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Quantitative Morphometric Analysis of Liver Biopsy: Problems and Perspectives

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1. Introduction

The liver biopsy has long been the gold standard for the evaluation of the state of liver diseases in patients, especially in chronic hepatitis. Hepatic fibrosis plays the most important role in the evolutionary process from chronic hepatitis to cirrhosis. Therefore, accurate assessment of the degree of hepatic fibrosis in chronic viral hepatitis is important to understand not only the clinical condition and prognosis of patients, but also the natural history of hepatitis. Information on a stage of chronic viral hepatitis C is essential to make prognosis and decide antiviral treatment. Semi quantitative scoring systems have been used in most studies that have relied upon liver biopsy to evaluate changes in fibrosis (Knodell et al., 1981, Desmet et al., 1994, Chevallier et al., 1994, French METAVIR, 1994, Ishak et al. 1995). Comparative characteristic of different scoring systems is presented by Brunt (2000) and Goodman (2007). However, these methods cannot completely avoid the observer's bias. Recent studies have reported that the estimation of fibrosis by semi quantitative scoring system is not always accurate and high rate of inter- and intraobsrever discrepancies takes place (Scheurer, 2003). The alternative to semi quantitative fibrosis scores is direct measurement of the amount of fibrosis, or necroinflammatory lesions, or portal zones in the biopsy by computer-assisted morphometric image analysis (O'Brien et al., 2000), or stereological morphometric analysis (Filimonova et al., 2010). Cell population analysis gives additional information about structure of parenchyma elements (liver plates and sinusoids).

2. Patients and methods

2.1 Patients

The different groups of patients with chronic viral hepatitis C (HCV) and chronic viral hepatitis B (HBV) were investigated. The patients with weak, moderate and expressed degree of fibrosis according to Ishak (Ishak et al., 1995) and METAVIR group classification (French METAVIR, 1994) participated. The diagnosis of chronic HCV or chronic HBV was established after careful examination of patients: the anamneses of diseases and life, laboratory analyses, virological and morphological studies. Serum level of ALT was expressed. The upper limit of normal (ULN) was 41 U/L for men and 31 U/L for women. The classification of chronic liver diseases, accepted by the International Congress of Gastroenterology (Los Angeles, 1994), was used during the formulation of the diagnosis.

2.2 Histological evaluation

All liver biopsies were performed according to the routine medical follow up program, using the standard Menghini procedure (Menghini, 1970, Mengini et al., 1975). Samples were formalin-fixed and paraffin-embedded. Serial paraffin sections were cut at 5 mcm. Criteria for adequacy of the biopsy specimens included a core length of 10 mm and at least 5-6 portal tracts. Hematoxylin-eosin stain was used. Each biopsy for necro-inflammatory activity and fibrosis was assessed by two hepatologists. For each biopsy specimen, a numerical score was established, both for the grading of necroinflammatory activity and to determine the stage of fibrosis. Knodel Histology Activity Index (HAI) was used to grade histopathological lesions (Knodell et al., 1981). HAI was graded according to 3 components: periportal inflammation with or without bridging necrosis (scale, 0-10), intralobular degeneration and focal necrosis (scale, 0-4), and portal inflammation (scale, 0-4). In accordance with the previously cited studies, the intensity of HAI was scaled as follows: A0 denoted no histological activity; A1, minimal activity (scale units, 1-3); A2, mild activity (scale units, 4-8); moderate activity (scale units, 9-12); and A4, severe activity (scale units, > 12). METAVIR group scoring system was used for detecting the stage of fibrosis (French METAVIR, 1994). Fibrosis was staged on a scale from F0 to F4, as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis. None of the included patients showed any signs of cirrhosis.

2.3 Computer digital analysis

Fibrosis ratio for each liver biopsy specimen was calculated using an image analysis system consisting of a microscope (Leica DM 2500) with attached digital camera (Leica DFC 320 R2) and a computer. Serial pictures of biopsy slices of patients with HBV were photographed by light microscope and saved electronically. The further process was performed with the computer program Adobe Photoshop CS 4. Image was converted into a binary image. The two-dimensional patterns were measured by direct pixel counting on the binary images under a simultaneous visual control of light microscopy. The total area of serial sections was the sum of the area of all microscopic fields including parenchyma and non-parenchymal areas. Fibrosis in the space of Disse was not calculated. We considered that non-parenchymal elements included the sum of portal areas and intralobular infiltrates and necroses.

2.4 Stereometric analysis

The stereometric analysis is based on the determination methods of the specific volumes of different structure (Hamilton and Allen, 1995). Samples of biopsy from the patients with HBV were investigated. The calculation was carried out using the standard graticule (400 squares). Morphometry was applicated on counting the points or intersections in the field of microscope at the magnification of x400. The field of vision of microscope at the magnification of x400 as a standard unit was accepted. In each field of sight a quantity of intersections of non-parenchymal liver structure – portal tracts, with nearby piecemeal and bridging necroses, venous vessels and intralobular necroses – were calculated. Other liver structures were studied together with parenchyma (liver plates and sinusoids). The measurement of portions (percentage) of portal areas, foci of intralobular necroses and vessels was estimated. The total area of the sections was the sum of the area of all

microscopic fields including parenchyma and non-parenchymal elements. Fibrosis in the space of Disse was not evaluated.

2.5 Cell population analysis

The comparative investigation was performed at the samples of biopsy from the patients with both chronic hepatitis, HCV and HBV. Proportion between the portion of the area occupated with liver plates and intralobular sinusoids was detected by stereometric morphometric analysis. The number of lytic necroses of hepatocytes, binucleate hepatocytes and polymorphous hepatocytes with large nuclei was calculated in composition of liver plates. The number of Kupffer cells and endoteliocytes was determined in composition of sinusoids. Calculation was performed in the standard field of vision of microscope at the magnification x400 in region of middle zone of liver lobule. Twenty standard fields of vision were investigated for each biopsy.

