Digestive Diseases and Sciences, Vol. 50, No. 3 (March 2005), pp. 547–551 (© 2005)
DOI: 10.1007/s10620-005-2472-5

Impact of Immunosuppressive Treatment on Liver Fibrosis in Autoimmune Hepatitis

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The impact of treatment on progression of fibrosis in autoimmune hepatitis (AIH) is unknown. We assessed the changes in liver fibrosis before and after treatment among these patients. Nineteen AIH patients who had paired liver biopsies were studied. Of these, seven had been treated with 6 months of cyclosporine A and the rest with 6 months of prednisolone for induction of remission. Thereafter all had been maintained on azathioprine. Biopsy specimens before and after treatment were reviewed by one pathologist and scored by the Ishak method. Mean fibrosis stages before and after treatment were compared. Also, factors predicting significant fibrosis (stage $\geq$3) and cirrhosis (stage $\geq$5) at presentation were assessed. Mean interval between biopsies was 3.38 years. Mean fibrosis stage decreased from 4.53 to 2.16 following treatment ($P < 0.001$). Mean decrement in inflammatory grade was 8 scores (range, 4–10) in patients in whom fibrosis improved, and 2 scores (range, 0–4) in patients in whom fibrosis did not decrease after treatment ($P < 0.001$). ALT-to-platelet ratio was the best predictor of significant fibrosis and also cirrhosis. Fibrosis commonly improves after immunosuppressive treatment in AIH. ALT-to-platelet ratio can predict accurately the presence of significant fibrosis and cirrhosis in AIH.

KEY WORDS: autoimmune hepatitis; liver fibrosis; therapeutics; immunosuppressive agents.

Liver fibrosis is an important prognostic factor in chronic liver diseases, as it gradually leads to cirrhosis. Most of the complications of chronic liver diseases are due to advanced cirrhosis. It has been shown that effective treatment of chronic hepatitis C affects progression of fibrosis favorably (1). Additionally, regression of hepatic fibrosis after treatment of the underlying cause has been reported in secondary biliary fibrosis (2). Regression of liver fibrosis in autoimmune hepatitis (AIH) has also been reported in a recent study (3) and a few case reports (4, 5).

The impact of treatment on the rate of progression of fibrosis in AIH is unknown. Additionally, factors associated with liver fibrosis before treatment and factors associated with regression of fibrosis after treatment in AIH have not been addressed previously. The aim of this study was to determine the fibrosis progression rate before and after treatment in AIH, as well as to determine factors associated with liver fibrosis at presentation and those associated with the absence of fibrosis after treatment.

MATERIALS AND METHODS

Data from patients with AIH who were treated at our center between 1995 and 2002 were retrieved. AIH was diagnosed if the patient had chronically elevated aminotransferases, hypergammaglobulinemia, positive auto-antibodies (antinuclear antibody, anti-smooth muscle antibody, anti liver–kidney microsomal antibody type 2), histopathological features compatible with autoimmune hepatitis on liver biopsy, and absence of viral (hepatitis B and C), metabolic (Wilson's disease, α-1 antitrypsin deficiency,

Manuscript received July 23, 2004; accepted August 27, 2004.

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hemochromatosis), and drug-induced liver disease (including alcohol). Patients with AIH were included if they had paired pre- and posttreatment liver biopsies. Liver biopsies were considered adequate if there were at least four portal tracts per high-power field. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from each patient before the study.

Nineteen patients were included. Seven patients were part of an open-label cyclosporine trial in the treatment of autoimmune hepatitis (6). Five of these seven patients had been treated initially with corticosteroids, however, they were switched to cyclosporine because of intolerable side effects of corticosteroids. Two of them were treated with cyclosporine at the start of treatment. These seven patients were treated with cyclosporine A (CSA; Neoral), 3 mg/kg body weight per day orally, for 6 months. Dose adjustments were made to maintain the cyclosporine level at 100–300 ng/ml. Then the treatment regimen was switched to tapering off low-dose prednisone together with azathioprine, and then azathioprine alone, 100 mg/day, was administered. The other 12 patients were treated with prednisolone, 1 mg/kg body weight for 3 months, then azathioprine, 100 mg/day, was added, prednisolone was gradually discontinued, and azathioprine alone was continued.

The following data were recorded: gender, age at first biopsy, age at the time of initiation of disease (the time of occurrence of the first symptoms ascribed to autoimmune hepatitis), and body mass index (BMI). Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum γ-globulin levels, and platelet count at the time of the first and second liver biopsies were also recorded.

