Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: https://www.tandfonline.com/loi/iplt20

Improvement of platelet in thrombocytopenic HCV patients after treatment with direct-acting antiviral agents and its relation to outcome

Zeinab Soliman, Mohamed El Kassas, Aisha Elsharkawy, Mohamed Elbadry, Yasser Hamada, Ramy ElHusseiny, Saeed M. El-Nahaas, Rabab Fouad, Gamal Esmat & Shereen Abdel Alem

To cite this article: Zeinab Soliman, Mohamed El Kassas, Aisha Elsharkawy, Mohamed Elbadry, Yasser Hamada, Ramy ElHusseiny, Saeed M. El-Nahaas, Rabab Fouad, Gamal Esmat & Shereen Abdel Alem (2020): Improvement of platelet in thrombocytopenic HCV patients after treatment with direct-acting antiviral agents and its relation to outcome, Platelets, DOI: 10.1080/09537104.2020.1742313

To link to this article: https://doi.org/10.1080/09537104.2020.1742313

Published online: 06 Apr 2020.

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

platelets

Platelets, Early Online: 1–8
 DOI: https://doi.org/10.1080/09537104.2020.1742313

Taylor & Francis
Taylor & Francis Group

Check for updates

Improvement of platelet in thrombocytopenic HCV patients after treatment with direct-acting antiviral agents and its relation to outcome

Zeinab Soliman[1], Mohamed El Kassas[2], Aisha Elsharkawy[1], Mohamed Elbadry[3], Yasser Hamada[1], Ramy ElHusseiny[4], Saeed M. El-Nahaas [1], Rabab Fouad[1], Gamal Esmat[1,5], & Shereen Abdel Alem [1]

[1]Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University, Cairo, Egypt, [2]Endemic Medicine Department, Faculty of Medicine, Helwan University, Cairo, Egypt, [3]Tropical Medicine and Gastroenterology Department, Aswan University, Aswan, Egypt, [4]Internal Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt, and [5]Department of research development, Badr University, Cairo, Egypt

Abstract

Little is known about evolution of platelet count after treatment with direct-acting antiviral agents (DAAs). The study aimed to evaluate the changes in platelet count after treatment with DAAs among thrombocytopenic patients with HCV-related advanced fibrosis and cirrhosis. A total of 915 chronic HCV patients with advanced fibrosis and cirrhosis who were treated with different DAAs-based regimens were retrospectively enrolled in final analysis. Included patients were those with thrombocytopenia (TCP). Platelet count was recorded at baseline, end of treatment (EOT) and 24-weeks after EOT (SVR24). Changes in platelet count and its relation to SVR were analyzed. The overall SVR24 rate was 98.8%. The platelet count showed statistically significant improvement from baseline to EOT (107 (84–127) $\times 10^3$/mm^3 vs. 120 (87–153) $\times 10^3$/mm^3(P = <0.0001) but remained unchanged thereafter to SVR24. Among responders, the platelet count significantly increased at SVR24 compared to baseline (P = <0.0001) but in relapsers, there was improvement in platelet count that didn't reach statistical significance (P = 0.9). Logistic regression analysis showed that higher Child-Pugh score and more advanced fibrosis at baseline were significant predictors of decreasing of platelet count and development of severe TCP at SVR24. Among thrombocytopenic patients with HCV-related advanced fibrosis and cirrhosis, the platelet count improved after treatment with DAAs regardless to treatment response.

Keywords

Advanced fibrosis, chronic hepatitis C virus, direct-acting antiviral agents, outcome, platelets, thrombocytopenia

History

Received 13 November 2019
Revised Vxx xxx xxxx
Accepted 21 February 2020
Published online xx xxx xxxx

Introduction

Chronic hepatitis C (HCV) infection has been associated with the development of a number of extrahepatic manifestations including thrombocytopenia (TCP) which is one of the most common hematological abnormalities. It is defined as a platelet count below 150 $\times 10^3$/mm^3 [1].

TCP in chronic HCV infection is complex and has a multifactorial pathogenesis as hypersplenism secondary to portal hypertension, decreased production or activity of thrombopoietin (TPO), autoimmune disarrangement and virus-induced bone marrow suppression [2–5]. Therefore, TCP is a frequent manifestation in chronic liver disease (CLD) and serves as a surrogate marker of disease severity and portal hypertension [6].

In the IFN-based therapy era, TCP represented one of the challenging obstacles to initiate or maintain antiviral therapy in chronic HCV (CHC) patients. Moreover, TCP is one of the well-known major side effects of IFN-based therapy, especially among patients with cirrhosis. This frequently necessitates dose reductions or early treatment discontinuation that could potentially decrease the likelihood of sustained virological response and increase the risk for hepatic decompensation or death [7,8].

Correspondence: Shereen Abdel Alem, Faculty of Medicine, Cairo University, Cairo 11562, Egypt. E-mail: shery_2424@yahoo.com

The recent introduction of directly acting antiviral agents (DAAs) in the treatment of chronic HCV offers a high cure rates and with nearly no adverse effects [9]. Minimal hematologic abnormalities including TCP was reported in not more than 1% of cases [10,11] compared to the previously occurring with the standard interferon-based treatments.

The aim of the study is to evaluate the changes in platelet count after treatment with different DAAs-based regimens among a large cohort of thrombocytopenic patients with chronic HCV-related advanced hepatic fibrosis and cirrhosis and its relation to outcome as well as the factors associated with an improvement or worsening of platelet count after treatment.