2.6 Statistical analysis

Variables, differing significantly according to the quantity of non-parencymal elements in the estimation group were identified by univariate analysis. Therefore, all variates were included in a multivariate forward stepwise regression analysis to determine the morphometric data. Pearson and Spearman correlation coefficients were used to evaluate whether changes in morphometric data were correlated with ALT. Student and Satterwhite criterions, Pearson and Spearman correlation coefficients were used to evaluate the structure of population. Statistical analysis was performed by tabulated processor Microsoft Excel 2003 and STATISTIKA 9.0.

3. Results

3.1 Quantitative image analysis for evaluation of pathological changes in liver structure

A biopsy is an important key factor for the prediction and tactics of treatment of patients with chronic viral hepatitis (Friedman, 2003, Friedman et al., 2007). Numerical fibrosis scoring systems (Knodell et al., 1981, French METAVIR, 1994, Ishak et al., 1995, Brunt, 2000) are usually used to assess the degree of activity and fibrosis stage. These systems are semi quantitative: the assessment of inflammatory, degenerative and fibrotic changes is determined in scale units. Many investigators recognize that there may be purely subjective error in numeral fibrosis scoring. Thus, the variability of fibrosis staged is estimated at 20% (Friedman, 2003). It is believed that the more indicators are presented in a given system, the more misunderstandings can occur.

Consequently, the assessment activity and the stage of process were usually performed by two independent experts. However, correct assessment of the degree of liver damage at the time of initial biopsy is extremely important for a treating physician. Thus, it effects the choice of treatment (antiviral or symptomatic treatment) and the ability to forecast progression of fibrosis.

Recently the computer morphometry has been used to assess the stage of fibrosis (Manabe et al., 1993; Chevallier et al., 1994; Kage et al., 1997; Pilette et al., 1998; Duchatelle et al., 1998; Masserolli et al., 2000; O'Brien et al., 2000). Chevallier et al. (1994) compared the computerized fibrosis ratio with a detailed subjective scoring system. That incorporated

evaluation of central veins, pericellular fibrosis, portal tracts, and the number and width of septa in patients with chronic liver diseases. Duchatelle et al. (1998) used computerized image analysis to measure liver fibrosis in groups of patients with chronic hepatitis C treated with interferon alfa. Significant correlation was observed between evaluations of fibrosis degree using two methods: a semi quantitative method with a staging scoring system and a computed image system (Kage et al., 1997).

Pilette et al. (1998) compared fibrosis ratios and subjective scores (METAVIR) in series of patients with chronic liver disease in a study of the validity of serum markers of fibrosis. The area of fibrosis, as determined by image analysis and the semi quantitative score was well correlated. However, for serum markers the correlation was higher with the area of fibrosis than with the semi quantitative score. Authors supposed that such characteristics as reproducibility, rapidity, simplicity, adaptability, and exhaustiveness also favored image analysis.

O'Brien et al. (2000) found an overall statistically significant correlation between fibrosis ratio and ordinal score, but subset analysis showed that this correlation was restricted to biopsy specimens with high scores (3-6, early bridging fibrosis to established cirrhosis). The fibrosis ratio was the total area of fibrosis divided by the total area of the section. Authors found that the fibrosis ratio of liver biopsy specimens calculated by digital image analysis was not always reflecting fibrosis in chronic hepatitis as indicated by subjective scoring classifications. This applied particularly to livers that were normal or in early fibrosis (stage 0-2).

Masserolli et al. (2000) described the design and validation of an original image analysis-based application, FibroQuant, for automatically and rapidly quantifying perisinusoidal, perivenular and portalperiportal and septal fibrosis in liver histological specimens. Sometime morphometric image analysis was used for evaluation of fibrosis progression (Goodman et al., 2007, 2009). Morphometry demonstrated complex, non-linear changes in fibrosis over time in heterogenous cohort of patients with interferon-refractory chronic hepatitis C (Goodman et al., 2009).

The purpose of this study was to explore the possibilities of using computer morphometry for quantitative assessment of histological parameters biopsies of patients with chronic hepatitis B. The group of patients with minimal, mild, moderate and severe activity, but without cirrhosis was studied. Two major indicators were selected for evaluation: the portal area or its fragments and intralobular infiltrates.

According to portal areas we calculated next variants:

- portal area without changes;
- expansion of portal area without damages of limiting plate;
- portal area with damage of limiting plate and development of short septa;
- portal area with damage of limiting plate and development of piecemeal necroses;
- portal area with marked bridging necroses;
- portal area with extensive fibrosis changes;
- portal area with extensive lymphoid infiltration;
- occasional fibrosis septa.

Intralobular infiltrates were divided into 2 groups:

- lymphohistiocyte infiltrates;
- lymphohistiocyte infiltrates with damaged hepatocytes.

So, our calculation included the evaluation of not only fibrosis changes, but also inflammatory area in portal and intralobular zones.

Different morphological pictures of samples of biopsy from patients with chronic viral hepatitis B are presented at Figures 1 – 4.

