Morphological Classification of Liver Cirrhosis Based upon Measurement of Per Cent of Interstitial Tissue in Liver Biopsy Specimens

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Per cent of interstitial tissue was measured in liver biopsy specimens of 62 patients with liver cirrhosis. The frequency curve of the per cent of interstitial tissue showed two peaks in 10–19 per cent and in 40–49 per cent, a dell being observed at 30 per cent. Therefore, liver cirrhosis could be classified into two groups, i.e., the group with less than 30 per cent of interstitial tissue and the group with 30 per cent or more of interstitial tissue.

Edema, liver enlargement, abnormalities in thymol flocculation test, in cephalin cholesterol flocculation test, and in serum γ-globulin concentration, and deformity of the hepatic veins in hepatic venograms were all significantly more frequently observed in the group with 30 per cent or more of interstitial tissue than in the group with less than 30 per cent of interstitial tissue. There was significant correlation between the per cent of interstitial tissue and the per cent of intrahepatic shunt.

As liver cirrhosis is diagnosed most correctly by morphological examinations, the establishment of a morphological classification of liver cirrhosis has long been desired. Though various classifications have been presented by many investigators, such as the Fifth Pan-American Congress of Gastroenterology, Popper and Elias, Gall, Steiner, Nagayo, and Miyake, we always encounter difficulties in actual performance of such proposed classifications. It is because individual cases cannot always be found to accord with the typical pictures which are proposed in the above-mentioned classifications. Recently Meister et al. and Suwa et al. pointed out that the mean diameters of regenerative nodules, the main factor in most of the classifications, showed only one peak in the frequency curve, and that it was impossible to classify liver cirrhosis into two or more types according to the size of nodules. The above-mentioned classifications are deemed to be subjective rather than objective, and depend upon the opinions

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Furthermore, the previously published classifications were made in necropsy materials, and have not been shown to be applicable to biopsy specimens. As it is desirable to establish morphological classification in liver biopsy specimens in which only the histology of the liver is clinically studied, the present authors, deriving suggestions from Suwa's histometrical studies of liver cirrhosis in necropsy materials, performed histometrical studies on interstitial tissue in liver biopsy specimens, and investigated the possibility of morphological classification of liver cirrhosis in liver biopsy specimens. Contrary to the conclusions of Suwa et al., the studies showed interesting results leading to the conclusion that morphological classification is possible by histometrical studies of interstitial tissue in liver biopsy specimens. This communication describes briefly the method and the results obtained in 62 patients with liver cirrhosis.

MATERIALS AND METHODS

Sixty-two patients with liver cirrhosis admitted from January, 1953, through January, 1964, comprise the substance of this study. Cases complicated with primary liver cancer, those showing an initial stage of liver cirrhosis, and those in which only small biopsy specimens were obtained were excluded. Forty-eight cases were males, and 14 cases were females. The ages ranged from 18 to 72 years.

Liver biopsy was performed by means of Vim-Silverman needle. Liver biopsy specimens were stained by Mallory's azan method. Measurement was made, by means of micrometer, of the length of interstitial tissue on parallel straight lines which were 0.5 mm apart. The parallel straight lines were got by shifting a mechanical stage. Not only connective tissue fibers between the regenerative nodules but also those within the regenerative nodules were measured. The measured total length including both parenchymal and interstitial tissue was mostly 8–15 mm. The per cent of interstitial tissue \( P_i \) was calculated according to the following equation:

\[
P_i = \frac{\sum \lambda}{L} \times 100
\]

where \( \lambda \) = length of interstitial tissue on the parallel lines

\( L \) = measured total length of both parenchymal and interstitial tissue on the parallel lines.

The per cent of interstitial tissue thus calculated expresses the per cent of interstitial tissue to the total liver volume, if it is assumed that interstitial tissue is evenly distributed throughout the liver.

For comparison, all the liver specimens were classified according to Gall. Etiology of liver cirrhosis was inferred from the previous history. Cases with previous histories of presumable icteric hepatitis were assumed to be due to viral
hepatitis. Cases in which over 140 ml of alcohol was drunk daily were assumed to be due to alcoholism.

The hepatic blood flow was measured by the sulfobromophthalein method. The intrahepatic shunted blood flow was measured by the galactose method. The extrahepatic shunted blood flow was measured by the method of Nakamura et al. Hepatic venography was performed by the method previously reported by us.

For the purpose of comparison, the per cent of interstitial tissue was measured also in 34 cirrhotic livers which were necropsied at the Department of Pathology, Tohoku University.