Materials and Methods

Patient Population

A total of 1790 patients with HCV-related CLD who received different DAAs–based treatment regimens were evaluated for eligibility for inclusion in this retrospective study. These patients were ≥18 years of age with seropositivity for HCV antibodies and detectable HCV RNA for more than 6 months. Included patients also had TCP, which was defined as a platelet count below 150 x 10^3/mm^3 in addition to advanced liver fibrosis (F3) or compensated cirrhosis (F4) (Child-Pugh score A5 up to Child-Pugh score B8). Only

patients with available platelet count at baseline, the end of treatment (EOT) and week 24 after the EOT (SVR24) were enrolled. Patients with hepatitis B virus or human immunodeficiency virus infection co-infection, patients with Child-Pugh score B9 and child-Pugh C cirrhosis, idiopathic thrombocytopenic purpura or other hematological disorders, and a history of hepatocellular carcinoma or extra–hepatic malignancies were excluded. Therefore, a total of 915 patients fulfilled the inclusion and exclusion criteria and were included in the statistical evaluation. Patients were further classified into two groups according to baseline platelet count: Group (1) patients with moderate TCP (platelet count $\leq 100 \times 10^3/mm^3$) (n = 402) and Group (2) patients with mild TCP (platelet count $>100 \times 10^3/mm^3$) (n = 513). None of our patients had severe TCP (platelet count $<50 \times 10^3/mm^3$) because these patients had either Child-Pugh B9, child-Pugh C cirrhosis or hepatic decompensation at baseline.

Patients underwent complete medical history and clinical examination with special emphasis on stigmata of liver cell failure such as jaundice, lower limb edema, ascites, and hepatic encephalopathy. Relevant laboratory parameters were measured and reported such as complete blood counts, liver biochemical profile (total serum bilirubin, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and international normalized ratio [INR]), renal function tests (serum urea and creatinine), alpha feto-protein (AFP), and HBsAg. Abdominal ultrasound was performed to assess the liver size and echopattern (ultrasonographic features of cirrhosis), portal vein diameter and to exclude ascites, hepatocellular carcinoma or other comorbidities. Ultrasound transient elastography (TE) (Echosense, Fibroscan 502, Paris, France) examination was done before treatment and according to the manufactures instructions [12] to assess the hepatic fibrosis stage taking into consideration the reliability criteria of at least 10 valid measurements with success rate >60% and interquartile range (IQR) <30%. Patients with liver stiffness measurements (LSM) of ≥9.5 kPa (advanced fibrosis F3) and ≥12.5 kPa (cirrhosis F4) were included in this study [13]. Liver cirrhosis was diagnosed depending on the clinical, laboratory, ultrasonographic criteria of liver cirrhosis and/or the results of TE.

Noninvasive biomarkers for liver fibrosis such as Fibrosis-4 score (FIB-4) and AST-to platelet ratio index (APRI) were calculated at baseline and SVR24 based on the following formula:

- FIB-4 score was calculated using Sterling's formula [14]:

Age (y) × AST (IU/l)/platelet count ($\times 10^9$/liter) × $\sqrt{}$ALT (IU/l)).

- APRI score was calculated using Wai's formula [15]:

(AST/upper limit of normal)/platelet count (expressed as platelets $\times 10^9$/L) × 100.

Serum HCV RNA level was measured at baseline, EOT and 24 weeks after EOT (SVR24) using PCR-based method (Cobas Amplicor, HCV Roche, Branchburg, NJ, USA, v 2.0, detection limit 15 IU/mL). Sustained virological response (SVR24) was defined as an undetectable HCV RNA at 24-week post-treatment follow-up, which was an indicative of therapeutic success.

Platelet count was assessed at baseline, EOT and 24-week after EOT (SVR24). The change in the platelet count at SVR24 was calculated as follows; Percent change of platelet = ((Platelet count_SVR24-Baseline Platelet count)/Baseline platelet) x100

The study was reviewed and approved by the Institutional Review Board (IRB) of Faculty of Medicine, Cairo University (N-38-2016). A written informed consent was taken from each patient before receiving treatment. The study was performed in compliance with the ethics principles of the 1975 Declaration of Helsinki and its later amendments with Good Clinical Practice (GCP) guidelines.

Statistical Analysis

Descriptive statistics were done; numerical data were presented as mean (SD), and categorical data as frequency and percentages. Numerical data were tested for normality using the Shapiro-Wilk normality test. Normally distributed paired samples were analyzed using the paired-samples T-test. Non-normally distributed paired samples were analyzed using the Wilcoxon signed-rank test. Correlation between the delta changes in platelet count with baseline parameters was done using the Spearman's rank correlation test. Logistic regression analysis evaluated which baseline parameters were associated with decrease of platelet count and development of severe TCP at SVR24. All statistical tests were two-sided, and a P-value < 0.05 was considered to be statistically significant. Statistical analysis was done using Statistics/Data Analysis (STATA) version 13.1 software.

Results

Out of 1790 chronic HCV patients with TCP enrolled for treatment, 915 patients were finally included in the analysis of our study according to the inclusion/exclusion criteria as shown in Figure 1. The demographic and laboratory features of patients are shown in Table I. There was a slight male predominance (52%)

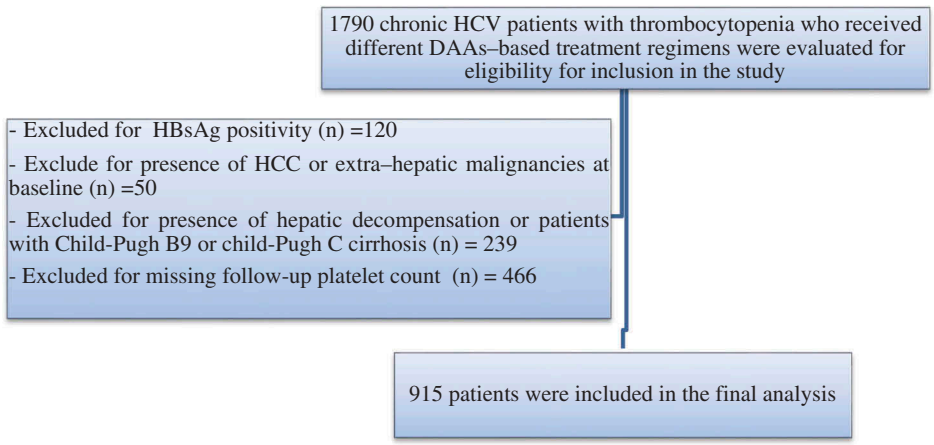

Figure 1. Flow chart of patient exclusion for this analysis.

Table I. Baseline characteristics of the whole study population (n = 915).