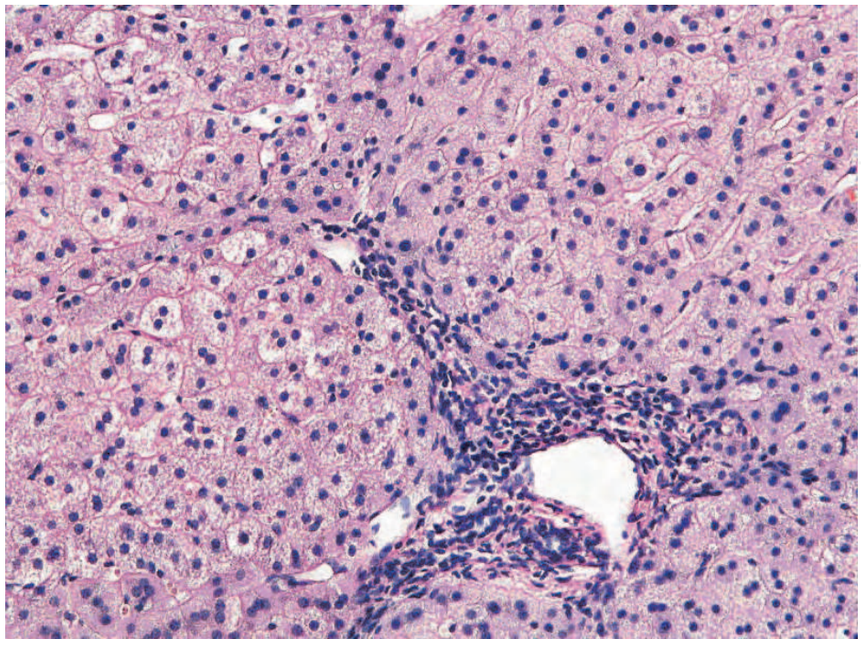

Fig. 1. Section of the liver biopsy specimen of patient with chronic hepatitis B. Portal area with damage of limiting plate and development of short septa. Hematoxylin-eosin. Obj.x20

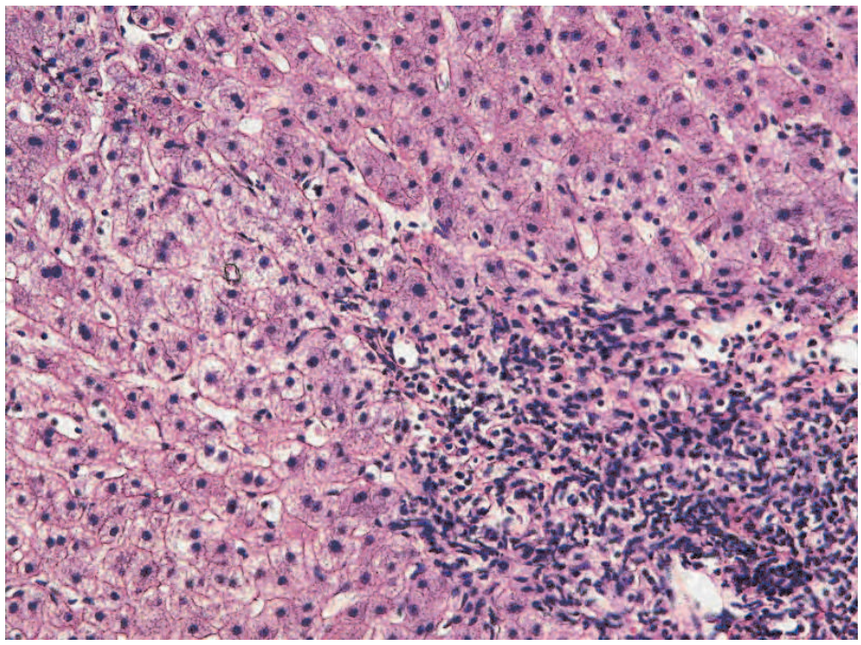

Fig. 2. Section of the liver biopsy specimen of patient with chronic hepatitis B. Portal tract with damage of limiting plate and development of piecemeal necroses (interface hepatitis). Hematoxylin-eosin. Obj.x20

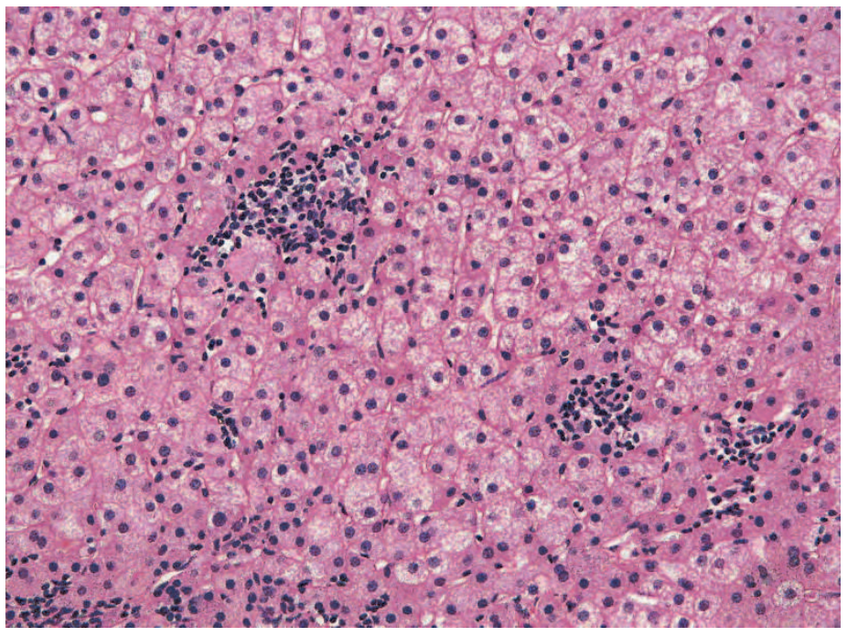

Fig. 3. Section of the liver biopsy specimen of a patient with chronic hepatitis B. Focal intralobular necroses in the middle zone of liver lobule. Hematoxylin-eosin. Obj.x20

