Comparison of intralobar non-parenchyma, subcapsular non-parenchyma, and liver capsule thickness

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SUMMARY  The intralobar and subcapsular areas of human liver were compared in terms of morphometry and morphology, and a correlation was made between the thickness of liver capsules and the amount of intralobar non-parenchyma in normal and diseased livers. Tissue was systematically sampled from 16 normal and 26 fibrotic livers taken at necropsy. The volume fraction of subcapsular and intralobar non-parenchyma and the capsule thickness were determined by morphometry on histological sections. The volume fraction of intralobar non-parenchyma not only correlated well ($\tau = 0.7$, $p < 0.001$) with but was also equivalent in each case to the actual volume fraction of subcapsular non-parenchyma. Morphologically, the intralobar and subcapsular areas were remarkably similar. The volume fraction of intralobar non-parenchyma also correlated well ($\tau = 0.5$, $p < 0.001$) with capsule thickness.

These results show that pathological changes in liver architecture were essentially similar in the intralobar and subcapsular areas and that capsule thickness reflected intralobar non-parenchymal changes.

Previous morphometric analyses of fibrosis in rat livers have shown that not only is total liver capsule volume remarkably and significantly increased from 0.27% to 1.4% of total liver volume in cirrhosis but that there is good correlation (correlation coefficient $= 0.87$) between histologically measured capsule thickness and the degree of fibrosis. Although capsule thickening in liver disease is known to occur, the correlation of capsule thickness with overall liver structure has not been studied. The continuity and ramification of the capsular stroma into the portal tracts and into the connective tissue of the hepatic veins makes such a correlation reasonable, but many pathologists are concerned about the use of a subcapsular sample for histological assessment of liver changes because non-parenchyma may be overly represented compared with the rest of the liver. Although the concern is often mollified, there is a need for a quantitative comparison of the amount of non-parenchyma in the intralobar and subcapsular areas. We report here the results of a study in which we made this comparison as well as measuring capsule thickness in human liver.

Material and methods

Liver tissue was sampled during consecutive necropsies at two medical centres except for cases with gross liver disease with neoplasia. A total of 42 livers was sampled from 35 men and seven women. The average (SD) age was 62 (15) years and average liver weight was 1600 (600) g. There were 16 normal and 26 fibrotic (14 cirrhotic) livers.

The right lobe was sliced parallel to the visceral surface (usually two slices) and fixed in a buffered 4% formaldehyde solution. The right lobe was chosen because it is the lobe that is usually sampled by needle biopsy. Three pieces of tissue measuring $2 \times 5 \times 10$ mm deep (from and perpendicular to the liver surface) were taken from each slice. The pieces were sampled at roughly 120° intervals around each cut surface, with the sampling at one slice offset 60° from the other slice. Areas of the coronary and falciform ligaments as well as adhesions were avoided. Three corresponding and similarly sized pieces of intralobar tissue (at least 2 cm away from the capsule or hilar area) were taken from a cut surface of each slice. The pieces of tissue were embedded in paraffin wax, sectioned perpendicular to the liver surface, and stained with a “liver trichome stain” (HISTO-LOGIC) for morphological and morphometric analysis.
Liver non-parenchyma and capsule

The volume fractions ($V_v$) of subcapsular and intralobar non-parenchyma for each liver were estimated for each case by point counting. A light microscope (at a magnification of 63 times) and with a camera lucida attachment was used to project a $4 \times 4$ square lattice grid on to the field of view, which covered a 3 mm diameter area of tissue. The 16 grid intersections were spaced at 0.4 mm intervals and were used to count points on parenchyma and non-parenchyma (segmental blood vessels and bile duct lumens with diameters larger than one third of the lattice spacing were not counted). For the subcapsular area, three consecutive fields, which started immediately beneath the capsule and extended perpendicularly to a depth of about 1 cm, were measured in a section from each of three randomly chosen blocks. For the intralobar area, three consecutive fields starting conventionally with the left lower corner of the section were similarly measured for each of three randomly chosen blocks.