	Mean (SD), n (%)
Age, years	55.5 (8.2)
Gender, n (%)	
Male	473 (51.7%)
Female	442 (48.3%)
BMI (kg/m^2)	30.41 (5.42)
Treatment status, n (%)	
Treatment-naïve	691 (75.5%)
Treatment experienced	224 (24.5%)
Co-morbidities, n (%)	
Hypertension	177 (19.3)
Diabetes	241 (26.3)
Stage of hepatic fibrosis by Liver stiffness measurement, n (%)	
Advanced fibrosis (F3)	226 (24.7)
Cirrhosis (F4)	689 (75.3)
Baseline TE values (kPa)	25.3 (14.1)
Baseline FIB-4	5.62 (3.30)
Baseline APRI	2 (1.45)
Child–Pugh Class, n (%)	
A	637 (92.5)
B (up to score B8)	52 (7.5)
DAAs-treatment regimens, n (%)	
Sofosbuvir/Ribavirin	447 (48.9)
Sofosbuvir/Dacltastavir/Ribavirin	294 (32.1)
Sofosbuvir/Simepriver	120 (13.1)
Sofosbuvir/Dacltastavir	32 (3.5)
Ritonavir-boosted paritaprevir, ombitasvir plus ribavirin	19 (2.1)
Sofosbuvir/Ledipasvir/Ribavirin	3 (0.3)
Response at SVR12 as well as SVR24, n (%)	
Sustained responders	904 (98.8)
Relapsers	11 (1.2)
Sustained responders at SVR24 according to stage of hepatic fibrosis, n (%)	
Advanced fibrosis (F3)	226/226 (100)
Liver cirrhosis (F4)	678/689 (98.4)

Unless otherwise stated numerical data are expressed as mean (SD). Abbreviations: APRI: Aspartate aminotransferase-to-platelet ratio index; BMI: Body mass index; DAAs: direct-acting antivirals; FIB-4: Fibrosis 4 score; kPa: kilopascals; TE: Transient elastography; SVR12: sustained virological response at week 12 after end of therapy; SVR24: sustained virological response at week 24 after end of therapy

with a mean age of 55.5 (8.2) years. The majority of patients were treatment naïve (75.5%). The mean TE value was 25.3 (14.1) kPa; 24.7% had F3, while 75.3% were F4 (Child-Pugh A, 92.5%). Baseline mean FIB-4 and APRI was 5.6 (3.3) and 2 (1.5), respectively. All patients were adherent to the prescribed DAAs treatment regimen with sofosbuvir representing the backbone antiviral therapy (97.7%) and had a minimum follow-up of 6 months after the EOT. All patients had undetectable HCV PCR at EOT. The overall SVR24 rate was 98.8% (904/915 patients); The SVR24 rate was higher in patients with F3 stage compared with F4 stage (226/226, 100% vs 678/689, 98.4% respectively). Around 1.2% (11/915) of patients were relapsers, and all of them were cirrhotic (F4 stage). Moreover, the SVR rate in those with baseline platelet count $\leq 100 \times 10^3$ mm^3 was 97.8% (393/402) compared to 99.6% (511/513) in those with baseline platelet count $>100 \times 10^3$ mm^3.

The median platelet count showed statistical significant improvement from baseline to EOT (107 (84–127) $\times 10^3$/mm^3 vs. 120 (87–153) $\times 10^3$/mm^3 (*P*-value = <0.0001) but remained unchanged from EOT to SVR24 (120 (87–153) $\times 10^3$/mm^3 vs. 120 (90–152) x10^3/mm^3 (*P*-value = 0.6) as shown in Table II and Figure 2. similarly, serum transaminases ALT and AST showed statistically significant improvement from baseline to EOT (*P*-value = <0.0001) but they remained unchanged from EOT to SVR24. Upon subgroup analysis of patients with baseline platelet count below and above 100 $\times 10^3$ mm^3, similar patterns of changes in the median platelet count, serum transaminases (ALT and AST) over the same time period was observed in both groups as presented in Table II.

Similarly, both FIB-4 score and APRI decreased significantly at EOT compared to baseline but they remained unchanged from EOT to SVR24 among the whole study population and whatever the baseline platelet count is as shown in Table II.

Subgroup analysis for the changes of the median platelet count among different groups is shown in Table III. There was statistical improvement of platelet count from baseline to EOT and remained unchanged from EOT to SVR24 among different age group, in relation to stage of hepatic fibrosis and use of ribavirin. Among responders, the median platelet count was significantly increased at SVR24 compared to baseline (*P* = <0.0001) however in relapsers, the improvement in platelet count didn't reach statistical significance (*P* = 0.9)

24-week after EOT (SVR24), platelet count improved in 64.8% (586/904) of patients achieved SVR, stayed the same in 1.9% (17/904) and decreased in 33.3% (301/904) as in Table IV. Moreover, 29.9% (270/904) patients who achieved SVR didn't have TCP while 70.1% (634/904) patients had TCP at SVR24. Of those who had TCP, a total of 31/634 (4.8%) patients developed severe TCP (platelet count <50 $\times 10^3$/mm^3) at EOT and continued to SVR24. Those patients had lower baseline platelet count (66 (55–82) $\times 10^3$ mm^3 vs. 108 (85–128) $\times 10^3$ mm^3, *P* = <0.001), lower baseline ALT levels (43 (33–63) U/L vs. 56 (36–85) U/L, *P* = 0.05), and lower baseline albumin levels (3.4 (0.52) mg/dL vs. 3.71 (0.52) mg/dL, *P* = 0.0008) but higher baseline AST levels (62 (40–74) U/L vs. 61 (41–90) U/L, *P* = 0.4), higher bilirubin (1.3 (1.1–1.6) mg/dL vs. 0.9 (0.7–1.2) mg/dL, *P* = <0.001), higher INR (1.2 (1.1–1.4) vs. 1.1 (1.1–1.3), *P* = 0.01), higher FIB-4 (7.93 (4.58–9.73) vs. 4.66 (3.36–6.82), *P* = <0.001) and higher APRI (2.27 (1.68–3.30) vs. 1.59 (1.005–2.49), *P* = 0.002) than did the other 873 patients, who did not develop severe TCP.