RESULTS

**Frequency curve of the per cent of interstitial tissue.** The frequency curve of the per cent of interstitial tissue is as shown in Fig. 1. There were two peaks in 10–19 per cent and in 40–49 per cent, a dell being observed at 30 per cent. Therefore, liver cirrhosis could be classified into the group with less than 30 per cent of interstitial tissue and into the group with 30 per cent or more of interstitial tissue. Thirty-five patients belonged to the former, and 26 patients belonged to the latter. Between these groups there was no significant difference in sex and age.

**Relations to morphological classification of Gall.** When liver cirrhosis was morphologically classified according to Gall, postnecrotic liver cirrhosis showed a frequency curve of the per cent of interstitial tissue with a peak in 40–49 per cent,
and posthepatitic liver cirrhosis showed a frequency curve with a peak in 10–19 per cent (Fig. 1). Nutritional liver cirrhosis belonged to the group with less than 30 per cent of interstitial tissue. Unclassified liver cirrhosis was similar to posthepatitic cirrhosis; it showed a frequency curve with a peak in 10–19 per cent of interstitial tissue. Accordingly, among the two peaks observed in the frequency curve of the per cent of interstitial tissue of the total patients, the peak in 10–19 per cent coincided with the peaks observed in posthepatitic cirrhosis, in nutritional cirrhosis, and in unclassified cirrhosis, and the peak in 40–49 per cent

![Frequency curves of per cent of interstitial tissue related to etiology of liver cirrhosis.](image)

Fig. 2. Frequency curves of per cent of interstitial tissue related to etiology of liver cirrhosis. —— shows liver cirrhosis due to viral hepatitis, —— liver cirrhosis due to alcoholism, —— liver cirrhosis due to hepatitis and alcoholism, and —— liver cirrhosis of unknown etiology.

![Relationship between per cent of interstitial tissue and clinical signs.](image)

Fig. 3. Relationship between per cent of interstitial tissue and clinical signs. An asterisk (*) indicates statistically significant difference at 5 per cent level.
coincided with the peak observed in postnecrotic cirrhosis.

Relation to etiology of liver cirrhosis. Liver cirrhosis of unknown etiology showed a frequency curve of the per cent of interstitial tissue with two peaks similar to those observed in the total cases. Liver cirrhosis due to other etiology showed no distinct tendency because of the small number of cases (Fig. 2).

Relations to clinical signs and to laboratory tests. Among clinical signs, edema and liver enlargement were significantly more frequently observed in the group

![Graph](image-url)

**Fig. 4.** Frequency curves of per cent of interstitial tissue related to liver function tests. ——— shows bromsulphalein test, ———— cephalin cholesterol flocculation test, ———— thymol turbidity test, and ———— thymol flocculation test.

![Graph](image-url)

**Fig. 5.** Relationship between per cent of interstitial tissue and liver function tests. An asterisk (*) indicates statistically significant difference at 5 per cent level, and a double asterisk (**) significant difference at 1 per cent level.
with 30 per cent or more of interstitial tissue than in the group with less than 30 per cent of interstitial tissue (Fig. 3).

There was a tendency for the abnormality in liver function tests to increase as the per cent of interstitial tissue became higher (Fig. 4). Among liver function tests, thymol flocculation test and cephalin cholesterol flocculation test showed significantly higher incidence of abnormality in the group with 30 per cent or more of interstitial tissue than in the group with less than 30 per cent of interstitial tissue (Fig. 5).

The mean serum albumin concentration in the group with less than 30 per cent of interstitial tissue was 3.33 g/dl, and that in the group with 30 per cent or more of interstitial tissue was 3.32 g/dl. The difference was not statistically significant. Thirty-five per cent of the group with less than 30 per cent of interstitial tissue and 59 per cent of the group with 30 per cent or more of interstitial tissue had less than 3.0 g/dl of serum albumin concentration (Fig. 5). The difference was not statistically significant. The mean serum γ-globulin concentration in the group with less than 30 per cent of interstitial tissue was 3.31 g/dl, and that in the group with 30 per cent or more of interstitial tissue was 3.90 g/dl. The difference was statistically significant (P<0.01). Nineteen per cent of the group with less than 30 per cent of interstitial tissue and 45 per cent of the group with 30 per cent or more of interstitial tissue had more than 3.0 g/dl of serum γ-globulin concentration (Fig. 5). The difference was statistically significant (P<0.01).

