Front. Med. 2019, 13(6): 658–666
https://doi.org/10.1007/s11684-019-0707-7

RESEARCH ARTICLE

Direct acting antiviral-induced dynamic reduction of serum α fetoprotein in hepatitis C patients without hepatocellular carcinoma

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Abstract **Direct acting antiviral (DAA) treatments may reduce the elevated α fetoprotein (AFP), but data on how these treatments affect elevated AFP in patients with chronic hepatitis C (CHC) remain insufficient. In the present study, the frequency of baseline AFP elevations and their related factors, AFP dynamics during and after DAA treatment, and factors associated with AFP reduction was assessed. This retrospective study included 141 patients with CHC without hepatocellular carcinoma who received DAA and achieved sustained virological response. The details are as follows: mean post-treatment follow-up was 99 weeks (12–213); mean age, 57.8 years old; 52%, males; 79%, genotype (GT) 1; and 47%, cirrhosis. Pre-treatment AFP elevation (> 5.5 ng/mL) was seen in 48.2% patients. On multivariate analysis, baseline AFP > 5.5 was associated with the presence of cirrhosis ($P = 0.001$), co-existing non-alcoholic steatohepatitis (NASH) ($P = 0.035$), and GT 1 ($P = 0.029$). AFP normalization was seen in 28.2% patients at treatment week 2, in 52% at the end of treatment, and in 73.4% at the end of follow-up. Post-treatment week 24 AFP normalization was associated with the absence of cirrhosis ($P = 0.003$), Child–Pugh score < 6 ($P = 0.015$), and baseline AFP < 10 ($P = 0.015$). AFP elevation is common in patients with CHC and independently associated with NASH, cirrhosis, and GT 1. DAA treatment resulted in AFP normalization as early as treatment week 2. Post-treatment week 24 AFP normalization is independently associated with the absence of cirrhosis, Child–Pugh score < 6, and baseline AFP < 10.**

Keywords chronic hepatitis C; α fetoprotein; direct acting antiviral treatment; cirrhosis

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease. Approximately 110 million people are HCV antibody positive, and 80 million people have chronic viremia HCV infection worldwide, which resulted in approximately 1.4 million deaths annually [1,2]. Approximately 10% to 20% of people with chronic HCV infection progress to end-stage disease, such as decompensated cirrhosis or hepatocellular carcinoma (HCC) [3]. The introduction of highly effective and well-tolerated direct acting antiviral (DAA) treatment regimens with extremely high rates of sustained virological response (SVR) has now revolutionized chronic hepatitis C (CHC) infection treatment [4].

α Fetoprotein (AFP) is a serum protein that exists during fetal life; it is replaced after birth by albumin as the major serum protein [5–7]. Serum AFP levels remain very low throughout life but can become elevated in certain germ cell tumors and HCC. This phenomenon is the reason why AFP levels have been widely used as a biomarker for HCC screening [8,9]. Serum AFP elevation has been reported during CHC infection, especially in patients with HCV cirrhosis [10–15]. The prevalence of elevated AFP levels in patients with CHC is between 10% and 43% [11,15–19]. Serum AFP level reduction after treatment completion using PEG-IFN based regimens has been reported [19–29]. Serum AFP re-elevation after PEG-IFN treatment is strongly associated with HCC development, regardless of whether or not SVR has been achieved [21,24,26,30].

DAAs are now the mainstay of HCV treatment. Serum AFP reduction during and after DAA treatment has been

Received March 14, 2019; accepted June 25, 2019
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reported. However, those studies included relatively small sample size, lacked treatment follow-up, had brief and short follow-up, and/or did not determine the clinical parameters related to AFP reduction [29,31–33].

The present study retrospectively investigates the baseline frequency and magnitude of AFP elevation in patients with CHC before initiating DAA treatment, the dynamic changes in AFP levels during and after DAA treatment, and independent biochemical and clinical variables that predict AFP normalization after DAA treatment.

Patients and methods

Patient enrollment and data collection

This was a single center retrospective study of all patients who received consecutive DAAs for HCV treatment at the University of California Irvine Liver Clinic from April 2014 to April 2017 and qualified for the following inclusion criteria. Institutional Review Board approval was obtained, and informed consent was waived.

