiation of the study. Of the 840 eligible women, 472 were contacted; these women had an available address that had not changed since the first visit at our institutions or had completed the survey on-site. Two hundred seventeen women responded adequately to the survey (on-site completion for 87 women); 16 were subsequently excluded because their liver biopsy sample was deemed inadequate (Fig. 1).

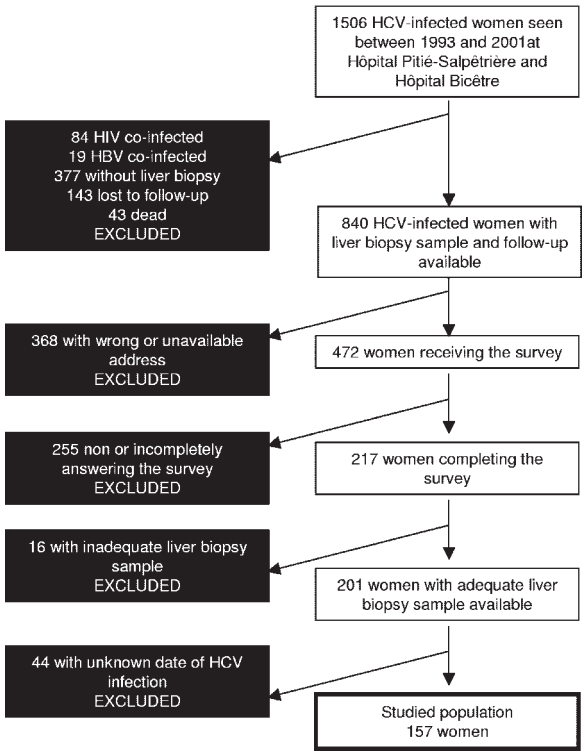

Fig. 1. Selection of the study population. HCV, hepatitis C virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus.

Among the 201 remaining patients (43% of the total eligible patients), 157 had an estimable date of infection. Their main characteristics are outlined in Table 1. The mean age at the time of the liver biopsy was 48 ± 1 years, 60% had a history of blood transfusion, and 18% were previous intravenous drug users; the mean age at contamination was 29 ± 1 years, and the mean duration of infection at the time of biopsy was 18 ± 1 years. HCV genotype 1 was present in the majority of patients (64%). Overall, alcohol consumption was minimal: only 9 (6%) of 157 patients responding to this portion of the survey reported daily alcohol consumption in excess of 20 g. Those characteristics were not different from those of the 840 HCV-infected women who underwent liver biopsy in our centers (see Table 1). The mean BMI was 23.6 kg/m^2; overweight as defined by a BMI greater than 25 kg/m^2 was found in 22% of the women. The events associated with estrogen exposure that occurred prior to liver biopsy are reported in Table 2. The mean ages at menarche and duration of fertility before the liver biopsy were 13 ± 0.5 and 29 ± 1 years, respectively. Between HCV infection and liver biopsy, 96 women (61%) had one or more past pregnancies (mean cumulative duration of pregnancies, 15 ± 1 months); 74 (47%) had one to five children (mean, 1.7 ± 0.1). Ninety-six women (61%) were menopausal at the time of the biopsy (natural in 55%). The mean age at menopause was 45 ± 1 years and, on average, occurred 11 ± 1 years prior to the biopsy. One hundred five women (67%) had taken oral contraceptives before the liver biopsy (mean cumulative duration of use, 4.8 ± 0.5 years), of whom 20% were taking oral contraceptives at the time of the biopsy. The type of oral contraceptive was available for 103 of the 105 women (estrogen and progesterone combination therapy in 94% and progesterone alone in 6%). The dosage of estrogens was 30 μg/d of ethinylestradiol in 70% of cases and 50 μg/d in 40% of cases; 36% of the women varied their oral contraceptive regimen. Among postmenopausal women, 51% had previously received HRT (mean duration before biopsy, 7 ± 1 yr); 82% were still receiving this therapy at the time of the biopsy. HRT consisted of a combination of transdermal estrogen and oral progesterone in all cases. Finally, only 7 of the 157 women were nulliparous, premenopausal, or had not received oral contraceptives between HCV infection and liver biopsy. The distribution of the studied population according to the estrogen-associated events prior to the liver biopsy was not randomly

Table 1. Patient Characteristics: Comparison Between the Studied Population and the Whole Population Seen in Our Centers

