Pathology Predictors of Response to Combined Therapy of Chronic HCV Patients; Is it Applicable in the DAA Era?

Abstract

Introduction & aim: Liver biopsy has been considered the “gold standard” of diagnosis, the most direct way of visualizing the necroinflammatory and architectural status of liver in HCV patients planned for therapy. We aimed to study the relation between pathological findings in liver biopsy and their impacts on sustained virological response (SVR) in HCV patients receiving pegylated interferon and ribavirin (Peg/Riba).

Methods: A retrospective analysis on data collected from 1486 HCV patients receiving Peg/Riba between May 2012 and May 2013 at the National Hepatology and Tropical Medicine Research Institute (NHTMRI). Initial labs before treatment including percutaneous liver biopsy were analyzed in relation to virological response.

Results: 982 (66.1%) of patients were males and 504 (33.9%) were females. The mean age was (43.72 ± 9.527y), with 1008 (67.8%) more than 40 years. Higher SVR achieved in patients with no or mild steatosis than moderate to severe degrees (96% vs 4%, p: 0.001). More than 60% of patients with no or mild fibrosis showed SVR (p: 0.04). Patients with mild activity showed much higher SVR than patients with severe or advanced activity (67.1% vs 32.9%, p: 0.001). Negative viral load at w12 was significantly higher in patients with no or mild fibrosis (96.7% vs 94.9%, p: 0.004), no or mild steatosis (95.7% vs 94%, p: 0.024), but was not related to activity (95.8% vs 95.7%, p: 0.869).

Conclusion: Steatosis, fibrosis and activity affected treatment outcome and could directly affect treatment decision. The response rate of peginterferon alpha 2a was comparable to alpha 2b. These findings are important to decide antiviral course in patients with multiple DAA RAVs leading to failure.

Keywords: HCV; Liver biopsy; Steatosis; Peginterferon; Viral response

Abbreviations: Peg/Riba; Pegylated Interferon and Ribavirin; SVR: Sustained Viral Response; AFP; Alpha Fetoprotein; NHTMRI: National Hepatology and Tropical Medicine Research Institute

Introduction

In nearly 50 years since Menghini popularized the use of percutaneous needle biopsy, microscopic evaluation of the liver has remained an important modality in the diagnosis and management of patients with liver disease. For patients with chronic hepatitis, liver biopsy has been considered the “gold standard” of diagnosis [1].

There are three primary reasons for performing a liver biopsy: it provides helpful information on the current status of the liver injury, it identifies features useful in the decision to embark on therapy, and it may reveal advanced fibrosis or cirrhosis that necessitates surveillance for hepatocellular carcinoma (HCC) and/or screening for varices [2].

We aimed to study the relation between histopathological findings in liver biopsy and their impacts on SVR in HCV patients receiving pegylated interferon and ribavirin.

Patients and Methods

This was a retrospective analysis done on data collected from 1486 HCV infected patients receiving combined treatment in the form of pegylated interferon and ribavirin (Peg/Riba) between May 2012 and May 2013 at the National Hepatology and Tropical Medicine Research Institute (NHTMRI). This study was approved by the Ethics Committee of the Centre and an informed consent (printed in Arabic) was obtained from all participants.

These patients fulfilled the criteria for antiviral therapy that included:
1. Male or female age 18 years or older
2. White blood cell count > 3000/mm^3
3. Neutrophil count > 1500/mm^3
4. Platelets > 80,000/mm^3
5. Hemoglobin > 12gm in females, 13gm in males
6. Prothrombin time < 2 seconds above ULN
7. Direct bilirubin 0.3 mg/dl or within 20% of ULN

Keywords: HCV; Liver biopsy; Steatosis; Peginterferon; Viral response

References

1. Menghini, et al. (1977) Liver biopsy in chronic hepatitis: A reappraisal. Gastroenterology 72, 359-367.
2. Ferrell RE, et al. (2010) The role of liver biopsy in chronic hepatitis. Gastroenterology 138, 2064-2074.