Inclusion criteria included patients with CHC treated with DAAs with or without ribavirin (RBV). Included patients underwent pre-treatment HCC screening by abdominal ultrasound, computed tomography, or magnetic resonance imaging to determine the occurrence of cirrhotic or elevated AFP. They received a full course of treatment, have minimal 12 week post-treatment follow-up. They attained SVR at 12 weeks of post-treatment (SVR12). Exclusion criteria included the following: (1) patients diagnosed with HCC before or during treatment; (2) patients with HCV relapse after DAA treatment; and (3) patients with a co-infection of human immunodeficiency virus (HIV) or chronic hepatitis B.

A total of 181 patients were reviewed. Forty patients were excluded from the study, 26 patients lacked AFP data, 3 patients underwent pre-treatment HCC, 8 patients exhibited HCV relapse after DAA treatment, and 3 patients were co-infected with HIV or hepatitis B. Consequently, 141 patients who satisfied the above inclusion criteria were included.

Baseline data collection included age, gender, ethnicity, liver disease stage, comorbidities, presence of steatosis on imaging or biopsy, non-alcoholic steatohepatitis (NASH) or autoimmune hepatitis (AIH) by liver biopsy and simplified scoring for AIH, diagnosis of cirrhosis, presence of hepatic decompensation, Child–Pugh (CP) class, model of end stage liver disease (MELD) score, body mass index (BMI), and history of prior treatment. Cirrhosis diagnosis was conducted on the basis of radiographic or histologic findings or the endoscopic finding of esophageal/gastric varices. Radiographic findings included presence of nodular liver, splenomegaly ($>$ 12.5 cm), and/or ascites. Histologic findings included the presence of stages 3–4 fibrosis.

Baseline and follow-up laboratory data included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, prothrombin time measured by international normalized ratio (INR), albumin, HCV RNA (sensitivity, 15 IU/mL), HCV genotype (GT), AFP levels (normal $<$ 5.5 ng/mL), creatinine, and platelets. To determine the dynamic changes, we also collected serum ALT, AST, AFP, and HCV RNA test results at treatment weeks 2, 4, end of treatment (EOT), post-treatment weeks 12, and 24, 48, 96, and 144, and end of follow-up (EOF).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software (Chicago, IL, USA). Categorical variables were reported as frequencies and percentages and compared using Pearson Chi-square (χ^2) test. ANOVA was used to compare the means. Both univariate and multivariate analyses were used to determine biochemical, virological, and clinical variables associated with elevated baseline serum AFP and post-treatment week 24 AFP normalization. All tests of significance were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Baseline demographics and laboratory values, and DAA regimens

The demographic characteristics of the study population are summarized in Table 1. The mean age of the cohort was 57.8 (20–85) years with 73 (52%) men and mean BMI of 27.3 (18–48). Among the 141 patients, 75 (53.1%), 27 (19.1%), 22 (15.6%), 7 (4.9%), and 10 (7%) were Caucasian, Asian, Hispanic, African American, and from other races, respectively. The mean post-treatment follow-up period was 99 (12–213) weeks. Hepatic steatosis was present in 79 (56%) patients, among which 41 (51.9%) were confirmed by biopsy, and 38 (48.1%) were based on imaging. The histological diagnosis of NASH and AIH was performed in 12 (8.5%) and 4 (2.8%) patients, respectively. Cirrhosis was found in 67/114 (47.5%) patients, and 34 (50.7%) patients underwent liver biopsy, which confirmed stages 3–4 fibrosis. The other 33 (49.3%) patients underwent the radiographic findings of nodular contour of the liver and splenomegaly, ascites, or endoscopy evidence of esophageal/gastric varices. A total of 6 (4.2%) patients had end-stage renal disease. Among the patients with cirrhosis, mean MELD and CP score were 9.6 (6–32) and 5.6 (5–9), respectively; 57 (85.1%) and 10 (14.9%) patients were CP classes A and B, respectively. Decompensation, ascites, hepatic

encephalopathy, and prior variceal bleeding were observed in 9 (13.4%), 6 (8.9%), 2 (3.0%), and 1 (1.4%) patients, respectively.