There was a statistically significant positive correlation between baseline hemoglobin and serum albumin with the changes in platelet count (rho = 0.1; *P* = 0.04 and rho = 0.14; *P* = 0.0001, respectively) as shown in Table V.

Logistic regression analysis was done to identify baseline parameters that predict decrease in platelet count and severe TCP at SVR24. In the multivariable regression model, the presence of cirrhosis and higher platelet count were independent predictors for decreasing platelet count. In the subgroup of cirrhosis, higher Child-Pugh class and higher platelet count were independent predictors of decreasing in platelet count. On the other hand, the independent predictors of developing severe TCP were cirrhosis and platelet count <100(10^3/mm^3). Again, in the cirrhosis group, both more advanced fibrosis and higher Child-Pugh score were independent predictors of developing severe TCP as shown in Table VI.

Discussion

TCP is one of the most important extra-hepatic manifestations of chronic hepatitis C virus (HCV) infection that occurs in 64–76% of HCV patients with advanced fibrosis and/or cirrhosis, compared with 6% of non-cirrhotic patients [6,16]. It represents a major problem as it may decrease both therapeutic adherence and success rate.

This study was conducted to elucidate the changes in platelet count after treatment with different DAAs-based regimens among a large cohort of chronic HCV-infected thrombocytopenic

Table II. Changes of laboratory parameters and fibrosis markers at baseline, EOT, and SVR24.

		Total population (n = 915)	Baseline platelet count $\leq 100 \times 10^3$ mm^3 (n = 402)	Baseline platelet count $>100 \times 10^3$ mm^3 (n = 513)
Sustained responder, n (%)		904 (98.8%)	393 (97.8%)	511 (99.6%)
Laboratory parameters				
Hemoglobin (g/dL)	Baseline	13.3 (12–14.5)	12.8 (11.7–14)	13.7 (12.4–14.8)
	EOT	11.6 (10.5–12.7)	11.5 (10.4–12.5)	11.65 (10.6–12.9)
	SVR24	12.4 (11.2–13.8)	11.9 (10.8–12.9)	12.8 (11.5–14.3)
	P -value	**<0.0001***	**<0.0001***	**<0.0001***
		<0.0001**	**<0.0001****	**<0.0001****
White Blood cell (10^3/mm^3)	Baseline	4.7 (3.7–6.1)	4.3 (3.4–5.4)	5.1 (4–6.5)
	EOT	4.5 (3.6–6)	4.23 (3.4–5.5)	4.8 (3.7–6.3)
	SVR24	4.7 (3.6–5.98)	4.1 (3.3–5.5)	5.1 (4–6.3)
	P-value	**0.009***	0.4*	**0.01***
		0.3**	0.3**	**0.05****
Platelets (10^3/mm^3)	Baseline	107 (84–127)	80.5 (68–90)	125 (114–139)
	EOT	120 (87–153)	91 (73–125)	136 (112–168)
	SVR24	120 (90–152)	91 (70–120)	140 (113–162)
	P-value	**<0.0001***	**<0.0001***	**<0.0001***
		<0.6**	<0.9**	<0.6**
ALT (U/L)	Baseline	55 (36–84)	55 (34–80)	56 (37–87)
	EOT	21 (16–30)	21 (16–30)	22 (16–30)
	SVR24	21 (16–29)	21 (16–30)	21 (15–29)
	P-value	**<0.0001***	**<0.0001***	**<0.0001***
		0.01**	0.1**	**0.03****
AST (U/L)	Baseline	61 (41–88)	64 (45–93)	58 (39–85)
	EOT	29 (22–37)	31 (24–37)	28 (21–36)
	SVR24	28 (22–35)	30 (23–37)	27 (21–34)
	P-value	**<0.0001***	**<0.0001***	**<0.0001***
		0.0004**	**0.05****	**0.002****
Total bilirubin (mg/dl)	Baseline	0.9 (0.7–1.2)	1 (0.8–1.3)	0.8 (0.64–1.1)
	EOT	1 (0.7–1.5)	1.2 (0.8–1.74)	0.9 (0.67–1.25)
	SVR24	0.85 (0.6–1.2)	1 (0.8–1.6)	0.7 (0.58–1)
	P-value	**<0.0001***	**<0.0001***	**<0.0001***
		<0.0001**	**0.001****	**<0.0001****
Albumin (g/dL)	Baseline	3.7 (0.51)	3.6 (0.52)	3.79 (0.48)
Mean (SD)	EOT	3.8 (0.53)	3.58 (0.5)	3.87 (0.51)
	SVR24	4 (1.49)	3.74 (0.52)	4.10 (1.89)
	P-value	**0.003***	0.2*	**0.01***
		0.01**	**<0.0001****	0.07**
INR	Baseline	1.12 (1.1–1.3)	1.2 (1.1–1.4)	1.1 (1–1.2)
	EOT	1.2 (1.07–1.28)	1.2 (1.1–1.35)	1.15 (1.04–1.24)
	SVR24	1.2 (1.09–1.29)	1.2 (1.12–1.34)	1.15 (1.1–1.24)
	P-value	0.3*	0.08*	0.8*
		0.3**	0.7**	0.3**
AFP (ng/ml)	Baseline	9 (5–18)	10 (5–20)	9 (5–17)
	SVR24	5.1 (3.3–7.6)	5.3 (3.6–8.8)	5.1 (3–7.4)
	P- value	**<0.0001**	**<0.0001**	**<0.0001**
Noninvasive parameters				
FIB-4	Baseline	4.71 (3.38–6.98)	6.7 (4.7–9.02)	3.84 (2.9–5.1)
	EOT	2.93 (1.96–4.25)	3.7 (2.66–5.24)	2.4 (1.72–3.36)
	SVR24	2.73 (1.94–4.10)	3.7 (2.64–5.2)	2.37 (1.8–3.2)
	P-value	**<0.0001***	**<0.0001***	**<0.0001***
		0.2**	0.9**	0.1**
APRI	Baseline	1.6 (1.01–2.52)	2.3 (1.58–3.20)	1.2 (0.87–1.84)
	EOT	0.7 (0.46–1.09)	0.9 (0.58–1.32)	0.56 (0.39–0.85)
	SVR24	0.6 (0.43–0.97)	0.85 (0.57–1.25)	0.53 (0.37–0.73)
	P- value	**<0.0001***	**<0.0001***	**<0.0001***
		0.0003**	**0.04****	**0.004****