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Basem El-Sayed El-Sayed1, Gamal El-Din Esmat2, Wahed Doss2, Ehsan Hassan Hassan1, Rasha Ahmed2 and Samar K Darweesh2

1Department of Pathology, National Hepatology and Tropical Medicine Research Institute, Egypt
2Department of Tropical Medicine and Hepato-gastroenterology, Cairo University, Egypt

*Corresponding author: Samar Kamal Darweesh, Department of Tropical Medicine and Hepato-gastroenterology, Faculty of Medicine, Cairo University, 63, Abo Dawood El-Thahery St, Nasr city, Cairo, Egypt, Tel: 002-01000702766; Email: samarkd@hotmail.com
Liver biopsy

Liver biopsies were ultrasound-guided, obtained percutaneously by a Menghini needle (14G) with 1.6mm internal diameter. All biopsies were fixed in formalin, embedded in paraffin and sectioned by microtome with a thickness of 5 μm. Slides were stained with Hematoxylin and Eosin (5 levels), Masson's trichrome (5 levels), for a total of 10 levels per specimen. All levels were screened. All specimens were examined by two pathologists and classified by consensus for all abnormal histological findings. The histological activity index (or histological grade) was determined using Ishak grading scheme expressed as a semi-quantitative score for portal inflammation (0–4), lobular activity sporadic lytic foci (0–4) and parenchymal confluent necrosis (0–6), and piecemeal necrosis (0–4). The extent of fibrosis (or histological stage) was determined using Ishak score (0–6). Steatosis was scored from grade 0 to 3.

Statistical analysis

Data were analyzed using PASW statistics 18. Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher’s exact test) was used to examine the relation between qualitative variables. For quantitative data normally distributed, comparison between two groups was done using t-test. Comparison between 3 groups or more was done using ANOVA test. For quantitative and qualitative data not normally distributed, comparison was done using Kruskal-Wallis test. Logistic regression model was done for predictors’ detection at 95% confidence level. Spearman-rho method was used to test correlation between numerical and different scoring variables. A p-value < 0.05 was considered significant.

Results

Basic characteristics

One thousand four hundreds and eighty six patients infected with HCV and experienced treatment with peginterferon and weight based ribavirin for 48 weeks; 982 (66.1%) of them were males and 504 (33.9%) were females. The mean age of them was (43.72 ± 9.527) years, with 1008 (67.8%) were more than 40 years old. The mean body mass index was (27.51 ± 3.231).

Liver biopsy pathological report, of fibrosis stage, activity grade and steatosis are found in Table 1. 819 (55.1%) patients had INF alpha 2a, and 667 (44.9%) had INF alpha 2b. The overall sustained virological response (SVR) was 60.6%. The SVR was 60.4% with alpha 2a; however it was 61% with alpha 2b type. This preceding data showed no statistical significance (p value: 0.735).

The mean age of patients who achieved SVR was (41.76 ± 7.778), and the mean age of relapsers was (46.94 ± 7.778), these findings were statistically highly significant denoting that older patients will be unlikely to achieve SVR (p value: 0.001, T: -11.166). However, the gender of the studied patients did not influence the response and showed statistical non significance (p value: 0.936).

Relation between SVR and laboratory findings

The baseline ALT and AST levels were higher in relapsers in comparison to patients who attained SVR, though it only showed highly significant relation with AST (p value: 0.207 and 0.001 respectively).
The total bilirubin and serum albumin were statistically significant with patients' response; however, there was no big difference between the values of relapers and patients with SVR (p values: 0.011 & 0.001 respectively). Also, the p value of pre-treatment serum creatinine in relation to SVR was 0.001.

HCV RNA by PCR before treatment illustrated that the mean of SVR group was 61,700 ± 12,210.5 IU/ml, on the other hand, the mean of relapers was 950,203.6 ± 510,934.5 IU/ml. That finding showed statistical significance (p value 0.03, T: -4.191).

Pre-enrollment fasting blood sugar, alkaline phosphatase, TSH, all of them had shown no statistical significance.

Out of 1486 enrolled patients, 1423 (95.8%) achieved negative HCV RNA after 12 weeks of treatment; of them 879 (61.8%) were negative for HCV RNA at week 72 of treatment and 544 (38.2%) were positive for HCV RNA at week 72. Nevertheless, only 63 (4.2%) of the treated patients attained only 2 log reduction at week 12; 63.5% of them could not achieve SVR. The preceding data showed high significance (p value: 0.001).