	Selected Population			Whole Population		
	N	n (%)	Mean ± SE	N	n (%)	Mean ± SE
Age at HCV infection	157		29 ± 1	570		30 ± 0.5
Route of infection	157			840		
Intravenous drug use		34 (22)			126 (15)	
Transfusion		95 (60)			370 (44)	
Other		28 (18)			109 (13)	
Unknown		–			235 (28)	
Age at liver biopsy	157		48 ± 1	840		47 ± 0.5
HCV duration (yr)	157		18 ± 1	570		17 ± 0.4
HCV genotype 1	157	100 (64)		509	331 (65)	
Self-reported alcohol consumption (g/d)	157			840		
0		113 (72)			622 (74)	
1–20		35 (22)			151 (18)	
21–50		8 (5)			42 (5)	
>50		1 (1)			25 (3)	
BMI (kg/m^2)	157		23.6 ± 0.4	NA	NA	NA
BMI > 25		35 (22)				
Cigarette smoking	157	74 (47)		NA	NA	NA
Histological activity index	157		1.18 ± 0.05	840		1.19 ± 0.02
A0		21 (13)			126 (15)	
A1		89 (57)			454 (54)	
A2		42 (27)			235 (28)	
A3		5 (3)			25 (3)	
Histological fibrosis score	157		1.58 ± 0.11	840		1.55 ± 0.04
F0		20 (13)			134 (16)	
F1		68 (43)			361 (43)	
F2		38 (24)			176 (21)	
F3		20 (13)			85 (10)	
F4		11 (7)			84 (10)	
Fibrosis progression rate ($\times 10^{-3}$ METAVIR units/yr)	157		108 ± 3	570		183 ± 21

NOTE. None of the observed differences reached statistical significance.
Abbreviation: NA, not available.

distributed. A prior history of pregnancy was more common in premenopausal women (97% vs. 39%; $P < .001$) and in women who had taken oral contraceptives (72% vs. 39%; $P = .01$). Moreover, postmenopausal women were less likely to report prior use of oral contraceptives (89% vs. 47%; $P < .001$).

Histological Features. Among the 157 women with a known date of HCV infection, the distribution of necroinflammatory lesions was as follows: A0, 13% (n = 21); A1, 57% (n = 89); A2, 27% (n = 42); and A3, 3% (n = 5). The mean grade of histological activity was 1.18 ± 0.05. Liver fibrosis was scored as follows: F0, 13% (n = 20); F1, 43% (n = 68); F2, 24% (n = 38); F3, 13% (n = 20); and F4, 7% (n = 11). The mean fibrosis score was 1.58 ± 0.11. The mean estimated rate of fibrosis progression was 108 ± 3 × 10^{-3} METAVIR units per year. The histological features of the study population were not different from those observed in the 840 HCV-infected women who underwent liver biopsy at our centers (see Table 1).

Influence of Past Pregnancies. The mean grade of necroinflammatory activity did not differ between nulliparous women and those with one or more past pregnancies (1.15 ± 0.08 vs. 1.17 ± 0.07; P value not significant); however, the stage of fibrosis (1.68 ± 0.13 vs. 1.20 ± 0.13; $P < .001$) and the mean estimated rate of fibrosis progression (138 ± 6 vs. 74 ± 8 × 10^{-3} METAVIR units/yr; $P < .01$) were significantly higher in nulliparous women. The comparisons of activity, fibrosis, and the estimated rate of fibrosis progression (Fig. 2) were similar regardless of pregnancy outcome (live birth or not) and the cumulative duration of the pregnancies (data not shown). Using the Kaplan-Meier method, women with previous pregnancies had a lower progression to septal fibrosis (F2, F3, or F4) compared with nulliparous women (log-rank test; $P = .02$) (Fig. 3). Multivariate regression analysis showed that a history of pregnancy was independently associated with a lower estimated rate of fibrosis progression ($P = .02$) (Table 3).

Influence of Menopause. Mean histological activity was similar regardless of menopausal status (premenopausal, 1.18 ± 0.08 vs. postmenopausal, 1.17 ± 0.08; P value not significant); however, postmenopausal women had higher mean fibrosis scores than premenopausal

Table 2. Summary of Life Events Associated With Estrogen Exposure

	N	n (%)	Mean ± SE
Age at first menarche (yr)	201		13 ± 0
Oral contraceptives*	157	105 (67)	
Duration of oral contraceptives (yr)*	105		4.8 ± 0.5
Pregnancies*†	157		
0		61 (39)	
1 or more		96 (61)	
Cumulative duration of pregnancies (mo)*	96		15 ± 1
Children*	157		
0		83 (53)	
1 or more		74 (47)	
Number of children	74		1.7 ± 0.1
Menopause*	157	96 (61)	
Age at menopause (yr)	96		45 ± 1
Duration of menopause (yr)*	96		11 ± 1
HRT	96	49 (51)	
Duration of HRT (yr)*	49		7 ± 1
Duration of fertility (yr)*	157		29 ± 1

