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• VIRAL HEPATITIS •

Is laparoscopy an advantage in the diagnosis of cirrhosis in chronic hepatitis C virus infection?

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Abstract

AIM: To evaluate the potential of laparoscopy in the diagnosis of cirrhosis and outcome of interferon treatment in HCV-infected patients.

METHODS: In this retrospective study, diagnostic laparoscopy with laparoscopic liver biopsy was performed in 72 consecutive patients with chronic HCV infection. The presence or absence of cirrhosis was analyzed macroscopically by laparoscopy and microscopically by liver biopsy specimens. Clinical and laboratory data and outcome of interferon-alfa treatment were compared between cirrhotic and noncirrhotic patients.

RESULTS: Laparoscopically, cirrhosis was seen in 29.2 % (21/72) and non-cirrhosis in 70.8 % (51/72) of patients. Cirrhotic patients were significantly older with a significant longer duration of HCV infection than noncirrhotic patients. Laboratory parameters (AST, y-GT, y-globulin fraction) were measured significantly higher as well as significantly lower (prothrombin index, platelet count) in cirrhotic patients than in non-cirrhotic patients. Histologically, cirrhosis was confirmed in 11.1 % (8/72) and non cirrhosis in 88.9 % (64/72). Patients with macroscopically confirmed cirrhosis (n=21) showed histologically cirrhosis in 38.1 % (8/21) and histologically non-cirrhosis in 61.9 % (13/21). In contrast, patients with macroscopically non-cirrhosis (n=51) showed histologically non cirrhosis in all cases (51/51). Thirty-nine of 72 patients were treated with interferon-alfa, resulting in 35.9 % (14/39) patients with sustained response and 64.1 % (25/39) with non response. Non-responders showed significantly more macroscopically cirrhosis than sustained responders. In contrast, there were no significant histological differences between non-responders and sustained responders.

CONCLUSION: Diagnostic laparoscopy is more accurate than liver biopsy in recognizing cirrhosis in patients with chronic HCV infection.Liver biopsy is the best way to assess inflammatory grade and fibrotic stage. The invasive marker for staging, prognosis and management, and treatment outcome of chronic HCV-infected patients need further research and clinical trials. Laparoscopy should be performed for recognition of cirrhosis if this parameter is found to be of prognostic and therapeutic relevance in patients with chronic HCV infection.

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INTRODUCTION

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease. Up to 85 % of HCV-infected patients develop chronic liver disease without elimination of the virus[1-3]. Chronic HCV infected patients develop cirrhosis in 7 % and 20 % after 20 and 40 years of infection[4], while symptoms and alarming biochemical markers appear late[5,6]. Patients with cirrhosis secondary to chronic HCV infection also have an increased risks for development of hepatocellular carcinoma (HCC), estimated to be between 1-4 % per year[7].

The diagnostic spectrum for chronic hepatitis C includes biochemical parameters, antibodies against HCV, qualitative and quantitative HCV RNA with genotyping, abdominal ultrasound and liver histology. Random core biopsy analysis can reveal information about the inflammatory grade and fibrotic stage of chronic HCV infection[8]. Diagnosis of compensated liver cirrhosis can be made with a high accuracy neither by percutaneous liver biopsy nor by ultrasound[9]. In comparison with percutaneous liver biopsy, laparoscopy allows macroscopic inspection of both liver lobes that might variate during progression of liver disease[10]. Percutaneous liver biopsy only allows the interpretation of a small biopsy. It has been reported that histological analysis fail with error ranges above 25 % in the diagnosis of cirrhosis in chronic liver disease[11,12], especially during the early phase of cirrhosis (Child A) and macronodular cirrhosis[13]. In a retrospective study, Poniachik *et al.* compared the presence of cirrhosis in 434 patients by liver biopsy and laparoscopy. Cirrhosis was seen laparoscopically in 169 patients and was confirmed in 115 patients histologically. In contrast, only 2 of 265 histologically confirmed cirrhotic livers were macroscopically without cirrhosis[14]. Ultrasound guided liver biopsy can result in a false negative histology due to the puncture of a regenerative nodule[15]. Cardi *et al.* showed superiority of laparoscopy over ultrasonography in diagnosis of widespread liver diseases[16]. Oberti *et al.* reported that only prothrombin index and serum hyalorunate were sensitive parameters for screening cirrhosis[17]. Early cirrhosis may often be missed due to clinical inappearance especially in patients with chronic HCV infection and accurate noninvasive markers of disease activity and fibrosis are not available[18]. The absence of early cirrhosis in chronic HCV infected patients might be a potential field for diagnostic laparoscopy that is not performed routinely and patients with early cirrhosis can be enrolled in the screening programs for HCC, too.