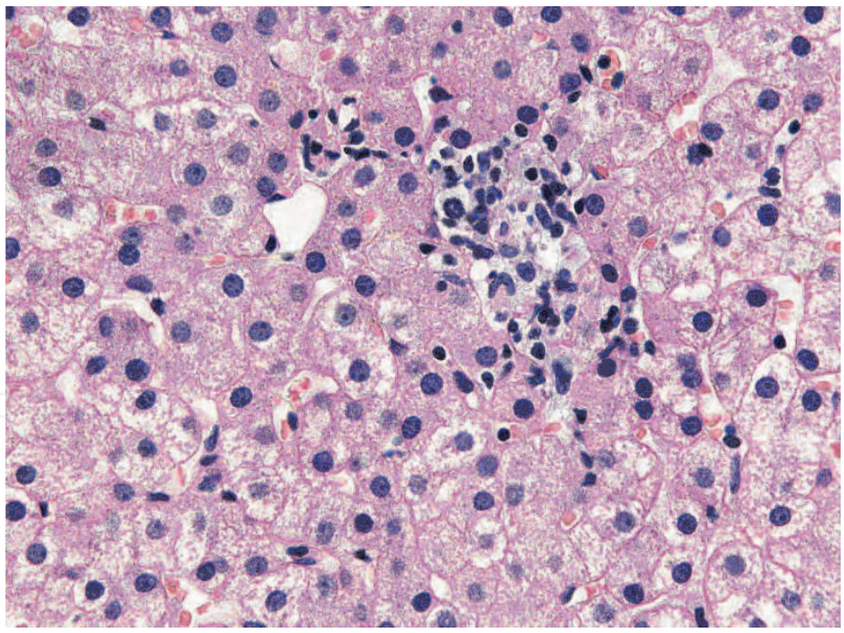

Fig. 4. Section of the liver biopsy specimen of a patient with chronic hepatitis B. Intralobular necrosis of hepatocytes with participation of lymphoid infiltration. Hematoxylin-eosin. Obj.x40

The special panoramic application of biopsy samples was performed by Photoshop CS4 program for computer analysis (Figure 5). This program allows you to connect serial micrographs of biopsy and get a computer image of the whole biopsy.

The program gives the possibility to unite serial micrographs of the single biopsy sections and create computer image of the total biopsy. The pixel count performs on the total square of the biopsy after the computer montage of individual micrographs. The squares of portal zones and intralobular infiltrates in pixels were determined separately, after that the special parts (percentage) of these components were counted. Further, after identification the areas of portal zones and intralobular infiltrates are calculated in pixels and transformed into percentage. The data obtained are summarized in Table 1.

Fig. 5. General picture of liver biopsy composed by computer microscopy (Obj.x20) using Adobe Photoshop CS4. This picture contains 142 standard computer microphotographs of biopsy. Total area of biopsy is 327342640 in pixels

Biopsy number	Total area of biopsy (in pixels)	Total area of parenchyma, %	Total area of non-parenchymal elements, %	Total area of portal zones and septa, %	Total area of intralobular necroses, %
1	126077500	99.05	0.95	0.65	0.3
2	151379671	98.50	1.50	1.32	0.18
3	63388283	98.35	1.65	1.22	0.43
4	80293713	98.24	1.76	1.59	0.17
5	88550390	97.65	2.35	2.20	0.15
6	127210818	97.63	2.37	2.13	0.24
7	257720901	97.50	2.50	2.14	0.36
8	99280000	97.05	2.95	2.77	0.18
9	62243476	95.81	4.19	3.03	1.16
10	96036721	95.13	4.87	4.45	0.42
11	327342640	88.85	11.15	9.75	1.40

Table 1. Computer morphometric analysis of the total area of parenchymal and non-parenchymal elements of liver biopsy in percents and total area of biopsy in chronic hepatitis B. The samples of biopsies are arranged in sequence of increasing of non-parenchymal elements independently from biopsy sizes

This study focuses on the consideration of parameters of liver non-parenchymal elements, which includes all types of portal zones (Figures 1 and 2) and different variants of intralobular infiltrates and necroses (Figures 3 and 4). Liver plates and sinusoids were attributed to the hepatic parenchyma. As can be seen from the Table 1, the total area of the samples of biopsies is variable. Thus, the maximum biopsy size in pixels was 327324640, whereas the minimum – only 6224347 pixels. It is already possible to determine the

suitability of a biopsy for further work at this stage. The absolute value of the area of any structures (in pixels) depends on the size of biopsy. Therefore, we have calculated the percentage of these structures.

Computer morphometric analysis discovered that the portions of the area of non-parenchymal elements of different biopsies of patients with HBV strongly varied from 0.95% to 11.5%. The area portions of the portal zones varied from 0.65% to 9.75%. Both indexes increased in biopsies of given group unidirectional and linearly. Intralobular necroses presented in all biopsies. Their specific parts varied from 0.15 % to 1.40 %. At the same time the percentage part of intralobular necroses did not depend on the indexes of percentage parts of portal zones. We supposed that separate quantitative evaluation of portal zones and intralobular necroses could be useful to indicate the severity and the progression of chronic hepatitis.

3.2 Histological evaluation of liver biopsies by stereometric analysis

The application of computer morphometry is not always possible, because it demands the complex expensive equipment (microscope, digital video camera and computer).

Nevertheless, the quantitative value of fibrosis development in liver is possible without using such complex equipment. In this case, usual light microscope may be effectively used for stereometric analysis (Filimonova et al., 2010).

We used this method for quantitative study of liver biopsies of patients with chronic viral hepatitis B. Information about the special group of patients selected for stereometric analysis is given in the Table 2.

Age (years)	20 ±1,3
Sex	male
Duration of hospitalization (days)	13,5±2,6
Duration of infection (years)	9,5±2,5
Initial ALT	371,5±277
Initial bilirubin	30,7±14,2
Drug user	1 person (10%)

Table 2. Characteristics of patients with chronic hepatitis B

The morphological investigation of liver biopsies of the patients of this group found the presence of some essential changes, such as moderate fibrosis, expansion of a part of portal zones, increasing of the numbers of the cells with vacuolated nuclei, the appearance of the intralobular infiltrates, dilatation of portal veins, pronounced polymorphism of the hepatocytes and its nuclei.

Morphometric analysis was used to study three indexes: determination of the area of portal zones, focal infiltrates, terminal hepatic venules and sublobular veins. At first we determined for each case the portion of selected indexes (in points of intersections), and then calculated the corresponding parts (in percentage) of the parenchyma, portal zones, focal infiltrates and vessels taken over the standard unit of microscopic fields (Table 3).

The sum of the area of portal zones, focal infiltrates and vessels was referred to non-parenchymal elements. The results of analysis are placed in the Tables in the sequence of increasing part of the non-parenchymal elements in the biopsy.