Numerical data presented as median (IQR) unless otherwise specified. Abbreviations: AFP: alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio index; EOT: End of treatment; FIB-4: Fibrosis 4 score; INR: International normalized ratio; SVR24: sustained virological response at week 24 after end of therapy. Statistically significant values are in bold.
**P*-value between baseline and EOT.
***P*-value between EOT and SVR24

patients with advanced hepatic fibrosis and/or cirrhosis, including those with mild and moderate TCP and its relation to outcome as well as the factors associated with an improvement or worsening of platelet count after treatment.

Adherence to antiviral therapy is crucial to achieve a therapeutic effect. In historical era of interferon, severe TCP was associated with premature discontinuation of antiviral therapy that might result in lower rate of SVR. In the present study,

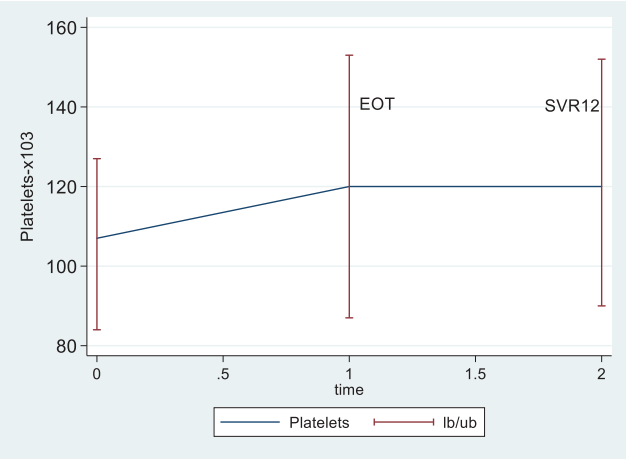

Figure 2. Changes of platelet count at different time point.

Table IV. Proportion of HCV-infected thrombocytopenic patients who achieved SVR with improvement, no change or worsening of platelet count at 24 weeks after end of treatment (SVR24).

	Platelet count improved at SVR24	Platelet count stayed the same at SVR24	Platelet count declined at SVR24	*P-value*
Total patients achieved SVR, n (%)	586 (64.8%)	17 (1.9%)	301 (33.3%)	-
Degree of fibrosis				
Advanced fibrosis (F3), n (%)	158 (27%)	6 (35.3%)	62 (20.6%)	0.07
Liver cirrhosis (F4), n (%)	428 (73%)	11 (64.7%)	239 (79.4%)	

our thrombocytopenic patients were adherent to the prescribed DAAs treatment regimen as well as treatment duration although 4.8% (31/634) of our patients experienced severe TCP during DAAs therapy but it wasn't associated with premature discontinuation of therapy. In addition, TCP in HCV-infected patients with advanced liver disease has been well-known as an independent risk factor for developing of hepatic decompensation and death [17]. However, none of our patients reported clinical decompensation during DAAs-based antiviral therapy and there were no deaths reported during treatment or follow-up periods which might also lead to premature discontinuation of therapy. Thus, our data confirm that TCP is no longer considered a limiting indication to initiate or maintain DAAs–based antiviral therapy. Therefore, DAAs–based therapy is generally safe and well tolerated in this population.

Sustained virological response (SVR) is the primary goal of antiviral therapy that has been associated with important clinical benefits and outcomes. Our results showed that TCP did not reduce the chance of achieving SVR rate as overall SVR24 was 98.8% and it was higher in patients with F3 stage compared to F4 stage (100% vs. 98.4%). Moreover, SVR24 rate was 97.8 and 99.6% among patients with baseline platelet count$\leq 100 \times 10^3$ mm^3 and patients with baseline platelet count$>100 \times 10^3$ mm^3, respectively. Therefore, Patients with TCP who are with advanced liver fibrosis and cirrhosis are likely to achieve a higher rate of SVR following DAAs-based therapy that will in turn result in lower rate of liver related mortality as well as a reduced risk of hepatocellular carcinoma [18–20].

TCP increases the risk of bleeding during invasive diagnostic procedures such as liver biopsy and percutaneous ethanol injection therapy or worsens the prognosis in patients with variceal bleeding so TCP considered an obstacle for both diagnosis and treatment. An increase in platelet count after antiviral therapy seems to be very important for diagnosis and treatment of HCV-related complications including HCC and gastroesophageal varices. Therefore, the main result of our study showed that there was a short-term significant improvement of platelet count after treatment with DAAs-based regimens among thrombocytopenic patients with advanced liver fibrosis or cirrhosis regardless to subgroup analysis. This was in agreement with earlier studies during interferon-based therapy which showed that platelet count significantly increased among CHC patients with advanced liver

Table III. Changes of platelet count among different groups at different time point.

	Baseline	EOT	SVR24	*P*-value
Age				
Age <60 years (n = 625)	105 (81–126)	116 (84.5–152)	118 (86.5–150)	**<0.0001*** 0.9**
Age ≥60 years (n = 290)	109 (90–133)	125 (91–154)	125 (94–157)	**<0.0001*** 00.5**
Stage of hepatic fibrosis				
Advanced fibrosis (F3), n = 226	128 (112–140)	147.5 (120–191)	146 (118–177)	**<0.0001*** 0.3**
Liver cirrhosis (F4), n = 689	98 (79–120)	111 (82–142)	110 (84–145)	**<0.0001*** 0.9**
Ribavirin_containing regimens				
Ribavirin_containing regimens, n = 763	106 (83–127)	120 (88–154)	120 (89–151)	**<0.0001*** 0.2**
Ribavirin_free regimens, n = 152	110.5 (87–131)	118.5 (83–148)	119.5 (91–152)	**0.0003*** 0.2**
Response				
Sustained responder, n = 904	107.5 (84–127.5)	-	120 (90–152)	**<0.0001**
Relapsers, n = 11	88 (79–100)	-	95 (69–120)	0.9

Numerical data presented as median (IQR) unless otherwise specified. Abbreviations: EOT: End of treatment; SVR24: sustained virological response at week 24 after end of therapy. Statistically significant values are in bold.
**P*-value between baseline and EOT.
***P*-value between EOT and SVR24

Table V.: Correlation between the changes in platelet count with baseline parameters.