In the treatment of chronic HCV infection has proved beneficial interferon-alfa in the last two decades[19-23]. Old age, high level of viraemia, HCV genotype II (1b), long duration of disease, high levels of hepatic iron store, especially the advanced liver damage represented by dimension of fibrosis were considered to be negative predictive factors in the outcome of interferon treatment[22,24-34]. Two studies focused exclusively on interferon treatment in patients with HCV related cirrhosis[35,36]. After introduction of pegylated interferon given only once a week, HCV-infected patients with cirrhosis or bridging fibrosis were treated in a clinical trial[37]. These data implicated that HCV infected patients with liver cirrhosis need different therapeutic schedules with longer duration and higher dose of interferon.

Since cirrhosis is a negative predictor for antiviral therapy in chronic HCV-infected patients and liver histology might underestimate the frequency of cirrhosis, the aim of this retrospective study was to evaluate the potential of laparoscopy in the diagnosis of cirrhosis and outcome of interferon treatment in this particular group of patients.

MATERIALS AND METHODS

Patients

Laparoscopy and laparoscopic liver biopsy were performed in 72 chronic HCV-infected patients. Diagnosis of chronic HCV infection was based on elevated liver enzymes for at least 6 months, detection of anti-HCV antibodies in ELISA (2nd generation) and HCV RNA by RT/PCR. Patients with active viral coinfections (HBV, HIV, CMV, EBV), evidence for autoimmune hepatitis with positive serologic constellation of specific autoantibodies (ANA, AMA, anti-SLA, SMA, anti-LKM), alcohol and/or i.v. drug abusers and patients with signs of liver cirrhosis and/or hepatic decompensation (e.g. ascites, gastrointestinal bleeding, etc.) were excluded. Each patient was asked to sign a written informing consent for diagnostic laparoscopy and liver biospy.

Biochemical and serological analysis

Serum levels of ALT, AST, γ-GT, AP and CHE, as well as concentrations of serum bilirubin, prothrombin index, γ-globuline fraction and platelet count were measured by established routine methods before laparoscopy, during and after interferon treatment. Anti-HCV antibodies were analyzed using an ELISA (2nd generation, Ortho Diagnostic Systems, Raritan, NJ, USA) according to the instructions of the manufacturer.

Detection of HCV RNA and determination of HCV genotypes

According to Okamoto *et al.* and Simmonds *et al.*[38,39] determination of serum HCV RNA by a nested RT/PCR technique and determination of genotypes by a restriction enzyme analysis were carried out as described previously[40].

Laparoscopy

Abdominal ultrasound, electrocardiogram and, in patients older than 60 years, a chest X-ray were performed before laparoscopy. The evening before laparoscopy, each patient received 75 mg promethazin orally. Two hours before examination, 50 mg promethazin and 30 minutes before exploration 50 mg pethidin and 0.5 mg atropine were injected intramascularly. If necessary, patients received sedativa or analgetics during the laparoscopic intervention. During laparoscopy, each patient was given continuously an isotonic electrolyte solution intravenously. Patients were monitored by pulsoxymetry. After local anesthesia, the pneumoperitoneum was installed by puncturing at Monros' point with the Verres needle followed by insufflation of 2-3 L nitrous oxide. After insertion of the laparoscope in a trocar with a safety shield at Kalks' point, macroscopic exploration of liver, spleen and peritoneum followed. Liver biopsies were taken generally from an area on the anterior surface of the left lobe of the liver or of macroscopic suspect areas using a Menghini needle, at least 2 cm from the liver edge, containing at least five portal areas. Macroscopic diagnosis of cirrhosis was made based on the following criteria: (1) diffuse nodules on the liver surface, or (2) shallow nodules (i.e., nodules usually of large diameter, slightly protruding from the liver surface) if the liver was hard on palpation and rigid on lifting with a blunt probe and if clearcut features of portal hypertension were observed[41,42].

Histopathological evaluation

Formalin-fixed and paraffin-embedded liver biopsies were stained with hematoxylin-eosin, trichrome and by applying the Prussian blue reaction as described previously[43]. One pathologist examined samples unblindedly. A modified "Histology Activity Index" (HAI) on the basis of reported staging scores served to assess the stage of fibrosis[44-46]. Absent portal fibrosis was staged as fibrosis score I (HAI^b=0, no fibrosis), mild to moderate fibrosis as stage II (HAI^b=1, fibrotic portal expansion), marked fibrosis as stage III (HAI^b=3, bridging fibrosis) and complete fibrosis as cirrhosis (HAI^b=4).