Paired biopsy samples were reviewed by a pathologist blinded to their sequence and to the clinical outcome of patients. The biopsy specimens were stained with hematoxylin and eosin, Masson's trichrome, and reticulin and were scored using the modified hepatitis activity index (7). Necroinflammation was graded from 0 to 18. Fibrosis was staged from 0 to 6 (0, no fibrosis; 1–2, portal fibrotic expansion; 3–4, bridging fibrosis; 5–6, cirrhosis).

Before treatment, the fibrosis progression rate (FPR) was calculated by dividing the HAI fibrosis stage (each stage = 1 unit) on pretreatment biopsy by the estimated duration of disease in years. The FPR after treatment was calculated by dividing the difference in fibrosis stage between the two biopsies (before and after treatment) by the interval between the two biopsies in years.

Various clinical and laboratory factors (age, BMI, serum γ-globulin, AST, ALT, platelet count, AST-to-platelet ratio, and ALT-to-platelet ratio) were looked at for possible ability to predict liver fibrosis at presentation. AST-to-platelet ratio was calculated using the following equation: [(AST/upper limit of normal of AST)/platelet count ($\times 10^9$/L)] $\times$ 100 previously used for prediction of fibrosis in chronic hepatitis C (8). ALT-to-platelet ratio was calculated in a similar manner. Finally, we tested which variable was associated with absence of fibrosis (e.g., stage 0 or 1) after treatment. The following variables were assessed: serum γ-globulin level of less than 4 g/dL at baseline, grading score of more than 9 at the first liver biopsy, BMI of more than 27 (which was the mean value), gender, age at first biopsy (younger than 40 years), duration of treatment, and type of medication used for induction of remission.

Statistical Analysis. Data are reported as means and ranges. Correlation of quantitative variables with fibrosis stage at presentation was assessed by the Spearman correlation coefficient.

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY PATIENTS

Factor	
Age, mean ± SD	24.16 ± 14.78
Gender, males/females	5/14
BMI (kg/m^2), mean ± SD	25.45 ± 5.36
Pretreatment stage	4.53 ± 1.35
Pretreatment grade	10.21 ± 2.57
Serum ALT (U/L), mean ± SD	724.06 ± 519.76

Paired t-test was used to compare variables before and after treatment. Univariate analysis (χ^2 test and Mann–Whitney test) was used to assess the association of different variables with the absence of posttreatment fibrosis. Logistic regression analysis was then used to assess the independent association of various variables with the absence of fibrosis. P values less than 0.05 were considered statistically significant. Statistical analyses were performed using the SPSS, version 10.1, software package (SPSS, Inc., Chicago, IL).

RESULTS

A total of 19 patients with AIH (5 men and 14 women) were enrolled. Characteristics of the patients at baseline are listed in Table 1. Mean duration of treatment and mean interval between biopsies for all patients were 3.38 ± 2.28 years. Mean duration of treatment was 4.67 ± 2.13 years in the cyclosporine group and 2.63 ± 2.08 years in the remaining patients ($P = 0.06$).

Mean serum AST decreased from 689.18 to 42.29 U/L ($P < 0.001$); mean serum ALT decreased from 724.06 to 39.76 U/L ($P < 0.001$); and mean serum γ-globulin decreased from 4.34 to 1.46 g/dL ($P < 0.001$). Mean inflammation grade decreased from 10.21 to 4.11 ($P < 0.001$). Mean fibrosis stage decreased from 4.53 to 2.16 ($P < 0.001$). Mean FPR decreased from 5.87 to −0.72 U/year ($P < 0.001$). There was a trend for more rapid fibrosis regression after treatment in the cyclosporine group than in the prednisolone group (FPR of −1.16 vs −0.46 U/year, respectively; $P = 0.06$).

Fibrosis scores decreased by 2 to 6 points in 13 patients (70%), remained unchanged in 5 (25%), and worsened by 1 point in one (5%) patient. Fibrosis improved in all seven patients treated with CSA. Of the 13 patients in whom fibrosis decreased, 3 had a 2-point improvement, and 10 had a 3-point improvement or more. In four of the seven patients who had histologically confirmed cirrhosis at baseline, fibrosis scores decreased to 0 or 1 at the follow-up liver biopsy.

