Grading and staging of hepatic fibrosis, and its relationship with noninvasive diagnostic parameters

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AIM: To explore the grade and stage of pathology and the relationship between grading and staging of hepatic fibrosis and noninvasive diagnostic parameters.

METHODS: Inflammatory activity and fibrosis of consecutive liver biopsies from 200 patients with chronic liver disease were determined according to the Diagnostic Criteria of Chronic Hepatitis in China, 1995. A comparative analysis was made in these patients comparing serum markers, Doppler ultrasonography, CT and/or MR imaging with the findings of liver biopsy.

RESULTS: With increase of inflammatory activity, the degree of fibrosis also rose. There was a close correlation between liver fibrosis and inflammatory activity. AST, GGT, albumin/globulin, ALP, AFP, hyaluronic acid, N-terminal procollagen III (P III NP), collagen type IV (Col IV), tissue inhibitors of metalloproteinases-1 (TIMP-1), alpha-2-macroglobulin, natural killer cells (NK), some parameters of Doppler ultrasonography, CT and/or MR imaging were all related to the degree of inflammatory activity. GGT, albumin/globulin, ALP, AFP, hyaluronic acid, Col IV, TIMP-1, alpha-2-macroglobulin, transforming growth factor-beta 1 (TGF-β1), NK, some parameters of Doppler ultrasonography, CT and/or MR imaging were all related to the staging of fibrosis. By regression analysis, the parameters used in combination to differentiate the presence or absence of fibrosis were age, GGT, the parameter of blood flow of portal vein per minute, the maximum oblique diameter of right liver by ultrasound, the wavy hepatic surface contour by CT and/or MR. The sensitivity, specificity and accuracy of the above parameters were 80.36 %, 86.67 %, and 81.10 %, respectively.

CONCLUSION: There is close correlation between liver fibrosis and inflammatory activity. The grading and staging of liver fibrosis are related to serum markers, Doppler ultrasonography, CT and/or MR imaging. The combination of the above mentioned noninvasive parameters are quite sensitive and specific in the diagnosis of hepatic fibrosis.

INTRODUCTION: Hepatic fibrosis has been a common response to chronic liver injury and might result in potentially lethal sequelae[1-3]. In chronic liver diseases, determination of stage and activity of the fibrotic process and evaluation of anti-fibrotic treatment required accurate variables, the commonly so-called ‘fibrotic markers’[4-11]. Since the value of laboratory test to diagnose liver fibrosis was limited, biopsy has been still the golden criterion of the diagnosis of liver fibrosis and cirrhosis at present[12,13]. But it is an invasive diagnostic method, so its application and further propagation are somewhat limited. Searching for a noninvasive diagnostic approach is an interesting subject both at home and abroad. Although some parameters have been found to have important values in iconography and laboratory tests, they are still far from satisfactory. So it is of great reasonable value to explore a credible, specific, and noninvasive diagnostic parameter of liver fibrosis for the prevention and treatment of chronic liver disease[14-28]. Therefore, on the basis of histology of chronic liver diseases, this study was designed to explore the relationship between the grade and stage of pathology, and noninvasive diagnostic parameters. We hoped that we could provide the basis for the noninvasive diagnosis of liver fibrosis, so as to improve the prevention and treatment of liver fibrosis.

MATERIALS AND METHODS

Selection of patients

The study was organized and carried out by Shanghai Cooperative Group of Hepatic Fibrosis Project. The Cooperative Group was led by Renji Hospital and Changhai Hospital in Shanghai. Cases collected by the Cooperative Group were 37 from Changhai Hospital, 36 from Renji Hospital, 30 from Putuo District Central Hospital, 22 from Shanghai Liver Disease Center of Nanjing Military Command, 20 from Changzheng Hospital, 14 from Zhongshan Hospital.
Histological examination
One week after admission, all patients underwent liver puncture biopsy under the guide of B type ultrasound with the 14G Quick-cut needle (8-Light Company, Japan) or Menghini needle. The length of liver specimen was more than 1 cm. The samples were fixed with 10 % formaldehyde, paraffin slides were made and stained with hematoxylin-eosin, reticular fiber and collagen fiber according to the grading and staging of Diagnostic Criteria of Chronic Hepatitis in China in 1995[20]. Eleven patients were graded and staged for inflammatory activity and liver fibrosis. Three pathologists read the slide independently. The results were checked with Kappa test by statistical experts. It was shown that the coherence of grading and staging of hepatitis fibrosis was excellent. The pathological diagnosis of liver biopsy was finally made by the Department of Pathology, Medical College of Fudan University.