Interferon treatment

Patients received recombinant interferon-alfa 2a (Roferon A, Hoffmann-La Roche, Basel, Switzerland) for initial treatment of chronic HCV infection. In our retrospective study, a dose of 6 MU three times a week was administered for 12 months according to Chemello *et al*[47]. Patients were classified as sustained responders if serum transaminases normalized (biochemical response) and serum HCV RNA became undetectable (virological response) during the treatment and at 6 months after the end of treatment (follow-up). During the follow-up, the re-emergence of both parameters after the end of treatment was defined as relapse. Patients with no improvement of biochemical or virological parameters within the first 3 months of treatment were classified as non-responders and treatment was stopped. For statistical analysis patients with relapse and non-response were classified as non-responders and compared with sustained responders. Patients who finished their course of treatment and 6 months of follow-up after the end of treatment were analysed.

Statistical analysis

Significant levels of differences between values were analyzed using the Chi-square test, Mann-Whitney test and student' s *t*-test as indicated.

RESULTS

Mean (M±SD) age of the 72 patients (49 % female, 51 % male) with chronic HCV infection was 46.8±12.1 years with a range of 27-72 years. Route and date of HCV infection were identified in 44 patients. The duration of HCV infection was 15.4±9.7 years and route of viral transmission was blood transfusion in 23 %, intravenous drug abuse in 18 % and anti-D prophylaxis in 3 %.

Laparoscopy found no severe complications. Liver biopsy caused mild bleeding in 6 (8 %) patients which was controlled during laparoscopy by local compression. None of the patients required blood transfusion. One patient developed a mesenteric emphysema after insufflation of nitrous oxide.

Cirrhosis was found by laparoscopy in 21/72 (29.2 %) patients and by histology in 8/72 (11.1 %) patients. According to HAI, patients with macroscopic cirrhosis by laparoscopy (n=21) showed histologically fibrosis stage I in 14.3 % (3/21), fibrosis stage II in 19 % (4/21), fibrosis stage III in 28.6 % (6/21) and cirrhosis in 38,1 % (8/21) (Table 1). Therefore 13/21 (61.9 %) macroscopic cirrhosis was not identified histologically. No cirrhosis was detected by laparoscopy in 51/72 (70.8 %) patients. According to HAI, patients without macroscopic cirrhosis (n=51) showed histologically fibrosis stage I in 58.8 % (30/51), fibrosis stage II in 31.4 % (16/51), fibrosis stage III in 9.8 % (5/51) and cirrhosis in 0 (0/51).

For statistical analysis, patients were divided into two groups. Group A represented patients with macroscopic cirrhosis (n=21) and group B patients without macroscopic cirrhosis (n=51) diagnosed by laparoscopic criteria. The mean (M±SD) age of patients in group A was 56.4±9.4 years, being significantly higher than in group B aged 41.7±11.2 years (P<0.02). There were 10 men and 11 women in group A and 27 men and 24 women in group B (n.s.). Mean (M±SD)

duration of chronic HCV infection in 44 patients of group A was 23.2±8.3 years, being significantly longer than in patients without macroscopic cirrhosis (group B) aged 10.4±5.7 years (P<0.02) (Table 2). In blood chemistry, patients of group A had significantly higher AST, γ-GT and γ-globulin fraction in serum electrophoresis as well as significant lower prothrombin index and platelet count than patients of group B. No significant differences were found for ALT, AP, bilirubin and cholinesterasis in both groups (Table 3). According to the Child-Pugh-Turcotte classification, 18 of the 21 patients with macroscopic cirrhosis were classified as Child A, 3 Child B and none Child C. In comparison of histological cirrhosis (8/21) and histologic non cirrhosis (13/21) of group A with macroscopic cirrhosis (n=21), significant differences were only found in AST and y-GT (Table 4). Differences were not significant in prothrombin index, y-globuline fraction and platelet count between cirrhosis (8/21) and histologic non-cirrhosis (13/21) of group A with macroscopic cirrhosis (n=21) (Table 4).

Table 1 Outcome of macroscopic laparoscopic exploration and histological analysis in 72 patients with chronic HCV infection

Histology (fibrosis stage score)	Laparoscopy	
	Patients without signs of cirrhosis (n=51)	Patients with signs of cirrhosis (n=21)
Fibrosis I	30	3
Fibrosis II	16	4
Fibrosis III	5	6
Cirrhosis	0	8

Absent portal fibrosis was judged as fibrosis score I (HAI[b]=0, no fibrosis), mild to moderate fibrosis as stage II (HAI[b]=1, fibros portal expansion), marked fibrosis as stage III (HAI[b]=3, bridging fibrosis), and complete fibrosis as cirrhosis (HAI[b]=4).