**Relation between SVR, EVR and pathologic findings**

The steatosis degree influenced SVR significantly with higher SVR achieved in patients with no or mild degrees of steatosis, also, higher rates of relapse were seen in patients with more severe degrees Table 2. Also, obviously steatosis was a dominant influencer for response during the course of treatment either at week 12 as shown in Table 3 or at week 72 as shown before in. That is to say, the more the steatosis degree the less chance to accomplish negative PCR for HCV at week 12.

### Table 1: Liver biopsy pathological findings.

| Degree of Fibrosis | No   | %    |
|--------------------|------|------|
| No                 | 28   | 1.9  |
| Mild               | 663  | 44.6 |
| Moderate           | 662  | 44.5 |
| Severe             | 133  | 9    |

| Degree of Activity | No   | %    |
|--------------------|------|------|
| Mild               | 946  | 63.7 |
| Moderate           | 493  | 33.2 |
| Severe             | 47   | 3.2  |

| Degree of Steatosis | No   | %    |
|--------------------|------|------|
| No                 | 554  | 37.3 |
| Mild               | 845  | 56.9 |
| Moderate           | 73   | 4.9  |
| Severe             | 14   | 0.9  |

| Total | 1486 | 100  |

**Table 2: Relation between the response of patients treated with Peg/Riba with fibrosis, activity and Steatosis.**

| Fibrosis (No/%) | Activity (No/%) | Steatosis (No/%) |
|----------------|----------------|-----------------|
| SVR            | RLP            | SVR             | RLP            |
| No             | 18 (64.3)      | 10 (35.7)       | 385 (69.5)     | 169 (30.5)    |
| Mild           | 420 (63.4)     | 243 (36.6)      | 635 (67.1)     | 311 (32.9)    |
| Moderate       | 392 (59.3)     | 270 (40.7)      | 255 (51.7)     | 238 (48.3)    |
| Severe         | 72 (54.2)      | 72 (54.2)       | 12 (25.5)      | 35 (74.5)     |

2χ   | 4.19 | 40.467 | 26.82 |

P value | 0.04 (S) | 0.001 (HS) | 0.001 (HS) |

R     | -0.853 | -0.134 | -0.134 |

SVR: sustained virologic response, RLP: relapse

**Table 3: Relation between PCR at WK12 and fibrosis, steatosis and grade of activity.**

| Fibrosis     | No to Mild | Mod to Severe |
|--------------|------------|---------------|
| No           | 668 (96.7) | 755 (94.9)    |
| 2 log decrease | 23 (3.3)   | 40 (5.1)      |

2χ   | 8.317 | 0.004 |

| Steatosis    | No to Mild | Mod to Severe |
|--------------|------------|---------------|
| No           | 1341 (95.7)| 82 (94)       |
| 2 log decrease | 58 (4.3)   | 5 (6)         |

2χ   | 5.604 | 0.024 |

| Activity     | No to Mild | Mod to Severe |
|--------------|------------|---------------|
| No           | (mild only) | 517 (95.7)    |
| 2 log decrease | 90 (95.8)   | 23 (4.2)      |

2χ   | 0.027 | 0.869 |
More than 60% of the patients with no or mild fibrosis stage showed SVR, however, patients with moderate and severe fibrosis showed less rates of SVR. Patients with mild activity showed much higher SVR than patients with severe or advanced activity Table 2.

Negative viral load after 12 weeks of treatment by PCR was significantly higher in patients with no or mild fibrosis stage (668/96.7%), it decreased when the patient had moderate to severe fibrosis stage (755/94.9%) and vice versa with patients who only attained 2 log reduction by week 12 (23/3.3% compared to 40/5.1%) (p value: 0.004). The relation between grade of activity and the early virological response was not of statistical significance Table 3.

By studying the non-matched baseline significant predictors of SVR by logistic regression model, it revealed that serum albumin and alpha fetoprotein were not significant predictors Table 4.