Laboratory tests
Blood and urine routine tests: α-fetoprotein(AFP) and prothrombin time were examined by the Cooperative Units.
Serum biochemical tests: Total bilirubin, indirect bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, γ-glutamyl transpeptidase(GGT), alkaline phosphatase(ALP), albumin, albumin/globulin, blood urea nitrogen(BUN), creatinine(Cr), triglyceride, cholesterol, high density lipoprotein and low density lipoprotein were all measured by Shanghai Institute of Digestive Disease.

Markers of hepatitis virus and immunological parameter:
HBsAg, Anti-HBs, HBeAg, Anti-HBe, Anti-HBC, HBV-DNA, Anti-HCV, HCV-RNA, CD3+, CD4+, CD8+, natural killer cell (NK), interleukin-2 (IL-2), and interferon-γ (IFN-γ) were detected by Shanghai Institute of Digestive Disease.

Related liver fibrosis markers: α-2-macroglobulin(α-MA), transferrin, apolipoproteina1, hyaluronic acid (HA), laminin, N-terminal procollagenIII(PNIIIP), 7S collagen IV (7S-IV), and transforming growth factor-β1(TGF-β1) were detected by the Clinical Immunology Center of Changzheng Hospital in Shanghai.

Tissue inhibitor of metalloproteinase-1(TIMP-1) were assayed by Shanghai Hongqiao Medical Reagent Institute.

B ultrasound examination
All B ultrasound examinations of the patients were carried out in Shanghai Institute of Digestive Disease. The patients had empty stomach for 14 hours before examination. Two skillful doctors performed the examination with color Doppler ultrasonic instrument(HDI 5000). The results were saved in compact disk, three experts judged the examination results and made the final reports.

CT and/or MR imaging
All CT and/or MR examinations were performed by Ruijin Hospital, Changzheng Hospital, Changhai Hospital, Zhongshan Hospital, and Shanghai No.6 Hospital in the Cooperative Group. CT scanners with PQ-2000 and/or PQ-5000 (Picker Company), Plus-s (Siemens Company), Hispeed Adv (GE Company), and MR scanners with Cyrosan T10-NT (Philips Company), Vision Plus and Magnetron Impact (Siemens Company) were used.

Statistical analysis
All the data were analyzed with SAS software by Statistical Department in Shanghai Second Medical University.

RESULTS
Histological examinations
It was revealed that there was a significantly positive correlation between the inflammatory activity and the staging of liver fibrosis. With increase of inflammatory activity, liver fibrosis became more serious (Table 1).

Table 1 Pathological diagnostic results of 200 liver biopsy samples

| Staging of fibrosis | Grading of inflammation | value |
|---------------------|-------------------------|-------|
|                     | 1                       | 2     | 3     | 4     | Total | χ²-value | P-value |
| 0                   | 18                      | 2     | 0     | 0     | 20    | 278.3    | 1E-04   |
| 1                   | 42                      | 42    | 0     | 0     | 64    |          |         |
| 2                   | 6                       | 33    | 26    | 0     | 64    |          |         |
| 3                   | 9                       | 2     | 19    | 4     | 25    |          |         |
| 4                   | 0                       | 0     | 3     | 23    | 26    |          |         |
| Total               | 66                      | 59    | 48    | 27    | 200   |          |         |

Laboratory examinations
Relationship between serum biochemical parameters and the grading of inflammation: Only serum biochemical parameters related to liver fibrosis are listed in Table 2.