The amount of the fields of view of biopsies of patients with HBV varied from 141 to 350 i.e. was 228.3±23.55 on the average. The sum amount of intersections by morphometric analysis varied from 43710 to 108500 number of points i.e. was 70773±7301.51 on the average.
The part of the non-parenchymal elements in liver biopsies of patients with chronic HBV varied from 2.39% to 12.46% (Table 3), but was essentially lower than in liver biopsies of patients with chronic HCV (Filimonova et al., 2010). The specific part of non-parenchymal elements did not exceed 4.62% in total, these indexes were essentially higher only from biopsies of two patients (8.41% *и* 12.46%, respectively).

Biopsy number	ALT	Total area of morphometry (points of intersections)	Parenchymal elements, %	Non-parenchymal elements, %	Portal zones, %	Focal intralobu-lar infiltrates, %	Hepatic veins, %
1	45	90520	97,61	2,39	1,39	0,25	0,75
2	16	92380	97,24	2,76	1,25	0,19	1,33
3	89	56420	97,22	2,78	1,09	0,66	1,04
4	71	108500	97,18	2,82	1,54	0,99	0,30
5	29	66960	97,14	2,86	1,64	0,13	1,10
6	15	43710	96,76	3,24	2,33	0,13	0,78
7	414	49290	9568	4,32	2,87	0,07	1,38
8	41	89590	95,38	4,62	2,79	0,08	1,75
9	-	43710	91,59	8,41	7,59	0,24	0,58
10	2842	66650	87,54	12,46	10,98	1,05	0,43

Table 3. Quantitative characteristic of liver biopsies of the patients with chronic hepatitis B according to morphometric analysis

The mean value of the non-parenchymal elements in liver biopsies of the patients of this group was 4.66%; the correlation of the portion of the non-parenchymal elements with the level of the ALT was less obvious than in chronic HCV (Filimonova et al., 2010). The high level of the ALT (2842 U/L) was observed only by maximal increasing of the specific part of the non-parenchymal elements (up to 12.46%).

Biopsy number	Total area of portal zones (points of intersections)	Area of portal zones, %	Number of portal zones in biopsy	Maximal area of portal zone (points of intersections)	Maximal area of portal zone at single microscopic fields, %
1	1262	1,39	16	236	76,13
2	1152	1,25	32	83	26,78
3	616	1,09	17	71	22,90
4	1674	1,54	48	110	35,48
5	1098	1,64	20	212	68,39
6	1017	2,33	30	99	31,94
7	1415	2,87	20	376	121,29
8	2497	2,79	39	335	108,06
9	3316	7,59	21	439	141,61
10	7319	10,98	42	532	171,61

Table 4. Quantitative characteristic of portal zones of liver biopsies of the patients with chronic hepatitis B according to morphometric analysis

The specific part of the portal zones (Table 4) of the liver of the most patients varies from 1.09% to 2.87%, these indexes were essentially higher only from biopsies of two patients (7.59% и 10.98%, respectively). The mean value of specific part of the portal zones varies from 33.9 points to 78.8 points in general, i.e. it occupies from 11% to 25% of the standard sight of view. Maximal areas of portal zones varied from 22.9% till 171, 61% (or from 71 till 532 points respectively) at single microscopic field (Table 4). The portal tracts of two patients with expressed activity of the process of the disease were increased to 157.9 and 174.26 points; it was corresponded with the 51% and 56% of the square of the standard field of the microscope view. The focal interlobular infiltrates were observed in liver of all the patients (Tables 3 and 5).

Biopsy number	Small focal infiltrates	Large focal infiltrates	Total number of infiltrates
1	6	6	12
2	5	5	10
3	6	15	21
4	15	24	39
5	5	4	9
6	9	4	13
7	1	3	4
8	2	2	4
9	3	9	12
10	6	24	30

Table 5. The number of focal infiltrates at one standard square unit (100 fields of sight under the magnification ×400) in liver biopsies of patients with chronic hepatitis B

The specific part of focal infiltrates varied from 0.07% to 1.05%. Large focal infiltrates predominated in liver of approximately the half of the patients (Table 5). It is characteristic that the specific part of focal infiltrates did not depend on the specific part of non-parenchymal elements and did not correlate with ALT level. The amount of focal necroses (15.4±3.6) in liver of these patients in 100 standard fields of the microscope view was two times less than in liver of patients with hepatitis C (29.6±5.2) (Filimonova et al., 2010).

The specific square of hepatic veins varied from 0.30% to 1.75% and did not depend on the specific part of non-parenchymal elements (Table 3). The mean specific square of vessels varied from 16.05 to 56.67 points. The growth of the venules from the portal tracts occurred often in liver of the majority of patients with chronic HBV.

On the whole the inflammation-necrotic changes in liver of the given selected group of the patients were manifested weakly; fibrosis indexes did not exceed F0 and F1, both in the METAVIR system and Ishak system. Bridging and piecemeal necroses presented in liver of only two patients (№ 7 and № 10), both of them had the high ALT index and strongly enlarged the specific part of non-parenchymal elements.

So the morphometric calculation of the non-parenchymal elements in liver biopsies of patients with HBV allows giving the complex evaluation of the replacement level of functional parenchyma by necro-inflammatory and fibrosis structures.

Stereological quantitative morphometry allows to getting more correct evaluation of some morphological parameters of pathologically changed liver in patients with chronic viral

hepatitis. It is very important for establishment of either positive of negative dynamic changes in liver, especially during estimation of efficiency of antiviral treatment. We supposed that the stereological morphometry is a suitable tool for quantitative evaluation of liver biopsies in therapeutics trials.

3.3 Cell population structure of liver biopsies from the patients with both chronic hepatitis, HCV and HBV

Only sufficiently large biopsies with 5-6 portal zones can be suitable for quantitative evaluation of parenchymal elements of liver by methods of stereological or computer morphometry. Nevertheless, biopsies used have frequently small sizes or consist of fine fragments without portal zones in clinical practice. Quantitative analysis of parenchyma elements (liver plates and sinusoids cells) is advisable in these cases.