Table 4: Predicted factors for response to Peg/Riba by logistic regression model.

| Predictor          | 95% CI       | Significance |
|--------------------|--------------|--------------|
| Age                | 0.008-0.14   | 0.01 (HS)    |
| Albumin            | - 0.059-0.057| 0.97 (NS)    |
| Platelets          | - 0.001-0.000| 0.016 (S)    |
| α fetoprotein      | - 0.001-0.007| 0.139 (NS)   |
| Creatinine         | - 0.484-0.270| 0.001 (HS)   |
| Stage of fibrosis  | - 0.040-0.005| 0.014 (S)    |
| Degree of Steatosis| 0.006-0.084  | 0.025 (S)    |
| RT PCR WK 12       | 0.078-0.308  | 0.001 (HS)   |
| T. Bil             | 0.021-0.188  | 0.014 (S)    |
| WBCs               | - 0.031-0.004| 0.012 (S)    |
| Hb                 | 0.010-0.043  | 0.001 (HS)   |
| AST (40)           | 0.000-0.001  | 0.001 (HS)   |
| RT-PCR baseline    | 0.030-0.143  | 0.024 (S)    |

Discussion

Because of logistical and economic issues, in Egypt, as in other resource-limited settings, also because of the availability or resistance to DAAs, decision makers should determine for which HCV patient treatment should be prioritized. For instance, immediate treatment of patients with mild to moderate fibrosis stage is less expensive and more effective than delaying treatment. However, immediate treatment at stage F1 is only slightly more effective than waiting for disease to progress to stage F2 before starting treatment and is sensitive to the forthcoming availability of new DAAs. On the other hand treating patients at stage F4 is less expensive and more effective than delaying treatment. Nevertheless, immediate treatment of patients with mild to moderate fibrosis is only slightly more effective than waiting for disease to progress to stage F2 before starting treatment and is sensitive to the forthcoming availability of new DAAs. On the other hand treating patients at stage F4 is highly effective and cost-effective [4].

Liver biopsy has been considered as the "gold standard" for defining liver disease status; it can be used to assess the degree of activity of an inflammatory process and the extent of fibrosis. The indications for this invasive technique must be weighed against the small, but not negligible, risk of a complication [5,6]. According to the study inclusion and exclusion criteria, we enrolled 1486 chronic HCV infected patients; all of them had completed the course of treatment (48 weeks).

Studying the pre-enrollment data revealed reliable and valuable information for treatment optimization. The age of the patient affected the response significantly in indirect manner; similar result was found in the review done by Reddy et al. [7]. However, in our results, gender did not influence the treatment outcome that was not the conclusion of the study done by Atsukawa et al. [8]. This controversy may be because the later study enrolled older patients [8].

Revising pre-treatment biochemical liver profile data has disclosed the significant inverse correlation between AST and the treatment outcome. As well, the serum Albumin and total bilirubin showed significant correlation with the patients’ response to treatment. Nevertheless, ALT was higher in relapsers group but that finding did not show statistical significance. Patients’ pretreatment hematological laboratory data showed significant relation with SVR. In particular, higher platelets count showed highly significant association with patient SVR. The Japanese researchers recognized that fact in their retrospective study, they found that the SVR rate of pretreatment platelet count < 130000/μL group was significantly lower than that of the pretreatment platelet count ≥ 130000/μL [9]. Pretreatment AFP showed lower levels in responders and higher levels in relapers. Zayed et al in 2013 published a decision tree model based on the pretreatment AFP value as an initial split variable at a cutoff of 8.08 ng/ml [10,11].

The type of interferon either alpha 2a or alpha 2b as a predictor of response was a point of debate most of the time [12]. In this study, we found that there was no statistical significance between patients who had either type of Interferon and their treatment outcome. On the contrary, El Raziky [13] and her colleagues found significantly higher EVR and SVR in patients treated with peginterferon alpha 2a [13]. Furthermore, Mauss et al. [14] in published a study to estimate the likelihood of achieving SVR and they found that if patients were matched by baseline characteristics, treatment with peginterferon alpha 2a may be a positive predictor of SVR when compared to peginterferon alpha 2b [14].

Baseline viral load was lower in our SVR group and it was higher in the relapsers, that observation showed statistical significance [14,15].

Early virological response is still one of the cornerstones for prediction and continuation of INF therapy in our study all of patients accomplished complete EVR and 4 % had only 2 log reduction. Around two thirds of the patients who achieved cEVR had SVR. These findings were statistically significant, which was in agreement with the study done by Chuang and his team [16]. The Saudi researcher who studied patients with HCV genotype 4, the predominant genotype in Saudi Arabia, she found that EVR is an excellent positive predictor factor for SVR [15].
Statistically, in our study, the cEVR was more achievable in patients who had no or mild stage of hepatic fibrosis. That finding was also observed in hepatic steatosis in which pEVR was related directly to the disease severity. On the contrary, cEVR and pEVR were almost the same in all grades of disease activity but that finding was not of statistical significance, that was in agreement of Tanta university; faculty of medicine investigators [17].