Table 2 Demographic and clinical data of patients with and without laparoscopically diagnosed cirrhosis

	Laparoscopy		P
	Patients with signs of Cirrhosis (n=21)	Patients without signs of Cirrhosis (n=51)	
Age (years)	56.36±9.42	41.7±11.2	<0.02[a]
Duration of disease (years) (n=44)	23.2±8.3	10.4±5.68	<0.02[a]
Sex ratio (m / f)	10 / 11	27 / 24	ns

[a]Student's t-test (unpaired); ns=not significant, years were showed as mean ± standard deviation. Duration of disease could be evaluated in 44 cases by patients' history.

Table 3 Laboratory data of patients with and without laparoscopic macroscopic evidence of cirrhosis

Laboratory data	Laparoscopy		P
	Patients with evidence of cirrhosis (n=21)	Patients without evidence of cirrhosis (n=51)	
AST (U / l)	59.7±54.2	29.3±19.6	< 0.02
ALT (U / l)	82.5±70.3	72.6±47.2	ns
γ-GT (U / l)	72.6±78.4	34.1±38.2	< 0.02
AP (U / l)	129.8±48.9	137.5±49.6	ns
Bilirubin (mg / dl)	1.1±0.93	1.86±6.23	ns
CHE (U / l)	5114±1331	5910±1001	ns
Prothrombin index (%)	93.8±12.6	102.2±6.3	<0.02
γ-globuline fraction (%)	19.9±6.5	13.7±2.9	<0.02
Platelet count (cell / µl)	172 940±65068	253 230±57096	<0.02

All data were showed as mean ± standard deviation. Statistical analysis was performed by applying Mann-Whitney test. ns=not significant.

Table 4 Comparing laboratory data of patients with macroscopic laparoscopic signs of cirrhosis

Laboratory data	Histological analysis		P
	No cirrhosis (n=13)	Cirrhosis (n=8)	
AST (U / l)	35.9±24.9	87.7±67.8	<0.02
ALT (U / l)	64.9±72.2	97.0±66.34	ns
γ-GT (U / l)	51.64±39.78	103.0±104.6	<0.02
AP (U / l)	126.3±54.6	144.6±39.6	ns
Bilirubin (mg / dl)	0.36±0.5	1.4±3.438	ns
CHE (U / l)	5129±1861	4685±1773	ns
Prothrombin index (%)	95.8±10.6	90.7±14.3	ns
γ-globuline fraction (%)	16.3±3.2	19.6±10.6	ns
Platelet count (cells / µl)	180 700±56 240	167 000±77 110	ns

All data were showed as mean ± standard deviation, statistical analysis were performed by Mann-Whitney test. ns=not significant.

Thirty-nine patients were treated with interferon-alfa 2a and followed up for 6 months after the end of treatment. HCV genotyping was performed in all 39 patients before start of therapy. HCV genotype II (1b) was the predominat genotype. The HCV genotypes of the 39 patients were 23 II (1b), 5 I (1a), 3 IV (2b), 5 V (3a) and 3 I (1a) combined with II (1b). Patients with macroscopic cirrhosis showed no significant difference of genotype distribution as compared with patients without cirrhosis. Interferon treatment resulted in 14/39 (35.9 %) sustained responders and 25/39 (64.1 %) non-responders including 2 relapsers. According to HCV genotype distribution, a significant higher rate of genotype II (1b) was observed in non-responders than sustained responders (P<0.02). Non-responders had a higher rate of macroscopic cirrhosis than sustained responders (P<0.02) (Table 5).

Table 5 Comparisons in pretreatment parameters between patients with sustained response and patients with non-response to interferon treatment (n=39)

	Sustained-responders	Non-responders	P
Number (%)	14 (35.9 %)	25 (64.1 %)	
Age (years)	50.6±13.9	47.2±10.6	ns
Genotype 1b (%)	5 (35.7 %)	18 (72 %)	<0.02[a]
Fibrosis staging score (%)			
I	7 (50.0 %)	9 (36.0 %)	
II	3 (21.4 %)	8 (32.0 %)	
III	3 (21.4 %)	3 (12.0 %)	
Cirrhosis	1 (7.0 %)	5 (20.0 %)	ns
Laparoscopic evidence of cirrhosis (%)	2 (14.2 %)	9 (36.0 %)	<0.02[a]

Data of age were showed as mean ± standard deviation; [a]Chi-square test, ns=not significant.