Relation to hepatic circulation. The mean wedged hepatic venous pressure

![Graph showing relationship between per cent of interstitial tissue and hepatic circulation. A double asterisk (**) indicates statistically significant difference at 1 per cent level.](image-url)
of the group with less than 30 per cent of interstitial tissue was 211 mm H₂O, and that in the group with 30 per cent or more of interstitial tissue was 249 mm H₂O. The difference was not statistically significant. Twenty-seven per cent of the group with less than 30 per cent of interstitial tissue and 52 per cent of the group with 30 per cent or more of interstitial tissue had 250 mm H₂O or more of wedged hepatic venous pressure (Fig. 6). The difference was not statistically significant. The mean hepatic blood flow of the group with less than 30 per cent of interstitial tissue was 640 ml/min/m², and that of the group with 30 per cent or more of interstitial tissue was 545 ml/min/m². The difference was not statistically significant. Thirty per cent of the group with less than 30 per cent of interstitial tissue and 41 per cent of the group with 30 per cent or more of interstitial tissue had less than 500 ml/min/m² of hepatic blood flow (Fig. 6). The difference was not statistically significant. Thirty-nine per cent of the group with less than 30 per cent of interstitial tissue and 28 per cent of the group with 30 per cent or more of interstitial tissue had 20 per cent or more of extrahepatic shunt (Fig. 6). The difference was not statistically significant.

On the other hand, there was a close relationship between the per cent of interstitial tissue and the per cent of intrahepatic shunt (Figs. 6 and 7). The correlation ratio was +0.89, and was statistically significant (P<0.01). Seventeen per cent of the group with less than 30 per cent of interstitial tissue and 48 per cent of the group with 30 per cent or more of interstitial tissue had 40 per cent or more of intrahepatic shunt. The correlation ratio is +0.89, and is statistically significant (P<0.01).
of intrahepatic shunt (Fig. 6). The difference was statistically significant (P<0.01).

Deformity of the hepatic vein branches in the hepatic venograms was found in 33 per cent of the group with less than 30 per cent of interstitial tissue and in all of the group with 30 per cent or more of interstitial tissue (Fig. 6). The difference was statistically significant (P<0.01).

Relation to causes of deaths and prognosis. Among 62 patients, 14 have died so far; 7 from hepatic coma, and 7 from esophageal bleeding. The relation between the cause of death and the per cent of interstitial tissue is as shown in Table I. Though deaths due to esophageal bleeding appeared to occur more frequently in the group with less than 30 per cent of interstitial tissue, and deaths due to hepatic coma appeared to occur more frequently in the group with 30 per cent or more of interstitial tissue, there was no significant difference because of the small number of the deceased patients.

The five-year survival rate in the group with less than 30 per cent of interstitial tissue was 30 per cent, and that in the group of 30 per cent or more of interstitial tissue was 73 per cent (Fig. 8).

Comparison between biopsy and necropsy. Comparison of the per cent of interstitial tissue found by biopsy and by necropsy was possible in 6 patients.

Table I. Relationship between Per Cent of Interstitial Tissue and Causes of Deaths

<table>
<thead>
<tr>
<th>Causes of deaths</th>
<th>Group with less than 30 per cent of interstitial tissue</th>
<th>Group with 30 per cent or more of interstitial tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic coma</td>
<td>2 (20%)</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>Esophageal bleeding</td>
<td>5 (71%)</td>
<td>2 (29%)</td>
</tr>
</tbody>
</table>

Fig. 8. Survival rate related to per cent of interstitial tissue. ——— shows group with less than 30 per cent of interstitial tissue, and ———— group with 30 per cent or more of interstitial tissue.
Fig. 9. Comparison of per cent of interstitial tissue between biopsy and necropsy. The correlation coefficient is +0.64, but is not statistically significant because of the small number of the cases compared.

The correlation coefficient of the per cent of interstitial tissue between biopsy specimens and necropsy materials was +0.64, but was not statistically significant because of the small number of the cases compared (Fig. 9).

The frequency curve of the per cent of interstitial tissue in necropsy materials is as shown in Fig. 10. In contrast to the frequency curve of the per cent of interstitial tissue in biopsy specimens, it did not show a curve with two peaks. However, it was also impossible to conclude that the curve had only one peak.

Comparison of biopsy results as successively carried out in the same patients
during relatively short periods. In 3 patients, biopsy was successively carried out during less than 2 months, and the per cent of interstitial tissue was compared. As shown in Table II, there was a general accordance.