The effect of liver biopsy findings on the SVR is a very crucial question that should be answered clearly. We studied here 1486 percutaneous liver biopsies done by experts at the same institution with the same techniques and examined by two independent pathologists to avoid either sample and or reading errors. The percent of F0 and F6 patients were low, that is because the national treatment program does not support those patients. However, these small numbers also added a significant statistical relation, with a strong impact on liver biopsy findings and the overall patient response to Peg/Riba. First, comparing the fibrosis stages with the treatment outcome at week 72, we found obviously a descending slope pattern of the SVR whereas the fibrosis stage increases. That finding is confirmed by doing Spearman-rho method, and revealed a negative value meaning an indirect correlation between SVR and hepatic fibrosis stage [18].

In our pathological data, the grade of activity showed clear negative relation with the patients SVR. On the other hand, the highest percent of relapers could be seen in patients with severe activity. These findings are the same conclusion of the study done by Saad et al. [19] on 100 patients with chronic HCV genotype 4 [19].

Lastly, the steatosis degree was severe in almost two thirds of the relapers and around 70% of patients who had no steatosis could achieve SVR. This indirect relation was also the finding of Fouad et al comparative study [20].

Logistic regression analyses were used to determine the effect of non-matched baseline variables and confounding factors on SVR. That model showed that age of the patient, pretreatment platelets counts, AST, baseline RT-PCR and w12 RT-PCR were true predictors of SVR. But serum albumin and AFP were not significant predictors. Furthermore, stage of fibrosis and degree of steatosis are negative predictors for SVR.

These study findings are important to decide antiviral course in patients with multiple DAA RAVs leading to failure. As for those who have NSSA RAVs but have no NS3 RAVs (eg, Q80K), simeprevir plus sofosbuvir plus ribavirin for 24 weeks is recommended. For those who can tolerate interferon, the addition of peginterferon may further enhance efficacy of these regimens [21]. For those with NSSA RAVs and NS3 RAVs, the only choice after DAA failure due to multiple mutations would be Peg/Riba with possible addition of another class of DAA.

Also, for patients with recurrent HCV infection following liver transplantation, sofosbuvir plus RBV and PEG-IFN are used. After week 12 of treatment, 91% of patients treated with sofosbuvir plus RBV and 75% of those treated with the addition of PEG-IFN achieved HCV RNA levels below the lower limit of quantification [21].

Conclusion

The liver biopsy findings namely steatosis, fibrosis and activity could directly affect the treatment decision as it could affect the treatment outcome, besides it helps decision makers to put a national wide treatment strategy. This strategy should be tailored for Egyptian patients and disease burden, and using available facilities to overcome HCV. These findings are important to decide antiviral course by Peg/Riba in patients with multiple DAA RAVs leading to failure.

The biopsy experiences in our study mostly belong to treatment with Peg/Riba regimen instead of DAA and currently although the SVR rate with DAA is more than 90%, but a notable difference is observed in SVR, duration of therapy and side effects between cirrhotics and non-cirrhotics.

Not only the HCV infection treatment offers blazing new trails but also non-invasive tests to evaluate liver stage of fibrosis and degree of steatosis do. Guidelines are on the way for approving those non-invasive tests, so the real role of liver biopsy is declining as an aggressive method.