In our investigation the cell population analysis of liver biopsies from the patients with both chronic hepatitis, HCV and HBV included the comparative evaluation of the specific part of non-parenchymal elements, analysis of the liver plates and sinusoids areas, cell population of liver plates and sinusoids.

3.3.1 Comparative analysis of the specific part of non-parenchymal elements

The specific part of non-parenchymal elements in liver biopsies of patients with HCV (Table 6) strongly varies: from 2.16% to11.93% (mean value is 6.9 ± 0.8%).

Biopsy number	Non-parenchymal elements, %	Liver plates, %	Sinusoids, %	Lytic necroses of hepatocytes	Binucleate hepatocytes	Polymorpho-us hepato-cytes	Endothelio-cytes	Kupffer cells
1	2,16	96,77	3,23	2,70	0,90	0,40	5,80	11,10
2	2,46	92,68	7,32	1,60	2,40	0,90	7,30	6,90
3	3,6	94,23	5,77	3,80	2,70	0,60	8,00	9,10
4	4,3	95,10	4,90	3,50	1,00	1,30	7,20	5,30
5	4,63	94,90	5,10	4,60	1,50	0,80	10,20	10,90
6	4,7	93,74	6,26	2,80	0,30	0,00	8,90	6,90
7	5,06	95,42	4,58	5,70	1,30	0,10	12,00	6,90
8	5,18	93,32	6,68	2,60	0,30	0,10	7,60	10,70
9	6,64	93,55	6,45	5,20	0,30	0,50	7,70	9,80
10	9,46	94,68	5,32	4,40	1,70	0,80	12,80	15,80
11	9,68	94,35	5,65	5,70	0,50	0,70	9,90	8,80
12	10,56	92,16	7,84	4,60	1,10	0,90	11,30	12,00
13	10,89	94,94	5,06	5,50	1,30	0,00	7,20	7,00
14	11,76	93,00	7,00	3,80	2,70	1,90	9,70	14,00
15	11,93	92,87	7,13	3,10	0,90	0,60	10,40	9,20

Table 6. Cell population structure of liver plates and sinusoids in patients with chronic hepatitis C

The piecemeal and bridging necroses are presented, as a rule, in liver biopsies of the patients with high index of non-parenchymal elements. The piecemeal necroses are described in 11 cases from 15, bridging necroses in 7 cases from 15. Such distribution shows that during the ordinary course of the disease the piecemeal necroses arise from the beginning, the bridging necroses are discovered later.

The specific part of non-parenchymal elements changes from 2.39% to 8.41% (mean value is 3.8 ± 0.9) in liver biopsies of patients with HBV (Table 7). The piecemeal necroses were observed only in one biopsy, the bridging necroses in two biopsies.

The data regarding the specific parts of non-parenchymal elements shows that liver damages of patients with HCV are more significant in comparison with the analogical indexes of patients with HBV.

Biopsy number	Non- paren-chymal elements, %	Liver plates, %	Sinusoids, %	Lytic necroses of hepatocytes	Binucleate hepatocytes	Poly-morphous hepato-cytes	Endothelio-cytes	Kupffer cells
1	2,39	91,65	8,35	6,75	1,00	1,00	6,25	7,15
2	2,76	91,81	8,19	5,35	3,85	2,15	5,50	8,40
3	2,78	93,29	6,71	2,05	1,80	1,15	8,05	8,45
4	2,82	93,65	6,35	3,70	0,85	0,90	6,90	6,10
5	2,86	85,77	14,23	1,30	4,10	2,35	7,35	6,15
6	3,24	91,16	8,84	5,70	1,20	1,20	5,10	5,15
7	4,32	93,87	6,13	4,91	1,36	0,91	8,64	5,73
8	4,62	91,39	8,61	3,20	1,95	1,60	8,30	6,50
9	8,41	86,26	13,74	3,55	4,90	1,70	8,45	8,40

Table 7. Cell population structure of liver plates and sinusoids in patients with chronic hepatitis B

3.3.2 Analysis of the liver plates and sinusoids

Morphometric investigation shows that the specific parts of liver plates of patients with HCV slightly vary from 92.16% to 96.77% (mean value is 94.1±0.31). The specific parts of sinusoids vary from 3.23% to 7.84% (mean value is 5.9±0.4). Such variations are more significant in livers of patients with HBV. The specific parts of liver plates in this case vary from 85.77% to 93.87% (mean value is 90.98±0.32), the specific parts of sinusoids vary from 6.13% to 14.23% (mean value is 9.02±0.32). Morphological and morphometric studies show (Figures 6 and 7; Tables 6 and 7) that the sinusoids of liver biopsies of patients with HCV are significantly narrowed than such of patients with HBV. Respectively the conditions of intralobular blood circulation are significantly differ under various types of hepatitis.

Thus, the connection between the disease severity and specific parts of liver plates and sinusoids are not established.

3.3.3 Cell population of liver plates

In liver biopsies of patients with chronic HCV (Table 6) the amount of lost hepatocytes (lytic necroses) in liver plates varies from 1.6% to 5.7% in standard field of vision (mean value is

3.97±04). The number of binucleate hepatocytes varies from 0.3 to 2.7 (mean value is 1.26±0.2). The number of polymorphous hepatocytes in standard field of vision varies from 0.0 to 1.9 (mean value 0.64±0.1).
In liver biopsies of patients with HBV (Table 7) the amount of lost cells varies from 1.3 to 6.75 (mean value is 4.06±0.59). The number of binucleate hepatocytes varies from 0.85 to 4.9 (mean value is 2.33±0.5). The number of polymorphous hepatocytes at standard field of vision varies from 0.9 to 2.35 (mean value is 1.44±0.3).
Thus, the amount of single lytic necroses of hepatocytes does not differ in liver biopsies of patients with HBV and with HCV. At that time the number of binucleate hepatocytes and polymorphous hepatocytes is certainly higher in liver biopsies of patients with HBV. This situation may be connected with the different level of processes of regeneration.