	Rho	*P*-value
Age	−0.03	0.4
Baseline HCV-RNA	0.01	0.8
Baseline ALT	−0.008	0.8
Baseline AST	−0.05	0.1
Baseline Hemoglobin	0.1	**0.04**
Baseline serum albumin	0.14	**0.0001**
Baseline serum bilirubin	−0.03	0.4
Baseline FIB-4	−0.05	0.1
Baseline liver stiffness	0.05	0.4
Baseline AFP	−0.01	0.7
Use of ribavirin	0.01	0.7

Abbreviations: AFP: alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FIB-4: Fibrosis 4 score. Statistically significant values are in bold.

disease who were successfully eradicated HCV infection and the maximum increase in platelet count was observed after 6 months of antiviral treatment [21–23]. In historical interferon era, elimination of HCV-associated bone marrow suppression, decrease in portal congestion brought by antiviral therapy, increasing TPO levels after HCV eradication and the abolishment of immune-mediated phenomenon might be responsible for the short-term improvement of platelet count following antiviral therapy [24]. TPO is produced mainly by the liver [25] and is involved at all stages of the process to regulate the maturation of megakaryocytes and release platelets [26]. It was also found that liver function is associated with TPO levels and the production of TPO has been negatively correlated with the degree of hepatic fibrosis [27,28]. Recently, a study by Honma et al demonstrated that platelet count increases significantly after HCV eradication in non-cirrhotic patients following DAAs therapy and it might be correlated with a decrease in platelet-associated immunoglobulin G (PA-IgG), thereby implicating an HCV-associated immune mechanism in the genesis of TCP [29]. Another recent study also reported that the increase in platelet count was only significant in HCV patients with cirrhosis and there was a tendency for platelet to increase in patients without cirrhosis following treatment with DAAs indicating that improvement in platelet count after HCV elimination is most likely due to viral elimination itself [30]. However, further studies are needed to determine the possible mechanisms of increasing platelets following DAAs-based therapy.

Despite ribavirin was one of the proposed mechanisms of treatment-related TCP in the era of interferon-based therapy and was used cautiously in thrombocytopenic patients [31]. However, the majority of our patients in this study were treated with ribavirin–containing regimens. We noticed that there was a significant improvement of platelet count from baseline to EOT and remained unchanged from EOT to SVR24 in these patients with not yet known mechanisms. Therefore, further studies with long term follow-up are needed to determine the impact of ribavirin on platelet count in thrombocytopenic HCV patients in DAAs-based therapy era.

The rapid improvement of platelet count after starting antiviral treatment has important clinical significance. Platelet-related indices (AST/platelet ratio index [APRI] or fibrosis-4 score [FIB-4]) have been developed to evaluate the degree of hepatic fibrosis and have been proposed as a noninvasive alternative to liver biopsy in patients with CHC [32]. Our study demonstrated that there was improvement of hepatic necroinflammation, which evidenced by decreased transaminase (ALT, AST) levels and it was correlated with increased platelet count over the same time period at EOT and 24-weeks after EOT (SVR24) in comparison

Table VI. Logistic regression analysis of baseline variables that predict decrease in platelet count and development of severe thrombocytopenia at SVR24.

	Decrease in platelet count				*Severe thrombocytopenia*			
	Univariable		*Multivariable*		*Univariable*		*Multivariable*	
	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*	OR (95% CI)	*P-value*
Age	1.01 (0.99–1.03)	0.3	-	-	0.98 (0.94–1.02)	0.3	-	-
Gender Female	1.17 (0.89–1.54)	0.2	-	-	0.76 (0.37–1.58)	0.5	-	-
Baseline AST (U/L)	1.001 (0.99–1.004)	0.4	-	-	0.99 (0.98–1.005)	0.4	-	-
Baseline ALT (U/L)	0.99 (0.99–1.002)	0.7	-	-	0.99 (0.98–0.99)	0.04	0.99 (0.97–1.001)	0.06
Baseline HCV RNA (IU/mL)	1.001 (0.85–1.18)	0.9	-	-	0.94 (0.61–1.46)	0.8	-	-
Baseline cirrhosis	1.47 (1.05–2.05)	**0.02**	1.86 (1.30–2.67)	**0.001**	4.92 (1.16–20.78)	**0.03**	2.46 (1.50–4.05)	**<0.0001**
Baseline Child_Pugh score	1.28 (1.01–1.62)	**0.04**	1.41 (1.10–1.81)	**0.006**	2.98 (1.83–4.86)	**>0.0001**	2.47 (1.51–4.05)	**<0.0001**
Baseline platelet count (per unit increase)/	1.005 (1.001–1.01)	**0.03**	1.01 (1.005–1.02)	**>0.0001**				
Baseline platelets >100 (10^3/mm^3)*	-	-	-	-	12.73 (3.84–42.18)	**>0.0001**	9.71 (2.27–41.62)	**0.002**
Ribavirin_containing regimens	0.87 (0.61–1.26)	0.5	-	-	1.89 (0.57–6.30)	0.3	-	-
Treatment duration 24 Weeks	0.76 (0.57–1.0005)	0.05	-	-	0.73 (0.36–1.50)	0.4	-	-

*Baseline platelet count <100 (10^3/mm^3) was predictive for severe thrombocytopenia only. In multivariable regression, this data represent 2 different models, one for platelet & cirrhosis and the other for platelet and Child-Pugh class in the subgroup of cirrhosis.
Statistically significant values are in bold.

to baseline. Concurrent with the changes in transaminase (ALT, AST) levels and platelet count, fibrosis scores such as FIB-4 and APRI values decreased over the same time period denoting improvement of hepatic fibrosis. Our finding was consistent with previous studies, which reported that early improvement of fibrosis scores such as FIB-4 and APRI due to significant improvement of platelet count and hepatic necroinflammation along with improvement of TE values following treatment with DAAs and this was more pronounced among cirrhotic patients [33–35] but the histological reversibility of liver fibrosis after HCV elimination by antiviral therapy was proven to be very slow [36]. Platelet count and platelet-related indices [APRI or FIB-4] may represent an easily accessible biomarker to assess fibrosis improvement after antiviral therapy, however, their short-term improvement after antiviral treatment does not indicate an actual improvement of histological liver fibrosis. Thus, we should consider that improvement in the liver fibrosis can be overestimated.