*Between HCV infection and liver biopsy.
†Includes abortions.

women (1.87 ± 0.16 vs. 1.17 ± 0.10; $P < .01$) and rates of fibrosis progression (119 ± 5 vs. 93 ± 12 × 10^{-3} METAVIR units/yr; $P < .05$) (see Fig. 2). Neither the delay between menopause and liver biopsy nor the etiology of menopause had a significant influence on the analysis of fibrosis stage or the estimated rate of fibrosis progression (data not shown). Multivariate logistic regression analysis controlling for age, BMI, and necroinflammatory activity indicated that menopausal status was an independent predictor of the presence of septal fibrosis (F2) or greater (odds ratio 9.3; 95% CI 1.4-62; $P = .02$). Similarly, multiple regression analysis (see Table 3) showed that menopause was associated with more rapid fibrosis progression independent of age, BMI, and necroinflammatory activity ($P < .05$). Among postmenopausal women, there was no significant difference in the mean activity score between those who had received HRT (n = 49; 1.13 ± 0.12) and those who had not (n = 47; 1.18 ± 0.10; P value not significant), whereas there was more advanced fibrosis in the latter group (1.79 ± 0.25 vs. 1.93 ± 0.20, $P < .05$). Moreover, women who received HRT had a significantly lower estimated rate of fibrosis progression than untreated women (99 ± 16 vs. 133 ± 6 × 10^{-3} METAVIR units/yr; $P = .02$). In fact, the mean estimated rate of fibrosis progression of the postmeno-

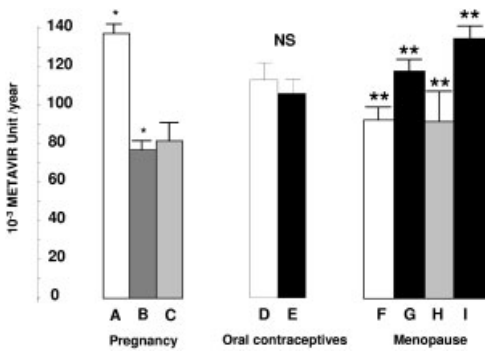

Fig. 2. Comparison of fibrosis progression rate (FPR) in HCV-infected women according to past history of pregnancies, oral contraceptives, menopause, and HRT. (A) Nulliparous women. (B) Women with one or more past pregnancies. (C) Women with one or more children. (D) Women not taking oral contraceptives. (E) Women with past or present use of oral contraceptives. (F) Nonmenopausal women. (G) Menopausal women (total). (H) Menopausal women receiving HRT. (I) Menopausal women not receiving HRT. The significant results are as follows: (1) FPR was significantly higher in nulliparous women, suggesting a long-term protective effect of pregnancies against liver fibrosis progression. (2) Postmenopausal women had a higher FPR compared with premenopausal women. Among postmenopausal women, the mean FPR was lower in users than nonusers of HRT, and similar to that of premenopausal women. These results suggest a deleterious effect of menopause on liver fibrosis progression that may be prevented by HRT. *$P < .001$; **$P < .02$. NS, not significant.

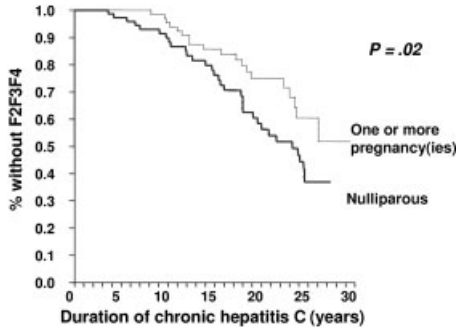

Fig. 3. Long-term progression to septal fibrosis (F2) or greater according to past history of pregnancies. Nulliparous women were more likely to progress to significant fibrosis ($P = .02$).

Table 3. Summary of the Multivariate Analyses of the Impact of Pregnancies, Oral Contraceptives, and Menopause on Liver Fibrosis Progression Using Stepwise Multiple Regression Analyses

	Multiple Regression Analyses of the Factors Increasing Fibrosis Progression Rate	
	r^2	P
Age ≥ 40	0.12	NS
Histological activity index ≥ A2	0.41	<.001
BMI ≥ 27	0.02	NS
Pregnancies*	0.24	.02
Oral contraceptives*	0.01	NS
Menopause*	0.23	<.05
Characteristics of the models	n = 157, r^2 = 0.19 to 0.21	

NOTE. The included independent variables were selected according to univariate analyses results and absence of colinearity.
Abbreviation: NS, not significant.
*Separately analyzed in 3 different models also including age ≥ 40, histological activity index ≥ A2, and BMI > 27.