3.3.4 Cell population of liver sinusoids

The amount of endotheliocytes in sinusoids of liver biopsies of patients with HCV (Table 6) strongly varies: from 5.8 to 12.8 cells in standard field of sight (mean value is 9.0±0.5). The amount of Kupffer cells is also significantly differing from 5.3 to 15.8 in the field of sight (main value is 9.6±0.7). Nevertheless, the precise linear dependence between the both of indexes is not revealing.
The amount of endotheliocytes in sinusoids of liver biopsies of patients with HBV (Table 7) weakly varies from 5.1 to 8.64 cells in standard field of sight (mean value is 7.17±0.13). The amount of Kupffer cells changes from 5.15 to 8.45 in the field of sight (mean value is 6.89±0.31).
Thus, the amount of endotheliocytes and Kupffer cells is essentially more in liver biopsies of patients with HCV. This circumstance is probably connected with the peculiarities of virus influence on the vessel component of liver parenchyma.
The cell population structure of liver biopsies of patients with HBV and HCV changes unequally. So, the using of Student and Satterwhite criteria allows discovering the statistically significant distinctions between mean values of all the indexes with the exception of the amount of lost hepatocytes in liver plates.
The coefficients of correlation between the indexes of specific parts of liver plates and the number of binucleate cells, between the indexes of specific parts of liver plates and the number of polymorphous cells are statistically significant for the cluster of liver biopsies of patients with HBV.
The statistically significant coefficients of correlation Pearson does not detected for the cluster of liver biopsies of patients with HCV.
The strong linear dependence between the indexes of specific parts of liver plates and the number of binucleate cells and the number of polymorphous cells was revealed in liver biopsies of patients with HBV.
The using of Spearmen coefficients of correlation allowed establishing the connection between the number of cells in sinusoids and the specific part of non-parenchymal elements in liver biopsies of patients with HBV. This circumstance could be used for indirect characteristics of the development of fibrotic changes in liver.
In total, the quantitative analysis of cell population structure in liver biopsies in the course of chronic hepatitis, especially in the case of defective biopsies, could be used for diagnostic and prognoses by expert evaluation.

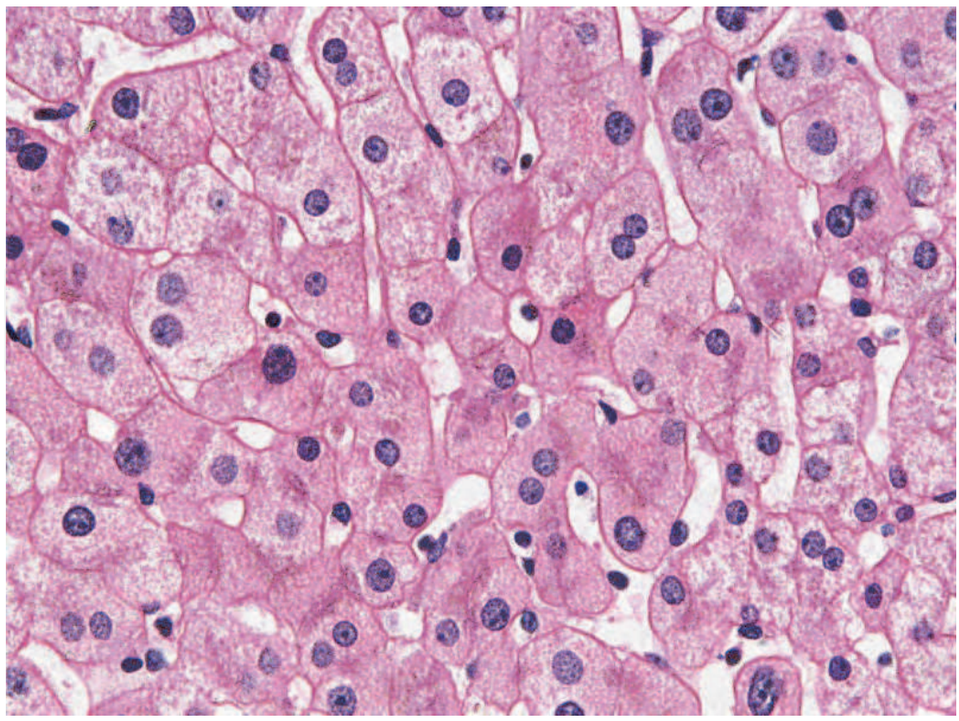

Fig. 6. The fragment of parenchyma with polymorphous hepatocytes and distorted sinusoids in liver of a patient with chronic hepatitis B. Hematoxylin-eosin. Obj.x40