References

1. Lefkowitch JH (2007) Liver biopsy assessment in chronic hepatitis. Arch Med Res 38(6): 634-643.
2. Kleiner DE (2005) The Liver Biopsy in Chronic Hepatitis C: A View from the Other Side of the Microscope. Semin Liver Dis 25(1): 52-64.
3. Zayed H (2014) Minister of Health announces new treatment guidelines for HCV patients: enrollment of patients with 3rd and 4th stages of hepatic fibrosis in 26 centers. Al-Ahram newspaper.
4. Obach D, Deuffic Burban S, Esmat G, Anwar W A, Dewedar S, et al. (2014) Effectiveness and Cost-effectiveness of Immediate Versus Delayed Treatment of Hepatitis C Virus-Infected Patients in a Country With Limited Resources: The Case of Egypt. Clin Infect Dis, 58(6).
5. Khair M, Abdel Rahman M, El Raziky M, El Akel W, Zayed, et al. (2012) Non-invasive prediction of hepatic fibrosis in patients with chronic HCV based on the routine pre-treatment workup. Hepat Mon 12(11): e6710.
6. Tannapfel A, Dienes HP, Lohse AW (2012) The indications for liver biopsy. Dtsch Arztebl Int 109(27-28): 477-483.
7. Reddy KR, Lin F, Zoulim F (2012) Response-guided and -unguided treatment of chronic hepatitis C. Liver Int 32 Suppl 1: 64-73.
8. Atsukawa M, Tsibota A, Shimada N, Kondo C, Itokawa N, et al. (2013) Efficacy of Alfalcacidol on PEG-IFN/ Ribavirin Combination Therapy for Elderly Patients With Chronic Hepatitis C: A Pilot Study. Hepat Mon 13(12): e14872.
9. Kanda T, Kato K, Tsibota A, Takada N, Nishino T, et al. (2013) Platelet count and sustained virological response in hepatitis C treatment. World J Hepatol 5(4): 182-188.
10. Mabrouk M, El Raziky M, Zayed N, Salama R, El Akel W, et al. (2013) Clinical, biochemical and pathological profiles of 5464 Egyptian chronic hepatitis C-infected patients. Hepatogastroenterology 60(127): 1731-1735.

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11. Zayed N, Awad AB, El Akel W, Doss W, Awad T, et al. (2013) The assessment of data mining for the prediction of therapeutic outcome in 3719 Egyptian patients with chronic hepatitis C. Clin Res Hepatol Gastroenterol 137(3): 254-261.

12. Esmat G, El Kassas M, Hassany M, Gamel M, El Raziky M (2014) Optimizing treatment for HCV genotype 4: PEG-IFN alfa 2a vs. PEG-IFN alfa 2b; the debate continues. Liver Int 34 (Suppl 1): 24-28.

13. El Raziky M, Fathalah WE, El Akel WA, Salama A, Esmat G, et al. (2013) The Effect of Peginterferon Alpha-2a vs. Peginterferon Alpha-2b in Treatment of Naive Chronic HCV Genotype-4 Patients: A Single Centre Egyptian Study. Hepat Mon 13(5): e10069.

14. Mauss S, Hueppe D, John C, Goelz J, Heyne R, et al. (2011) Estimating the likelihood of sustained virological response in chronic hepatitis C therapy. J Viral Hepat 18(4): e81-e90.

15. Ismail MH (2013) Prediction of sustained virologic responses to combination therapy of pegylated interferon-alpha and ribavirin in patients with chronic hepatitis C infection. J Family Community Med 20(1): 35-40.

16. Chuang WL, Yu ML (2013). Host factors determining the efficacy of hepatitis C treatment. J Gastroenterol Hepatol 48(1): 22-30.

17. Ziada DH, El Saadany S, Enaba M, Ghazy M, Hasan A (2012) The interaction between insulin resistance, liver fibrosis and early virological response in Egyptian patients with chronic hepatitis C. Can J Gastroenterol 26(6): 325-329.

18. El Raziky M, Attia D, El Akel W, Shaker O, Khatab H, et al. (2013) Hepatic fibrosis and serum alpha-fetoprotein (AFP) as predictors of response to HCV treatment and factors associated with serum AFP normalisation after treatment. Arab J Gastroenterol 14(3): 94-98.

19. Saad Y, Ahmed A, Saleh DA, Doss W (2013) Adipokines and insulin resistance, predictors of response to therapy in Egyptian patients with chronic hepatitis C virus genotype 4. Eur J Gastroenterol Hepatol 25(8): 920-925.

20. Fouad A, Sabry D, Ahmed R, Kamal M, Allah SA, et al. (2013) Comparative diagnostic study of biomarkers using FibroMax TM and pathology for prediction of liver steatosis in patients with chronic hepatitis C virus infection: an Egyptian study. Int J Gen Med 6 127-134.

21. AASLD and IDSA guidelines (2016) Recommendations for Testing, Managing, and Treating Hepatitis C.