Our data demonstrate that the presence of higher Child-Pugh score and more advanced fibrosis at baseline were significant predictors of decreasing of platelet count and development of severe TCP at SVR24. Thus, the degree of liver disease is considered to be the most important predictive factor either for improvement or worsening of platelet count following antiviral treatment.

Limitations of the study including the retrospective nature of the study, the impact of portal pressure or splenomegaly on platelet count wasn't investigated, and laboratory parameters such as TPO and autoantibodies to platelet weren't measured to assess their relationships with platelet count following DAAs therapy. Thus, further studies with long term follow-up are needed to determine the potential mechanisms of increasing platelet count following DAAs-based therapy in thrombocytopenic patients.

In conclusion, DAAs-based therapy is well tolerated as well as highly effective in thrombocytopenic patients with HCV-related advanced hepatic fibrosis and cirrhosis and this results in improvement of platelet count. This improvement of platelet count is most likely due to viral elimination itself. However, long-term effects of DAAs therapy on platelet count in this population need further study.

Conflict of interest

Gamal Esmat: speaker, advisory board member and investigator for Gilead Science while all other authors: nothing to be declared.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ORCID

Saeed M. El-Nahaas http://orcid.org/0000-0002-8548-8978
Shereen Abdel Alem http://orcid.org/0000-0002-3612-0130

References

1. Karasu Z, Tekin F, Ersoz G, Gunsar F, Batur Y, Ilter T, Akarca US. Liver fibrosis is associated with decreased peripheral platelet count in patients with chronic hepatitis B and C. Dig Dis Sci 2007;52 (6):1535–1539. doi:10.1007/s10620-006-9144-y.
2. Giannini E, Borro P, Botta F, Fumagalli A, Malfatti F, Podesta E, Romagnoli P, Testa E, Chiarbonello B, Polegato S. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. J Hepatol 2002;37 (5):572–577. doi:10.1016/S0168-8278(02)00274-X.
3. Olariu M, Olariu C, Olteanu D. Thrombocytopenia in chronic hepatitis C. J Gastrointest Liver Dis 2010;19:381–385.
4. Pradella P, Bonetto S, Turchetto S, Uxa L, Comar C, Zorat F, De Angelis V, Pozzato G. Platelet production and destruction in liver cirrhosis. J Hepatol 2011;54(5):894–900. doi:10.1016/j.jhep.2010.08.018.
5. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, Esteban R. Thrombocytopenia associated with chronic liver disease. J Hepatol 2008;48(6):1000–1007. doi:10.1016/j.jhep.2008.03.009.
6. Giannini EG. Review article: thrombocytopenia in chronic liver disease and pharmacologic treatment options. Aliment Pharmacol Ther 2006;23(8):1055–1065. doi:10.1111/apt.2006.23.issue-8.
7. Reiberger T, Rutter K, Ferlitsch A, Payer BA, Hofer H, Beinhardt S, Kundi M, Ferenci P, Gangl A, Trauner M. Portal pressure predicts outcome and safety of antiviral therapy in cirrhotic patients with hepatitis C virus infection. Clin Gastroenterol Hepatol 2011;9 (7):602–608. doi:10.1016/j.cgh.2011.03.002.
8. Maan R, van der Meer AJ, Hansen BE, Feld JJ, Wedemeyer H, Dufour J-F, Zangneh HF, Lammert F, Manns MP, Zeuzem S. Effect of thrombocytopenia on treatment tolerability and outcome in patients with chronic HCV infection and advanced hepatic fibrosis. J Hepatol 2014 Sep;61(3):482–491. doi:10.1016/j.jhep.2014.04.021.
9. Faria R, Woods B, Griffin S, Palmer S, Sculpher M, Ryder SD. Prevention of progression to cirrhosis in hepatitis C with fibrosis: effectiveness and cost effectiveness of sequential therapy with new direct-acting antivirals. Aliment Pharmacol Ther 2016 Oct;44 (8):866–876. doi:10.1111/apt.2016.44.issue-8.
10. Feld JJ, Jacobson IM, Hezode C, Asselah T, Ruane PJ, Gruener N, Abergel A, Mangia A, Lai C-L, Chan HLY. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. N Engl J Med 2015;373(27):2599–2607. doi:10.1056/NEJMoa1512610.
11. Foster GR, Afdhal N, Roberts SK, Brau N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 infection. N Engl J Med 2015;373(27):2608–2617. doi:10.1056/NEJMoa1512612.
12. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705–1713. doi:10.1016/j.ultrasmedbio.2003.07.001.
13. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, de Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128 (2):343–350. doi:10.1053/j.gastro.2004.11.018.
14. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner JS, Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL. Development of a simple noninvasive index to predict significant fibrosis inpatients with HIV/HCV co-infection. Hepatology 2006;43(6):1317–1325. doi:10.1002/()1527-3350.
15. Wai,C.-T., Greenson,J.K., Fontana, R.J., Kalbfleisch,J.D., Marrero,J.A., Conjeevaram, H.S., and Lok, A.S.-F. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38(2):518–526. doi:10.1053/jhep.2003.50346.
16. Louie KS, Micallef JM, Pimenta JM, Forssen UM. Prevalence of thrombocytopenia among patients with chronic hepatitis C: a systematic review. J Viral Hepat 2011;18(1):1–7. doi:10.1111/jvh.2010.18.issue-1.
17. Liangpunsakul S, Ulmer BJ, Chalasani N. Predictors and implications of severe hypersplenism in patients with cirrhosis. Am J Med Sci 2003;326(3):111–116. doi:10.1097/00000441-200309000-00001.
18. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA 2012;308:2584–2593. doi:10.1001/jama.2012.144878.
19. Morgan TR, Ghany MG, Kim HY, Snow KK, Shiffman ML, De Santo JL, Lee WM, Di Bisceglie AM, Bonkovsky HL, Dienstag JL. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. Hepatology 2010;52:833–844. doi:10.1002/hep.23744.
20. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Manns MP, Zeuzem S, Hofmann WP.