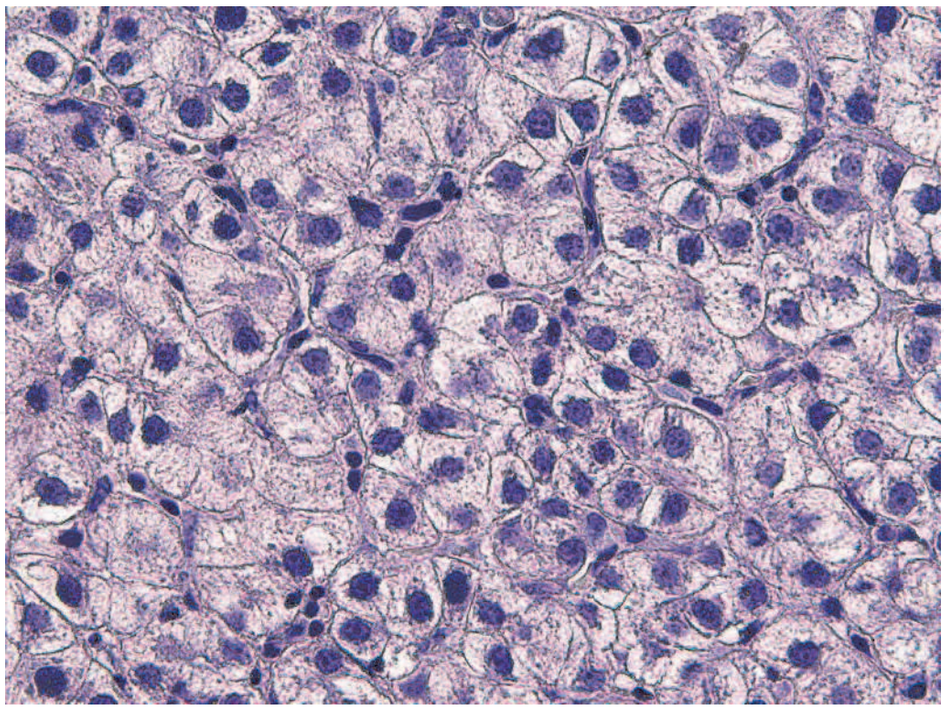

Fig. 7. The fragment of parenchyma with unimorphous hepatocytes and narrow sinusoids in liver of a patient with chronic hepatitis C. Alcian blue. Obj.x 40

4. Conclusion

Evaluation of fibrosis in liver biopsy remains as a point for the diagnosis, monitoring of disease prognosis, and response to treatment. Quantitative stereological and computer morphometric analysis as well as cell population analysis was used for more correct evaluation of dynamic of liver damages in patients with chronic hepatitis B and chronic hepatitis C.

Stereometric analysis of quantitative morphological changes in liver biopsies using conventional light microscopy yields good results. Especially effective is the application of quantitative analysis of elements in liver parenchyma (ratio between liver plates and sinusoids, counting the number of hepatocytes and sinusoidal cells in a standard field of view) for the analysis of non-standard biopsies (small size, fragmentation). These data are essential for predicting disease and an overall assessment of the liver in each case. However stereometric analysis is time consuming and requires special skills in work.

Colorimetric detection of zones of differential staining of collagen (Manabe et al., 1993; Duchatelle et al., 1998) or immunohistochemical markers of fibrogenesis (Kweon et al., 2001) with subsequent determination of the area of fibrosis in the percentage of the total field is commonly used in computer morphometry.

In our investigation the total registration of non-parenchymal elements in liver biopsies of patients with chronic viral hepatitis carried out using a special program that allowed quantifying the presence of fibrotic, inflammatory and necrotic changes in the liver.

It is particularly important to evaluate separately the number and the area of focal necroses, which characterize the activity of intralobular hepatocyte death. We believe that the increase of the area of non-parenchymal elements of liver demonstrates a degree of replacement of functioning parenchyma by inflammatory infiltrates, necroses and scars. Our studies show the advisability of separate accounting bridging necroses and septa, as this may serve as a prognostic sign of distortion of the architectonics of liver lobules, usually preceded by cirrhosis. In some biopsies we observed the early development of septa without noticeable changes in portal tracts. Computer and stereological morphometry can also registrate the degree of expansion of portal tracts.

Our research suggests that four percents or more increasing of the portion of portal zones area and increasing of specific parts of intralobular focal infiltrates are unfavorable factors for development of chronic liver diseases.

Thus, computer morphometry is a modern and reliable method for quantifying inflammatory, necrotic and fibrotic changes in liver biopsies of chronic diseases. At the same time this method requires the creation of a single algorithm to study the reproducibility and easy comparison of results obtained in different laboratories.

Some of the perspectives and problems of computer analysis of liver biopsies are summarized below.

Perspectives:

1. Computer microscopy and morphometry are ideal for digital archiving of samples of biopsies for comparison with repeated biopsies.
2. Quantitative information about the parameters of the biopsy improves the accuracy in diagnosing of disease and allows to optimize the treatment tactics and to adjust quickly the course of treatment.
3. Digital image of a biopsy can be sent by e-mail and be immediately consulted. It is also gives the possibility of participation of a large number of specialists for analysis of digital images biopsy in complicated cases, thus avoiding medical errors.

4. Quantitative analysis of digital images of biopsies is indispensable to study the effectiveness of testing new drugs developed by pharmaceutical companies. The effect can be calculated as a percentage.
5. Quantitative indicators of morphological parameters of biopsies may serve as a basis for developing mathematical models of the dynamics of chronic liver disease.

Problems:

1. It is necessary to work out some common standards of the quantitative computer evaluations of biopsy morphological changes, including the development of fibrosis. That will make possible the correct comparison of the results in different studies.
2. Comprehensive assessment and computer analysis of biopsies require the participation of medical specialists having the practice with chronic viral hepatitis patients and combining the ability to analyze the morphological changes in the liver with high skills as computer users.
3. A digital image of microscopic slides has a large amount of files (up to 2 - 3 gigabytes per biopsy), so high-power computers are necessary for the digital processing of biopsies.

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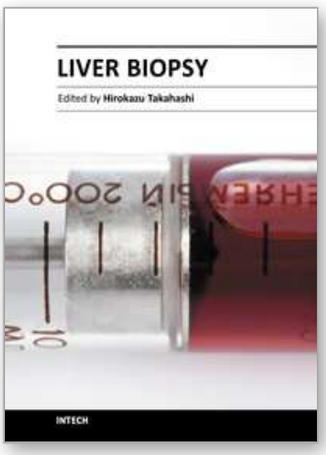

Liver Biopsy

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Liver biopsy is recommended as the gold standard method to determine diagnosis, fibrosis staging, prognosis and therapeutic indications in patients with chronic liver disease. However, liver biopsy is an invasive procedure with a risk of complications which can be serious. This book provides the management of the complications in liver biopsy. Additionally, this book provides also the references for the new technology of liver biopsy including the non-invasive elastography, imaging methods and blood panels which could be the alternatives to liver biopsy. The non-invasive methods, especially the elastography, which is the new procedure in hot topics, which were frequently reported in these years. In this book, the professionals of elastography show the mechanism, availability and how to use this technology in a clinical field of elastography. The comprehension of elastography could be a great help for better dealing and for understanding of liver biopsy.

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