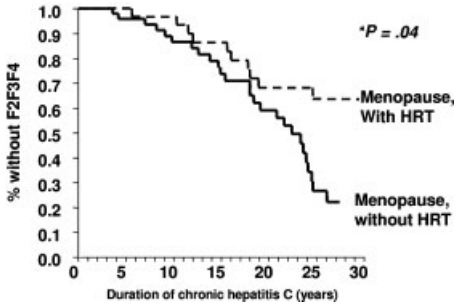

Fig. 4. Long-term progression to septal fibrosis (F2) or greater according to past history of HRT in postmenopausal, HCV-infected women. The significant difference in liver fibrosis progression that appears 20 years after HCV infection ($P = .04$) strongly suggests a protective effect of HRT. HRT, hormone replacement therapy.

pausal women who had received HRT was similar to that of premenopausal women ($93 \pm 12 \times 10^{-3}$ METAVIR units/yr; *P* value not significant) (see Fig. 2). Using the Kaplan-Meier method, the progression to septal fibrosis (F2) or greater was significantly greater in postmenopausal women who did not receive HRT ($P = .04$) (Fig. 4). The small number of postmenopausal women did not permit multivariate analysis.

Influence of Oral Contraceptives. Oral contraceptive therapy had no impact on the grade of necroinflammatory activity (mean activity grade in those receiving vs. not receiving oral contraceptives, 1.18 ± 0.09 vs. 1.18 ± 0.07; *P* value not significant); however, the mean fibrosis score was lower in users of oral contraceptives than in nonusers (1.38 ± 0.14 vs. 1.80 ± 0.14; $P = .02$). The estimated rate of fibrosis progression was not significantly different in users versus nonusers of oral contraceptives in univariate (108 ± 8 vs. 115 ± 1; *P* value not significant) (see Fig. 2) nor multiple regression analysis (see Table 3). Similarly, in multivariate logistic regression analysis controlling for age, BMI, and necroinflammatory activity, oral contraceptive use was not an independent predictor of the presence of F2 fibrosis or greater.

Discussion

Our study of the events of reproductive life and exogenous estrogen therapy provides consistent evidence that estrogens may have a protective effect on the long-term course of chronic hepatitis C in females.

Several limitations must be acknowledged. First, the concept of dynamic fibrosis progression[1] restricts the analyses to patients with a "known" duration of infection and is limited by the assumption of linearity in the progression of fibrosis. It also restricts the analysis to one liver biopsy sample to eliminate posttreatment biopsies from analyses and to avoid a selection bias if considering only patients with serial liver biopsy samples available without anti-HCV therapy. The concept of linear fibrosis progression does not account for late acceleration of liver fibrosis that was recently suggested in various forms of chronic liver diseases and may be influenced by menopause.[19] Second, our retrospective study was built on the basis of self-administered questionnaires of a small proportion of our general population. However, we did not find any significant difference between the studied population and the general population with respect to demographic, virological, or histological findings (see Table 1). The use of self-administered questionnaires may also have introduced recall bias that may have introduced inaccuracies, especially regarding the use of oral contraceptives. The absence of a significant impact of the use of oral contraceptives on the variability of HCV-related liver fibrosis progression may partially result from such a bias. However, we can assume that the records of past pregnancies, menopause, and the use of HRT that are either major or recent estrogen-associated events were not hampered by recall bias. Third, we are unable to provide definitive evidence of a protective effect of HRT on liver fibrogenesis. Only an appropriately powered, randomized trial with serial liver biopsy specimens could resolve this issue. Moreover, the small number of postmenopausal women receiving this therapy did not permit multivariate analysis. However, the comparison of fibrosis progression rates between premenopausal women and postmenopausal women with or without HRT (see Fig. 2) is intriguing. Nevertheless, our findings should be considered hypothesis-generating and in need of confirmation.

The most relevant finding was that the menopausal state was independently associated with accelerated fibrosis progression. It is unlikely that this effect is due simply to aging, because this variable was controlled for in our multivariate analyses. Moreover, the deleterious effect of menopause was not apparent in women who had received HRT, suggesting that hormone deficiency is indeed the culprit. This finding has important clinical implications for the management of postmenopausal women with chronic hepatitis C. Although osteoporosis may be encountered in HCV-infected women,[14] many physicians are reluctant to prescribe HRT because of concerns regarding potential hepatotoxicity.