Mean interval between biopsies for patients in whom fibrosis regressed was 4.2 years, while for those in whom fibrosis did not regress this interval was 1.6 years ($P = 0.003$). Seven of 19 patients had interim liver biopsies. In all of them the stages of interim biopsy specimens were

TABLE 2. CORRELATION OF FACTORS WITH FIBROSIS STAGE AT PRESENTATION

Factor	*Correlation coefficient*	P *value*
AST	0.62	0.009
ALT	0.65	0.004
Plt	−0.63	0.005
AST-to-Plt ratio	0.64	0.017
ALT-to-Plt ratio	0.62	0.024
γ-Globulin	−0.06	0.83
AST/ALT ratio	0.09	0.74
Age	0.27	0.26

Note. AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; Plt, platelet count.

between the first and the last biopsies (stages of interim biopsies were lower than for the first corresponding biopsies and higher than for the last one).

Factors Associated with Advanced Fibrosis and Cirrhosis at Presentation. Fibrosis stage at presentation was significantly correlated with serum AST, serum ALT, platelet counts, AST-to-platelet ratio, and ALT-to-platelet ratio, but it was not associated with age, AST-to-ALT ratio, serum γ-globulin and BMI (Table 2). Serum AST, serum ALT, platelet count, AST-to-platelet ratio, and ALT-to-platelet ratio were plotted against stage of fibrosis in receiver operating characteristic (ROC) curves, and the cutoff point best predicting significant fibrosis (stage $\geq$3) and cirrhosis (stage $\geq$5) was determined (Tables 3 and 4). The best predictor of significant fibrosis was an ALT-to-platelet ratio $\geq$2.61 with an area under the curve of 100% and 100% positive and negative predictive values. ALT-to-platelet ratio $\geq$6.55 could predict cirrhosis with 100% sensitivity and specificity (Tables 3 and 4, Figure 1).

Relationship Between Inflammation and Fibrosis (HAI Grade and Stage). All 13 patients in whom fibrosis had regressed showed at least two grades of improvement in inflammation, while of the 6 patients with no change or worsening of liver fibrosis, 3 had at least a two-grade improvement in inflammation (100 vs 50%, respectively; $P = 0.02$). Mean decrement in inflammatory grade was 8 scores (range: 4–10) in patients in whom fibrosis improved and 2 scores (range: 0–4) in patients in whom fibrosis did not decrease after treatment ($P < 0.001$).

Factors Associated with Absence of Fibrosis After Treatment. On univariate analysis longer duration of treatment ($\geq$3 years) and use of CSA were associated with absence of fibrosis after treatment ($P = 0.03$ and 0.02, respectively). On logistic regression analysis only the use of CSA was associated with absence of fibrosis after treatment ($P = 0.045$; 95% CI, 1.05–136.70).

DISCUSSION

Our data clearly show that liver fibrosis regresses with treatment in AIH. In a recent report, Czaja *et al*. (3) showed that prednisolone monotherapy led to an improvement of liver fibrosis in AIH. In our series, AIH patients who received induction therapy with either prednisolone or CSA and maintained on azathioprine had regression of their liver fibrosis.

Improvement in fibrosis scores was typically parallel to reduction in inflammatory grading scores. This suggests that liver inflammation is an important trigger for liver fibrosis in AIH, and removing this inciting stimulus by immunosuppressive treatment may lead to regression of liver fibrosis. Sampling error is always a potential threat in comparing paired liver biopsy specimens, but the parallel improvement in clinical and biochemical variables is a reassurance that this is probably not the case in this instance. Additionally, all liver biopsy samples had at least four portal tracts and many of them had six or more portal tracts. This also reduces the probability of sampling errors.

We calculated the rate of fibrosis progression before and after treatment. Our patients had a very rapid rate of fibrosis progression before treatment. According to our data these patients would have become cirrhotic within less than 1.5 years of presentation if left untreated. This should be interpreted with caution, as it is always difficult to determine when the disease has started. This is because in many instances, appearance of symptoms is not the beginning of the disease and the actual hepatic fibro-inflammatory process may have started much earlier than the first symptom (9). However, our data suggest that patients with AIH who have high transaminase

TABLE 3. FACTORS PREDICTING SIGNIFICANT FIBROSIS (STAGE $\geq$3) AT PRESENTATION

Factor	*AUC*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
AST $\geq$257 U/L	0.97	93.30	100	100	66.60
ALT $\geq$264 U/L	0.93	93.30	100	100	66.60
Plt $\leq$313,500/μL	0.75	100	50	94.10	100
AST-to-Plt ratio $\geq$2.5	0.93	93.30	100	100	66.60
ALT-to-Plt ratio $\geq$2.61	1	100	100	100	100

Note. AUC, Area under ROC curves; PPV, positive predictive value; NPV, negative predictive value; AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; Plt, platelet counts.