Capillary thickness was defined as the perpendicular distance between the liver surface and lobular or nodular parenchyma. Areas of local extra-capsular fibrosis like adhesions were not included in the measurements. In the case of nodular parenchyma the distance was measured from the liver surface to a line drawn more or less tangential to and successively from the outermost aspects of each nodule in the particular section. A light microscope (at a magnification of 63 times) with a camera lucida attachment over an x-y digitising tablet was used to project an image of four parallel lines from a drawing placed on the tablet on to the field of view in the microscope (line spacing at 0.4 mm). The lines were randomly oriented perpendicular to the capsular surface; capsule thickness was measured along each line on the digitising tablet with a light-emitting diode cursor connected to a microcomputer (BioQuant II, R&M Biometrics, Nashville, Tennessee, USA). Sections from each of three randomly chosen blocks were measured for each case.

The Non-parametric Statistics Program of BMDP Statistical Software (Los Angeles, California, USA) was used to find the Kendall's coefficient of rank correlation between variables.

Results

The volume fraction of intralobar non-parenchyma correlated well ($\tau = 0.7$, $p < 0.001$) with the volume fraction of subcapsular non-parenchyma. Moreover, there was little, if any, difference in the actual volume fraction between the subcapsular and intralobar areas in each case; this equality was apparent from the distribution of the data points for each case around a line with a slope (1) (fig 1).

![Fig 1] Scatter plot of volume fractions ($V_v$) of subcapsular versus intralobar non-parenchyma for each of the 42 cases studied. The line indicates a slope of 1, which represents a one-to-one correspondence between the intralobar and subcapsular values.

![Fig 2] Subcapsular area of a non-cirrhotic liver shows apparent nodules (N) immediately beneath the liver capsule. Most of the subcapsular area, however, is without nodules. ("Liver trichrome stain".)
In accord with the morphometric data, histological examination showed no apparent differences in the amount of fibrosis and in the prevalence of nodules between intralobar and subcapsular areas. The only potential source of error in cases of fibrosis without cirrhosis was the occasional small area of parenchyma that was immediately beneath the capsule and walled off by non-parenchymatous tracts (fig 2), and which gave the impression of nodulation. This resembled intralobar nodules adjacent to major portal tracts (fig 3). In these cases the greater part of the intralobar and subcapsular samples were clearly without nodules, and consequently the livers were evidently not cirrhotic.

Capsule thickness was also correlated well \((r = 0.5, p < 0.001)\) with the volume fraction of intralobar non-parenchyma (fig 4). Morphological details of the capsules tended to show that the major thickening was
is representative of the rest of the liver. We have also shown that in man there is a significant correlation between capsule thickness and the amount of intralobar non-parenchyma, a correlation that was first shown in our studies on animals. Our observation that capsule thickening is mainly attributable to the proliferation of portal tract components conforms to a picture of normal liver anatomy, which shows that there is ramification and continuity of capsular stroma adjacent to hepatic veins and portal tracts.

These data justify the use of subcapsular samples of liver. Even the occasional impression of nodulation arising from small areas of parenchyma, which are immediately beneath the capsule and walled off by fibrosis, can be easily excluded as cirrhotic nodules when the overall subcapsular samples (which can be less than 1 cm deep) are examined. Given that occasional intralobar nodules can be found adjacent to major portal tracts, we are convinced that both quantitatively and qualitatively there are no essential differences between the subcapsular and intralobar areas of the liver in man.

We considered the possible effect that peritonitis may have had on capsule thickness. Among our cases there were five (four with and one without cirrhosis) that had confirmed peritonitis. Retrospective examination of the data showed that the capsule thicknesses in those livers were no greater than in livers with comparable intralobar fibrosis.

For the purpose of measuring the thickness of the liver capsule, we defined it as the non-parenchymatous area between the liver surface and the hepatocellular parenchyma. There was invariably a layer of dense, collagenous connective tissue and, at times, there was also a layer just below composed of portal tract elements. The thickness of this layer changed in relation to the amount of intralobar non-parenchyma.

Although some may argue that the "true" capsule is represented only by the invariable, outer connective tissue layer, we consider both layers to comprise the capsule, not only by definition in this study, but because of the embryological and anatomical continuity between Glisson's capsule and the portal tracts. Our data conform to the concept that the amount of diffuse portal expansion like that seen in pre-cirrhotic conditions may be estimated from the amount of capsular thickening. With the rapid and substantial improvements in non-invasive imaging techniques like ultrasonography, a technique for assessing liver non-parenchyma by detecting capsule thickness may eventually be accepted.

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References


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