Rev. Inst. Med. Trop. Sao Paulo
2016;58:10
http://dx.doi.org/10.1590/S1678-9946201658010

LIVER BIOPSY: IMPORTANCE OF SPECIMEN SIZE IN THE DIAGNOSIS AND STAGING OF CHRONIC VIRAL HEPATITIS

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SUMMARY

Liver biopsy is the gold standard method for the grading and staging of chronic viral hepatitis, but optimal biopsy specimen size remains controversial. The aim of this study was to evaluate the quality of liver specimen (number of portal tracts) and to evaluate the impact of the number of portal tracts in the staging of chronic hepatitis. **Material and Methods:** 468 liver biopsies from consecutive patients with hepatitis C virus and hepatitis B virus infection from 2009 to 2010 were evaluated. **Results:** The length of fragment was less than 10 mm in 43 cases (9.3%), between 10 and 14 mm in 114 (24.3%), and ≥ 15 mm in 311 (64.4%); of these, in 39 (8.3%) cases were ≥ 20 mm. The mean representation of portal tracts was 17.6 ± 2.1 (5-40); in specimens ≥ 15 mm the mean portal tract was 13.5 ± 4.7 and in cases ≤ 15 mm was 11.4 ± 5.0 ($p = 0.002$). Cases with less than 11 portal tracts were associated with F3, and cases with 11 or more portal tracts with F2 ($p = 0.001$). **Conclusion:** this study demonstrated the good quality of liver biopsy and a relationship between the macroscopic size of the fragment and the number of portal tracts.

KEYWORDS: Chronic viral hepatitis; Liver biopsy.

INTRODUCTION

The emergence of hepatology as a defined clinical medical specialty is coincident with the introduction of liver biopsy and Menghini's needle in 1958[1]. Clinical hepatologists were trained in liver pathology and were responsible for the foundation of the main schools of hepatology in Europe and United States[2]. In the past, the only way to diagnose liver disease was by liver biopsy.

Grading and staging chronic hepatitis are essential for assessing prognosis and deciding which patients should be given priority for treatment[3]. Liver biopsy is also helpful to rule out other diagnoses and to evaluate steatosis and iron[4]. Although biopsy is an invasive procedure, the risk of major complications is very low (0.22-0.75%)[5,6]. The mortality rate attributed to the procedure is estimated in one per 10 thousand biopsies and is usually secondary to bleeding; this is even lower when the biopsy is not performed for evaluation of liver tumors[7,8].

Recently, noninvasive methods were developed to assess liver fibrosis[9]. In this setting, measure of liver stiffness by elastography is widely used for selection of patients eligible for drug therapy[3]. However, liver biopsy remains the gold standard and the most used method in our setting[10]. In a recent study that evaluated 1,202 patients with chronic hepatitis C virus (HCV), elastography showed false positive results in patients with high levels of aminotransferases and obesity[11]. Usually, a body mass index (BMI) greater than 28 results in failure of liver stiffness measurement[12]. Moreover, fibroscan is a diagnostic test that depends on the experience of the operator[13]. Recent meta-analysis demonstrated 83% of sensitivity and 89% of specificity for the diagnosis of cirrhosis by elastography. On the other hand, in patients with moderate fibrosis, (F2 of METAVIR) sensitivity was 79% and specificity 78%[14]. It's noteworthy that the guidelines for hepatitis C treatment of the AASLD (American Association for the Study of Liver Diseases) and the EASL (European Association for the Study of the Liver) accepted noninvasive methods for therapeutic decision[3,15].

Liver biopsy is subjected to sampling error, since a standard specimen represents only about 0.0002% of the whole liver. Then, it is essential that the sample be representative for limiting the risk of inappropriate results, but optimal biopsy specimen size remains controversial[9,16,17,18].

Therefore, the aim of this study was to evaluate the quality of liver specimen [number of portal tracts (PT)], to correlate the sample size with the number of PT and to evaluate the impact of the number of PT in the staging of chronic viral hepatitis.