Our findings suggest that this treatment—or at least the combination of transdermal estrogen and oral progesterone—is safe and potentially beneficial with respect to liver fibrosis. We also found that pregnancy did not have a deleterious effect on HCV-related necroinflammatory activity or fibrosis when assessed over the long term. Indeed, women with a prior history of pregnancy, regardless of the outcome or duration, had a lower estimated rate of

fibrosis progression. This effect was independent of age, BMI, and histological activity. Prior studies examining the impact of pregnancy on chronic hepatitis C have reported an increase of serum aminotransferases and HCV viral load following delivery[20–23]; pregnancy-induced changes in the immune system have been implicated. This was not a focus of our study. To our knowledge, only one prior study has examined the impact of pregnancy on histopathological lesions in women with chronic hepatitis C. In a case–control study of 12 women biopsied before and after delivery, Fontaine and colleagues[13] reported an increase in necroinflammatory activity in 83% of cases versus only 25% of nonpregnant controls ($P = .02$); fibrosis also tended to progress post partum. The authors concluded that pathological exacerbation could occur following delivery in HCV-infected women and that in rare cases irreversible deterioration may be observed. Our study suggests that in general, the long-term impact of pregnancy on HCV-related histological lesions is benign and potentially protective with respect to fibrosis progression.

Two thirds of the women in our study reported prior therapy with oral contraceptives. The majority had taken low-dose preparations of ethinylestradiol and progesterone; the mean duration of therapy was approximately 5 years. Oral contraceptive therapy had no impact on the grade of necroinflammatory activity or estimated rate of fibrosis progression, although mean fibrosis scores were higher in nonusers. However, in multivariate analyses, the effect of oral contraceptives on both fibrosis score and fibrosis progression rate was not statistically significant. This may be a true finding; alternatively, the low doses of ethinylestradiol may have been insufficient to increase serum estrogen levels to a level at which a significant antifibrotic effect could be observed.[24] The apparent protective effect of oral contraceptives on the stage of fibrosis seen in the univariate analysis may have been due to a higher proportion of postmenopausal and nulliparous women (both having a deleterious impact) in the group that did not receive this therapy. It also should have been influenced by the younger age of women who received oral contraceptives compared with others. Nevertheless, our data suggest that oral contraceptives are well tolerated from a long-term hepatic perspective in women with chronic hepatitis C. However, rare cases of acute cholestasis secondary to these agents warrant careful observation following their initiation.[25]

In our study, the long-term effects of pregnancy, oral contraceptives, menopause, and HRT on liver fibrosis progression were assessed via multivariate analyses considering the factors known to influence HCV-related fibrosis (age, BMI, and hepatic necroinflammatory activity). We employed the concept of liver fibrosis progression[1] that considers the duration of HCV infection and is more sensitive than the fibrosis score for assessing significant differences between small groups. Alcohol consumption was not included in our multivariate models because only 6% of the studied women reported daily alcohol consumption in excess of 20 g, and this variable did not influence liver fibrosis in the univariate analyses. Moreover, we evaluated separately the impact of previous pregnancies, oral contraceptives, and menopause on fibrosis and the estimated rate of fibrosis progression. These analyses were designed to decrease the probability of a type II error and to take into account colinearity between the three studied variables.

Our results from clinical observation support experimental data showing an antifibrotic effect of estrogens in liver, lung, kidney, and skin tissue.[12,26–29] In a rat model of liver fibrosis induced by dimethylnitrosamine, Yasuda and colleagues[12] compared liver fibrosis in males and females administered exogenous estrogen versus those with hypoestrogenemia induced by antiestrogen antibodies or ovariectomy. Compared with females with normal levels of estrogen, increased fibrosis was observed in males and females with hypoestrogenemia. An *in vitro* study showed that proliferation of hepatic stellate cells in primary culture and fibrogenesis were blocked by estrogens.[12]

In conclusion, our study suggests that estrogens may have a protective effect on histopathological lesions over the long-term in women with chronic hepatitis C. Postmenopausal status is characterized by accelerated fibrosis progression. In contrast to previous reports,[13] pregnancy does not appear to have a deleterious impact on hepatic histology and may in fact be protective against the progression of fibrosis. Oral contraceptives appear safe with respect to liver fibrosis progression in women with chronic hepatitis C. Finally, HRT may have an antifibrotic effect, but its potential benefits on liver fibrosis in postmenopausal women need to be confirmed and must be balanced against other health risks.

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