The number needed to treat to prevent mortality and cirrhosis-related complications among patients with cirrhosis and HCV genotype 1 infection. J Viral Hepat 2014;21(8):568–577. doi:10.1111/jvh.2014.21.issue-8.
21. Iga D, Tomimatsu M, Endo H, Ohkawa S-I, Yamada O. Improvement of thrombocytopenia with disappearance of HCV RNA in patients treated by interferon-alpha therapy: possible etiology of HCV-associated immune thrombocytopenia. Eur J Haematol 2005;75(5):417–423. doi:10.1111/ejh.2005.75.issue-5.
22. Karagozian R, Grace ND, Qamar AA. Hematologic indices improve with eradication of HCV in patients with cirrhosis and predict decompensation. Acta Gastroenterol Belg 2014;77(4):425–432.
23. van der Meer AJ, Maan R, Veldt BJ, Feld JJ, Wedemeyer H, Dufour J-F, Lammert F, Duarte-Rojo A, Manns MP, Zeuzem S. Improvement of platelets after SVR among patients with chronic HCV infection and advanced hepatic fibrosis. J Gastroenterol Hepatol 2016 Jun;31(6):1168–1176. doi:10.1111/jgh.2016.31.issue-6.
24. Mandorfer M, Kozbial K, Schwabl P, Freissmuth C, Schwarzer R, Stern R, Chromy D, Stättermayer AF, Reiberger T, Beinhardt S. Sustained virologic response to interferon-free therapies ameliorates HCVinduced portal hypertension. J Hepatol 2016;65(4):692–699. doi:10.1016/j.jhep.2016.05.027.
25. Wolber EM, Jelkmann W. Thrombopoietin: the novel hepatic hormone. News Physiol Sci 2002;17:6–10. doi:10.1152/physiologyonline.2002.17.1.6.
26. Kaushansky K. Thrombopoietin. N Engl J Med 1998;339:746–754. doi:10.1056/NEJM199809103391107.
27. Giannini E, Botta F, Borro P, Malfatti F, Fumagalli A, Testa E, Podesta E, Chiarbonello B, Polegato S, Mamone M. Relationship between thrombopoietin serum levels and liver function in patients with chronic liver disease related to hepatitis C virus infection. Am J Gastroenterol [Comparative Study] 2003 Nov;98(11):2516–2520. doi:10.1111/j.1572-0241.2003.08665.x.
28. Kawasaki T, Takeshita A, Souda K, Kobayashi Y, Kikuyama M, Suzuki F, Kageyama F, Sasada Y, Shimizu E, Murohisa G. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis. Am J Gastroenterol [Research Support, Non-U.S. Gov't] 1999 Jul;94(7):1918–1922. doi:10.1111/ajg.1999.94.issue-7.
29. Honma Y, Shibata M, Hayashi T, Kusanaga M, Ogino N, Minami S, Kumei S, Oe S, Miyagawa K, Senju M. Effect of direct-acting antivirals on platelet-associated immunoglobulin G and thrombocytopenia in hepatitis C virus-related chronic liver disease. Liver Int 2019 Sep;39(9):1641–1651. doi:10.1111/liv.v39.9.
30. Sayyar M, Saidi M, Zapatka S, Deng Y, Ciarleglio M, Garcia-Tsao G. Platelet count increases after viral elimination in chronic HCV, independent of the presence or absence of cirrhosis. Liver Int 2019 Nov;39(11):2061–2065. doi:10.1111/liv.v39.11.
31. Lin KH, Hsu PI, Yu HC, Lin CK, Tsai WL, Chen WC, Chan -H-H, Lai K-H. Factors linked to severe thrombocytopenia during antiviral therapy in patients with chronic hepatitis c and pretreatment low platelet counts. BMC Gastroenterol 2012 Jan;18(12):7. doi:10.1186/1471-230X-12-7.
32. Stasi C, Milani S. Non-invasive assessment of liver fibrosis: between prediction/prevention of outcomes and cost effectiveness. World J Gastroenterol 2016;22(4):1711–1720. doi:10.3748/wjg.v22.i4.1711.
33. Elsharkawy A, Abdel Alem S, Fouad R, El Raziky M, El Akel W, Abdo M, Tantawi O, AbdAllah M, Bourliere M, Esmat G. Changes in Liver stiffness measurements and Fibrosis scores following Sofosbuvir based treatment regimens without Interferon. J Gastroenterol Hepatol 2017 Sep;32(9):1624–1630. doi:10.1111/jgh.13758.
34. Bachofner JA, Valli PV, Kröger A, Bergamin I, Künzler P, Baserga A, Braun D, Seifert B, Moncsek A, Fehr J. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. Liver Int 2017 Mar;37(3):369–376. doi:10.1111/liv.2017.37.issue-3.
35. Fouad R, Elsharkawy A, Abdel Alem S, El Kassas M, Alboraie M, Sweedy A, Afify S, Abdellatif Z, Khairy M, Esmat G. Clinical impact of serum α-fetoprotein and its relation on changes in liver fibrosis in hepatitis C virus patients receiving direct-acting antivirals. Eur J Gastroenterol Hepatol 2019 Sep;31(9):1129–1134. doi:10.1097/MEG.0000000000001400.
36. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132(7):517–524. doi:10.7326/0003-4819-132-7-200004040-00002.