DISCUSSION

Diagnostic laparoscopy is recommended in the diagnosis of peritoneal diseases, evaluation of ascites of unknown origin, staging of abdominal cancer and chronic and focal liver disease[48]. Laparoscopy is commonly not performed for the diagnosis of cirrhosis in patients with chronic HCV. The availability of percutaneous liver biospy guided by ultrasound, CT- or MRI-scan offers selected biopsy of an suspicious area in the liver. The rate of laparoscopic liver biopsies in gastroenterology declined and also the number of training programs for this procedure[49]. However, all imaging procedures do not allow a direct viewing of the liver. The direct

visual inspection of the liver and the abdomen is the privilege of laparoscopy.

In this study, more chronic HCV infected patients had been diagnosed of cirrhosis macroscopically by laparoscopic inspection of the liver than histologically by using the Menghini needle for laparoscopic liver biopsy. All patients with histological cirrhosis also had cirrhosis macroscopically, but 13/21 patients with macroscopic cirrhosis had no cirrhosis histologically. This discrepancy implicates an underestimation of cirrhosis in chronic HCV-infected patients, if diagnosis of cirrhosis is based only on liver biopsy using the Menghini needle. Poniachik *et al.* investigated the role of laparoscopy and laparoscopic liver biopsy in the diagnosis of cirrhosis in 434 patients with chronic liver disease including 169 patients with laparoscopic evidence of cirrhosis. The histological sampling error was 32 % among patients documented to have cirrhosis by laparoscopy[14]. The selection of a suction or cutting needle for liver biopsy has a major impact at the stage of cirrhosis. The use of a cutting needle like Vim-Silverman or Tru-Cut is reported to give a more representive histology than suction needles like Menghini, Klatskin or Jamshidi, because fibrotic tissue tends to fragment with the use of suction needle[49,50]. In a randomized study on 1192 patients with diffuse liver disease, Colombo *et al* investigated percutaneous liver biopsies comparing the Tru-Cut and the Menghini needle. For diagnosing cirrhosis accuracy of the Tru-Cut needle is significantly better (89.5 %) than the Menghini needle (65.5 %). Complication rates were very low with both needles[51].

Comparing laparoscopy and laparoscopic biopsy for diagnosis of cirrhosis, laparoscopic diagnosis of macroscopic cirrhosis was of higher value than histological diagnosis of microscopic cirrhosis regarding blood chemistry and the response to interferon therapy. The rate of non-response to interferon therapy was 64.1 % in our study. Significant parameters for non-response were genotype II (1b) and laparoscopic evidence of cirrhosis, but not histological diagnosis of cirrhosis. Nowadays, new therapeutic regimes have replaced interferon monotherapy. The combination of interferon-alfa with ribavirin improved sustained response rate for chronic HCV-infected patients[52] and for relapsers and non-responders after initially interferon monotherapy[53-55]. Increased efficacy was shown with pegylated interferon as compared with standard interferons in cirrhotic and noncirrhotic patients with chronic hepatitis C[37,56,57] and currently standard therapy is pegylated interferon in combination with ribavirin[8]. In this treatment situation, the role of liver biopsy has increasingly been discussed[58]. If patients with positive predictors of virological response, such as low viral load and infection with genotype 2 or 3, can be treated and have very high chances of response, a biopsy that reveals mild histologic changes may do little to dissuade the clinician and patients from immediate treatment[8]. Liver biopsy may become recommended only in those patients whose pretreatment characteristics predict the lowest success rate[8]. Paired liver biopsy specimens enable staging of inflammation and fibrosis before and after treatment to define histological response in those patients. Compaired with our data, the rate of cirrhosis before treatment will be underestimated without performing laparoscopy in those patients. Both procedures, liver biopsy and laparoscopy, are invasive, but accurate noninvasive markers for staging disease activity, fibrosis and cirrhosis are not available[18]. Our rate of complications related to laparoscopy and laparoscopic liver biopsy was 10 % and no severe complication was observed. These data are in accordance with other reports[11,42,59-62]. The rate of severe complications using blind percutaneous liver biopsy is reported to be 0.3-1.5 % and is almost comparable with laparoscopic liver biopsy[41,63-67]. Diagnostic laparoscopy and laparoscopic liver biopsy are a safe and invasive procedure in patients with compensated liver disease. Minilaparoscopy has increasingly emerged as a less invasive diagnostic method in this field[68].

Based on our data, diagnostic laparoscopy is indicated for recognition of early cirrhosis in patients with chronic HCV infection. In fact, as early diagnosis of cirrhosis affects management of chronic HCV infected patients, it should be the key factor in the decision-making process.

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