MATERIALS AND METHODS

Liver biopsies from consecutive patients with HCV and HBV infection from 2009 to 2010 were included in this study conducted

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Table 1
Portal tracts according fibrosis staging

Fibrosis	Number of EP					
	N	Mean	Std Deviation	Median10.50	Minimum	Maximum
F0	67	11.48	4.56	11.00	5	23
F1	192	11.90	5.18	13.00	5	40
F2	114	13.70	4.46	10.00	5	24
F3	56	11.04	1.93		5	23

One way analysis of variance-(F^2_{calc} = 5.366; p = 0.001)

in a tertiary hospital in southern Brazil (*Irmandade Santa Casa de Misericórdia de Porto Alegre*-ISCMPA).

Patients under the age of 18 were excluded, as well as those with other causes of chronic liver disease.

All biopsies were guided by ultrasonography using an 18 G disposable Tru-Cut needle. The specimens were fixed in 10% formalin and evaluated with respect to size (mm). When two or more fragments were obtained, the larger was analyzed. Fragmentation of the specimen was also assessed. Samples were classified as ≥ 15 mm and < 15 mm for statistical analysis.

Liver biopsies were routinely stained with Hematoxylin-eosin, Perls and Masson's trichrome, and evaluated by the same hepatopathologist who was blinded to the size of specimen and clinical data. The number of PT was evaluated in all cases, excepted in those with cirrhosis, because of changes in liver architecture, annulling the individualization of PT[19]. Specimens with less than five PT were considered inadequate for analysis[20]. Biopsies were classified according to METAVIR score[21].

This study was approved by the Institutional Review Board of ISCMPA.

Statistical analysis was performed using SPSS software (Statistical Package for Social Sciences) version 17.0. Quantitative variables were described using mean and standard deviation (symmetrical distribution) or median (asymmetric distribution). Categorical variables were described by absolute and relative frequencies. The Chi-square and Pearson, Fisher's exact test and One Way analysis of variance test were used for statistical analysis. A p value ≤ 0.05 was considered statistically significant.

RESULTS

Four hundred and sixty-eight liver biopsies were evaluated. The mean age of patients was 57.7 years (18-78). Two hundred and thirty-eight patients were female (50.8%).

Fragment size was less than 10 mm in 43 cases (9.3%), range between 10 and 14 mm in 114 (24.3%), and was ≥ 15 mm in 311 (64.4%); of these, in 39 (8.3%) cases were ≥ 20 mm. The mean length of fragments was 13.3 ± 4.2 mm. The fragmented specimens were 21/468 (4.5%), and in these cases the report pointed out the possible limitation of the staging performed.

Of the 468 cases, 39 (8.3%) had a diagnosis of liver cirrhosis; therefore, the specimens considered for assessment of number of PT was 429. The mean representation of PT per biopsy was 17.6 ± 2.1 (5-40) and the median was 15. Moreover, in specimens ≥ 15 mm the mean PT was 13.5 ± 4.7, and in cases < 15 mm it was 11.4 ± 5.0 (p = 0.002). The number of PT per sample was as follows: 5 PT in 20 cases (4.7%), 6 to 8 in 88 cases (20.6%), 9 to 10 in 67 cases (15.5%), and ≥ 11 PT in 254 (59.3%).

Fibrosis stage was scored as follows: F0 in 67 patients (14.3%), F1 in 192 (41.0%), F2 in 114 (24.4%), F3 in 56 (12.0%), and F4 in 39 (8.3%). The necroinflammatory activity was classified as absent (A0) in 32 cases (6.8%), mild (A1) in 215 cases (45.5%), moderate (A2) in 185 (39.1%), and severe (A3) in 36 (7.6%). Steatosis was present in 41.6% and iron in 20.5%.

The association between fibrosis staging and the number of PT is shown in Table 1. There was a statistically significant difference indicating that the mean of PT in samples with F2 (13.7 ± 4.4) was significantly higher when compared to other stages (F0: 11.5 ± 4.6 – p < 0.01; F1: 11.9 ± 5.2 – p < 0.05; F3: 11.0 ± 4.9 – p < 0.001).