Table 2 Relationship between serum biochemical parameters and grading of inflammation

| Parameter               | 1-2 | 1-3 | 1-4 | 2-3 | 2-4 | 3-4 |
|-------------------------|-----|-----|-----|-----|-----|-----|
| RBC                     | b   | b   | b   |     |     |     |
| PLT                     | b   | b   | b   |     |     |     |
| AST                     | a   | b   | b   | b   | b   | b   |
| GGT                     | b   | b   | b   |     |     |     |
| Albumin                 | a   | b   | b   | b   | b   | b   |
| Albumin/ globulin       | b   | b   | b   |     |     |     |
| ALP                     | b   | b   | b   |     |     |     |
| AFP                     | b   | b   | b   | b   | b   | b   |
| HA                      | b   | b   | b   | b   | b   | b   |
| PIINP                   | b   | b   |     |     |     |     |
| 7S-IV                   | a   | b   | a   |     |     |     |
| TIMP-1                  | a   | a   |     |     |     |     |
| α-MA                    | b   | a   | a   |     |     |     |
| IgG                     | b   | b   | b   |     |     |     |
| IgG+IgA+IgM             | b   | b   | b   |     |     |     |

Relationship between serum biochemical parameters and staging of liver fibrosis: Only the serum biochemical parameters related to liver fibrosis are listed in Table 3.

B ultrasound examinations
Comparison between parameters of ultrasonic 2-D and color Doppler flow image and the groups of inflammation grading: Only the parameters of ultrasonic 2-D and color Doppler flow image related with the groups of inflammation grading are listed in Table 4.

Table 3 Comparison between parameters of ultrasonic 2-D and color Doppler flow image related with the groups of inflammation grading

| Parameter | 1-2 | 1-3 | 1-4 | 2-3 | 2-4 | 3-4 |
|-----------|-----|-----|-----|-----|-----|-----|
| RBC       |     |     |     |     |     |     |
| PLT       |     |     |     |     |     |     |
| AST       |     |     |     |     |     |     |
| GGT       |     |     |     |     |     |     |
| Albumin   |     |     |     |     |     |     |
| Albumin/ globulin |     |     |     |     |     |     |
| ALP       |     |     |     |     |     |     |
| AFP       |     |     |     |     |     |     |
| HA        |     |     |     |     |     |     |
| PIINP     |     |     |     |     |     |     |
| 7S-IV     |     |     |     |     |     |     |
| TIMP-1    |     |     |     |     |     |     |
| α-MA      |     |     |     |     |     |     |
| IgG       |     |     |     |     |     |     |
| IgG+IgA+IgM |     |     |     |     |     |     |

Comparison between parameters of ultrasonic 2-D and color Doppler flow image related with the groups of inflammation grading: Only the parameters of ultrasonic 2-D and color Doppler flow image related with the groups of inflammation grading are listed in Table 4.
Comparison between parameters of ultrasonic 2-D and color Doppler flow image and groups of liver fibrosis staging: Only the parameters of ultrasonic 2-D and color Doppler flow image related with the groups of liver fibrosis staging are listed in Table 5.

CT and/or MR imaging examination
Among the 200 patients who received liver biopsy, 192 patients had CT and/or MR imaging examination. Twenty cases (10.4 %) received both CT and MR imaging examination, 92 cases (47.9 %) received only CT examination, 80 cases (41.7 %) received only MR examination.

Table 3  Relationship between serum biochemical parameters and staging of liver fibrosis

| Parameter            | 0-1 | 0-2 | 0-3 | 0-4 | 1-2 | 1-3 | 1-4 | 2-3 | 2-4 | 3-4 |
|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| RBC                  | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| GGT                  | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |
| Albumin              | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |
| Albumin/ globulin    | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |
| ALP                  | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |
| ALT                  | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| TIMP-1               | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| α-MA                 | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| TGFβ-1               | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| IgG                  | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |
| IgG+IgA+IgM          | b   | b   | b   | b   | b   | b   | a   | a   | a   | a   |

*<0.05, *<0.01.