DISCUSSION

As clinicians, we always have a desire to classify liver cirrhosis morphologically in liver biopsy specimens in which only the histology of the liver can be clinically studied. However, when classification is made in liver biopsy specimens according to the previously published methods, many cases are usually found which are difficult to be classified. Though this is partly because of the small specimens obtained by liver biopsy, it is mainly based upon the fact that there is no established rational or objective method of classification of liver cirrhosis. Most previously published classifications were proposed by presenting two or more typical pictures by a combination of several factors, such as the size of regenerative nodules and the width of interstitial tissue. However, individual cases cannot always be found to accord with the typical pictures. It is easily understood when we consider that it is impossible to classify liver cirrhosis into two or three types by combination of two or more factors. Moreover, until now no one has investigated methods by which criteria rational classification is possible. Recently Suwa and his colleagues pointed out that a classification is possible when a distinct qualitative indicator is present, and that when a continuous quantity is introduced as an indicator for classification its frequency distribution must be separated into two or more well-defined parts with independent peaks. However, their studies showed that the largest axes of nodules, the total nodule volume in a unit volume of the liver, and the mean thickness of internodular septa did not reveal discontinuity in their frequency distribution, and a classification of liver cirrhosis based upon the difference of the above quantities was unsuccessful in necropsied materials. Therefore, it is natural that there have been many controversies in the classification of liver cirrhosis. The previously published classifications are deemed to be subjective rather than objective.
Histometrical studies are useful for obviation of the subjective errors. They have been introduced in the studies for classification of liver cirrhosis by Meister et al.\textsuperscript{7} and Suwa et al.,\textsuperscript{8} though they both concluded that the rational classification of liver cirrhosis was impossible from the measurement of diameters of nodules in necropsied cirrhotic livers. The present authors measured the per cent of interstitial tissue in liver biopsy specimens by a histometrical method, and were successful in classification of liver cirrhosis.

In the evaluation of the per cent of interstitial tissue, the first problem is whether the per cent of interstitial tissue which is measured in small biopsy specimens can represent the per cent of interstitial tissue in the total liver. In this respect, the fairly good accordance of the per cent of interstitial tissue between two liver biopsies which were successively carried out during short periods supports the reliability of liver biopsy specimens. Liver biopsy specimens seem to be clinically useful for the evaluation of interstitial tissue, though their limits must be considered. Parallelism was observed also between liver biopsy specimens and necropsy materials. The lower per cent of interstitial tissue in liver biopsy specimens as compared with that in necropsy materials may be due to the loss of a part of the fibrous tissue in performing biopsy procedures and to the progression of fibrosis after the performance of biopsy up to the time of death.

In evaluation of the frequency curve of the per cent of interstitial tissue, the small size of biopsy specimens offers no problem when many cases are examined. The frequency curve of the per cent of interstitial tissue in the total cases showed two peaks which also accorded with the peak of postnecrotic cirrhosis and the peak of posthepatitic cirrhosis. This clearly shows that liver cirrhosis can be classified by measuring the per cent of interstitial tissue in liver biopsy specimens into the group with less than 30 per cent of interstitial tissue and into the group with 30 per cent or more of interstitial tissue. That these two groups showed significant difference in clinical signs and laboratory examinations reveals the usefulness of this classification.

Unlike the frequency curve of the per cent of interstitial tissue in liver biopsy specimens, the frequency curve in necropsy materials did not clearly show two peaks. However, it did not have one peak, either. The difference between liver biopsy specimens and necropsy materials may be explained by the fact that random sampling is possible in liver biopsy while a sort of selection is inevitable in necropsy. The difference of method of measurement of interstitial tissue — i.e., fine connective fibers within the regenerative nodules were measured in the present investigation but were not measured in the study of Suwa et al. — may also contribute to the difference between the results of liver biopsy specimens in the present investigation and those of necropsy materials in the study of Suwa et al.

In the classification of liver cirrhosis proposed in the present investigation,
not only posthepatitic cirrhosis but also nutritional cirrhosis as defined by Gall are mostly classified into the group with less than 30 per cent of interstitial tissue. However, nutritional cirrhosis is relatively rarely found in Japan, and classification of liver cirrhosis into two groups seems to be justified.

The correlations of the per cent of interstitial tissue with clinical signs and with liver function tests are easily understood. But it is unexpected that liver enlargement which was reported to be well correlated with good prognosis was observed more frequently in the group with 30 per cent or more of interstitial tissue.

The significant correlation between the per cent of interstitial tissue and the per cent of intrahepatic shunt is in accord with the fact that the intrahepatic shunts in liver cirrhosis are morphologically found in connective tissue bands. It also suggests that fairly exact measurement is possible by the galactose method of the shunted blood flow in interstitial tissue, namely, the anatomical intrahepatic shunted blood flow.

The frequent occurrence of deaths from hepatic coma in the group with 30 per cent or more of interstitial tissue and that from esophageal bleeding in the group with less than 30 per cent of interstitial tissue is well explained by the close relationship between hepatic circulation and prognosis. Hepatic coma occurs more frequently in cases with higher per cent of intrahepatic shunt, and esophageal bleeding occurs more frequently in cases with higher per cent of extrahepatic shunt. As shown in the present investigation, higher per cent of intrahepatic shunt was observed in cases with higher per cent of interstitial tissue, and, though not so clearly shown, higher per cent of extrahepatic shunt appears to be more frequently seen in patients with less than 30 per cent of interstitial tissue. Therefore, the association between causes of deaths and the per cent of interstitial tissue can be understood.

References

4) Steiner, P.E. ibid., 1960, 37, 21.