There was a significant association between fibrosis stage F3 and representation of less than 11 PT, and an association between F2 and 11 or more PT (p = 0.001) (Table 2).

Table 2
Fibrosis staging according portal tracts: stratified sample

Fibrosis	Portal Tracts		Total
	< 11	≥ 11	
F0	32 (18.5%)	32 (12.6%)	64 (15%)
F1	81 (46.8%)	111 (43.9%)	192 (45.1%)
F2	30 (17.3%)	84 (33.2%)	114 (26.8%)
F3	30 (17.3%)	26 (10.3%)	56 (13.1%)
Total	173 (100%)	253 (100%)	426 (100%)

Chi-square and Pearson (p = 0.001)

DISCUSSION

Liver biopsy is a diagnostic method widely used for staging of chronic hepatitis, despite the rising of noninvasive methods. However, one of its limitations is sampling variability. In order to minimize sampling error, biopsy needs to be representative of the whole liver[7].

The size considered ideal for histological analysis is under debate in the literature[22]. Some studies suggest that a biopsy of 10-15 mm in length, with 4-6 PT is sufficient for staging of chronic hepatitis[17,18,23] whereas, other authors suggest a minimum size of 20 to 25 mm and at least 11 PT[9,16].

SCHIANO *et al*.[18] analyzed 100 biopsies and didn't find a significant difference in fibrosis stage when evaluating different sizes of the same liver specimen. Ninety-four per cent of the specimens of 10 mm received the same staging or just one degree of difference when compared with the 20 mm. On the other hand, COLLOREDO *et al*.[9] studied 161 biopsies from patients with chronic hepatitis considering only specimens of 30 mm or more. These fragments were first examined in their original size and then reduced in size to be reanalyzed (20 mm, 15 mm and 10 mm). The necroinflammatory activity and fibrosis were more often considered as discrete when smaller size was analyzed. A sample larger than or equal to 20 mm and representation of at least 11 full PT was considered necessary for accurate evaluation.

BEDOSSA *et al*.[16] also compared the results of staging the same biopsy in two different sizes: 25 mm and 15 mm. In agreement with the previous study, they demonstrated that larger fragments allowed to assess staging more accurately.

In the present study, in 90.7% cases the fragment had more than 10 mm, the minimum size recommended by BEDOSSA *et al*., in the study that validated the METAVIR classification[21]. Similarly, in this study, the average number of PT was 17.6 and the median was 15, demonstrating a good quality of liver biopsy.

Importantly, a systematic review evaluating the quality of liver biopsies demonstrated that the mean PT represented was 7.5 ± 3.4 in 10,027 biopsies. In this systematic review, the correlation between fragment size and number of PT was low (Spearman r = 0:45)[22]. In this study, we found a correlation between PT and size of fragment.

In the present study, 40.7% of the sample had less than 11 PT. However, it was shown that advanced fibrosis (F3) was even linked to the representation of less than 11 PT. Thus, although it has been shown that 11 PT would be ideal minimum number for staging[9,16], this study is consistent with other studies that suggest that less than 11 PT may be suitable[17,18,23].

Regarding the liver biopsy sample size , attention should be drawn to the fact that the vast majority of clinical trials in chronic hepatitis did not mention the number of PT represented[22,24].

We conclude that liver biopsy in real life, in a general hospital, has good quality, showing in almost all cases, the minimum size suggested for the diagnosis and staging. A relationship between the macroscopic size of the fragment and the number of PT represented has also been demonstrated. Furthermore, in this study, representation of less than 11 and more than 5 PT probably did not adversely influence the staging.

Abbreviations:
PT- Portal tracts
HCV - Hepatitis C virus
BMI- Body Mass Index
AASLD- American Association for the Study of Liver Diseases
EASL- European Association for the Study of the Liver
ISCMPA- *Irmandade Santa Casa de Misericórdia de Porto Alegre*

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Received: 03 March 2015
Accepted: 19 May 2015