Table 4  Comparison between parameters of ultrasonic 2-D and color Doppler flow image and groups of inflammation grading

| Parameter                          | 1-2 | 1-3 | 1-4 | 2-3 | 2-4 | 3-4 |
|------------------------------------|-----|-----|-----|-----|-----|-----|
| Inner diameter of left portal vein | a   | a   | a   | a   | a   | a   |
| Inner diameter of middle liver vein| a   | a   | a   | a   | a   | a   |
| Inner diameter of right liver vein | a   | a   | a   | a   | a   | a   |
| Thickness of gallbladder wall      | a   | b   | a   | b   | a   | a   |
| Shape of gallbladder               | a   | b   | a   | b   | a   | a   |
| Vertical diameter of spleen        | a   | b   | a   | a   | a   | a   |
| Thickness of spleen                | a   | b   | a   | a   | a   | a   |
| Diameter of spleen vein            | b   | a   | a   | a   | a   | a   |
| Thickness of the light dots in liver substance | b   | b   | b   | b   | b   | b   |
| Movement degree along with breath  | a   | a   | a   | a   | a   | a   |
| Movement degree along with heart beat| b   | b   | b   | b   | b   | b   |
| Blood stream velocity in constriction phase of liver artery | a   | a   | a   | a   | a   | a   |
| Blood stream velocity in dilation phase of liver artery | a   | a   | a   | a   | a   | a   |

*<0.05, *<0.01.

Table 5  Comparison between parameters of ultrasonic 2-D and color Doppler flow image and groups of liver fibrosis staging

| Parameter                          | 0-1 | 0-2 | 0-3 | 0-4 | 1-2 | 1-3 | 1-4 | 2-3 | 2-4 | 3-4 |
|------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thickness of liver capsule         | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Maximum oblique diameter of right liver | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Tube diameter of portal vein trunk| a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Inner diameter of left portal vein | a   | b   | b   | b   | a   | a   | a   | a   | a   | a   |
| Inner diameter of right portal vein| a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Thickness of gallbladder wall      | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| Shape of gallbladder               | b   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Diameter of splenic vein           | b   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Vertical diameter of spleen        | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| Thickness of spleen                | a   | b   | a   | a   | a   | a   | a   | a   | a   | a   |
| Thickening of the light dots in liver substance | a   | b   | b   | b   | b   | b   | b   | b   | b   | b   |
| Movement degree along with breath  | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |
| Movement degree along with heart beat| b   | b   | b   | b   | b   | b   | b   | b   | b   | b   |
| Parameter of blood flow of portal vein per minute | a   | a   | a   | a   | a   | a   | a   | a   | a   | a   |

*<0.05, *<0.01.
These methods could significantly improve the diagnosis and differential diagnosis of liver disease[17,18,20-26]. In this study, ultrasonography examination indicated that the thickness of liver capsule, maximum oblique diameter of right liver, tube diameter of portal vein trunk, diameter of left portal vein, diameter of right portal vein, thickness of gallbladder wall, thickness of spleen, diameter of splenic vein, parameter of blood stream quantity per minute in portal vein, light dot shape, and shape of gallbladder were correlated with the staging of liver fibrosis. CT and/or MR imaging only revealed that the volume of spleen was correlated with liver fibrosis. The results demonstrated that B ultrasound had more value than CT and/or MR imaging in the diagnosis of liver fibrosis. This of course needs further study. It should be noted that factors such as individual variation, nature of the instrument used, patient’s condition at the time of examination and difference of the performer’s skill might affect the evaluation of the result[20-26].

At the same time, we should carry out quantitative and/or semi-quantitative research on ultrasonic two-dimensional imaging and Doppler blood stream, so as to increase the sensitivity, specificity and accuracy of the diagnostic parameters.

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Edited by Xu JY and Wang XL