TABLE 4. FACTORS PREDICTING CIRRHOSIS (STAGE ≥5) AT PRESENTATION

Factor	*AUC*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
AST ≥515 U/L	0.82	90.90	83.30	90.9	83.3
ALT ≥503 U/L	0.83	90.9	83.3	90.9	83.3
Plt ≤206,000/μL	0.95	90.90	85.70	90.9	85.7
AST-to-Plt ratio ≥5.76	0.89	100	83.30	91.60	100
ALT-to-Plt ratio ≥6.55	1	100	100	100	100

Note. AUC, area under ROC curves; PPV, positive predictive value; NPV, negative predictive value; AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; Plt, platelet counts.

and high γ-globulin levels should be treated promptly following diagnosis. Since even a few months of delay in the diagnosis or treatment after presentation can lead to rapid progression to advanced fibrosis and cirrhosis. Rate of fibrosis regression with treatment was also relatively high (−0.70 U/year), especially in the cyclosporine group (−1.16 U/year). This means that in the latter group, fibrosis regressed approximately one stage per year with treatment. Our data suggest that in AIH patients with advanced fibrosis, performing posttreatment liver biopsies to look for histologic remission is probably better postponed for years after clinical and biochemical remission.

We found that use of CSA and longer duration of treatment are associated with absence of fibrosis after treatment. On logistic regression analysis, only use of CSA was associated with absence of fibrosis after treatment, albeit with a rather wide confidence interval (due to our relatively small sample size). Some points should be kept in mind while interpreting these data: This beneficial effect may be due either to the longer duration of treatment in the cyclosporine group than in those treated with prednisolone (4.67 vs 2.63 years, respectively; $P = 0.06$) or to different mechanisms of immunosuppression of CSA and its possible antifibrotic effects (10, 11). Therefore, although this is encouraging information about the potential long-term benefits of induction of remission with CSA, it awaits confirmation in larger studies. Currently a head-to-head trial comparing CSA and prednisolone for induction of remission in AIH is ongoing at our center.

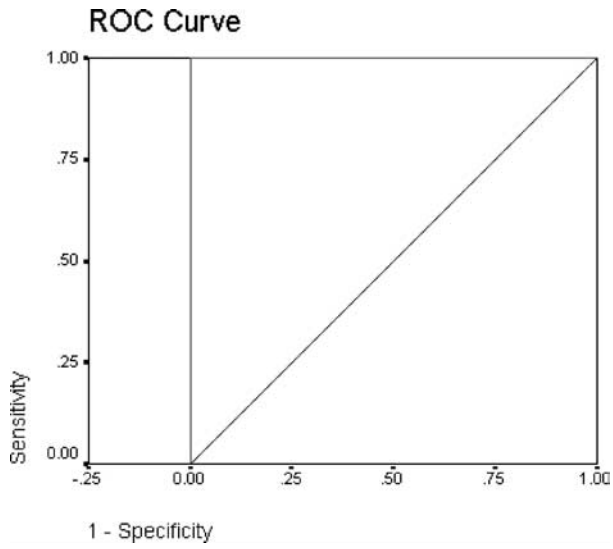

Fig 1. ROC curves of ALT-to-platelet ratio in the prediction of cirrhosis in the studied patients. An AUC of 1.0 is characteristic of an ideal test.

We also found that ALT-to-platelet ratio is a useful noninvasive marker of liver fibrosis in AIH. Since liver fibrosis is correlated significantly with an increase in ALT level and a decrease in platelet count, ALT-to-platelet ratio can amplify the difference in ALT and platelet values among patients with different stages of fibrosis (8). This awaits prospective confirmation in another set of patients with AIH. In addition, if the above index proves useful for prediction of fibrosis in posttreatment liver biopsies, it may be useful for follow-up of these patients and prediction of the time of posttreatment liver biopsy.

Another limitation of our study (and any other study using the modified HAI score) is that ordinal numerical scores are used to describe a continuous variable (i.e., fibrosis). When using this system, it should be kept in mind that progression from one stage to another does not necessarily represent an ordinal progression in matrix accumulation (12). An added limitation in any fibrosis reversibility study is the fact that with the Ishak staging system, once a biopsy is stage 6, the histology can only remain stable or improve. It cannot get worse. Hence, stability or regression is expectable in cirrhotic patients.

In conclusion, liver fibrosis in AIH clearly regresses after treatment; and a 6-month course of CSA for inducing remission in AIH may have beneficial antifibrotic effects. ALT-to-platelet ratio seems to be a good noninvasive marker of liver fibrosis in AIH. All these findings await further careful testing in future studies.

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