Baseline laboratory variables are also shown in Table 1. A total of 112 (79.4%), 12 (8.5%), 11 (7.8%), 2 (1.4%), 1 (0.7%), and 3 (2.1%) patients exhibited GT-1, -2, -3, -4, -5, and -6 infection, respectively. Mean serum AFP was 13.3 (1.1–197) ng/mL, and the mean log 10 HCV RNA was 5.88 (2.6–7.26) IU/mL. Mean serum ALT and AST were 71.7 (8–496) and 63.5 (10–244) IU/L, respectively. Other baseline laboratory variables were as follows: total bilirubin 0.8 (0.2–3.9) mg/dL, INR 1.08 (0.88–2.23), albumin concentration 3.9 (2.3–5.2) g/dL, platelet count 171 (32–467) $\times 10^3/\mu L$, and serum creatinine 1.16 (0.3–8.0) mg/dL.

The following DAA treatment regimens were used in the study: 81 (57.4%), 16 (11.3%), 6 (4.2%), 16 (11.3%), 11 (7.8%), 3 (2.1%), 4 (2.8%), and 4 (2.8%) patients were treated with Ledipasvir–Sofosbuvir +/− RBV; Sofosbuvir + RBV; Ombitasvir–Paritaprevir–Ritonavir/Dasabuvir +/− RBV; Sofosbuvir and Simeprevir; Elbasvir–Grazoprevir; Sofosbuvir and Daclatasvir; Sofosbuvir–Velpatasvir; and other PEG–IFN/RBV/Sofosbuvir, respectively. The majority of the regimens were Sofosbuvir-based. Ledipasvir-Sofosbuvir +/− RBV was the most commonly used with 81 (57.4%) patients. A total of 60 (42.5%) patients were treatment naïve.

Frequency of baseline AFP elevation and clinical variables associated with baseline AFP elevation

As shown in Table 2, in 141 patients, 68 (48.2%) had a baseline serum AFP elevation (> 5.5 ng/mL), 35 (24.8%) with > 10 ng/mL, 9 (6.3%) with > 25 ng/mL, and 5 (3.5%) with > 50 ng/mL. Among the 67 patients with cirrhosis, 47 (70%) exhibited baseline serum AFP elevation compared with 21 (28.4%) baseline serum AFP elevation in 74 patients with non-cirrhotic disease ($P = 0.0001$). In patients with baseline AFP elevation, the mean baseline AFP was 15.9 (5.5–197) ng/mL. In patients with cirrhosis, the mean AFP, ALT, and AST were 20.4 (2–197)

Table 1 Baseline clinical demographics and laboratory values in 141 study subjects

Characteristics	*n* (% or range)
Mean age	57.8 (20–85)
Male:female	73:68 (51.7:48.3)
Ethnicity	
Caucasian	75 (53.1)
Asian	27 (19.1)
Hispanic	22 (15.6)
African American	7 (4.9)
Other	10 (7)
Mean BMI	27.3 (18–48)
Mean post-Rx follow-up (week)	99 (12–213)
Cirrhosis	67 (47.5)
Mean MELD	9.6 (6–32)
Mean Child–Pugh score	5.6 (5–9)
Child–Pugh class A	57 (85.1)
Child–Pugh class B	10 (14.9)
Decompensation	9 (13.4)
Steatosis	79 (56)
Co-existing NASH	12 (8.5)
Co-existing AIH	4 (2.8)
Genotype 1	112 (79.4)
Genotype 2	12 (8.5)
Genotype 3	11 (7.8)
Genotype 4	2 (1.4)
Genotype 5	1 (0.7)
Genotype 6	3 (2.1)
Treatment naïve	60 (42.5)
Mean AFP (ng/mL)	13.3 (1.1–197)
Mean log 10 HCV RNA (IU/mL)	5.88 (2.6–7.26)
Mean baseline ALT (IU/L)	71.7 (8–496)
Mean baseline AST (IU/L)	63.5 (10–244)
Mean total bilirubin (mg/dL)	0.8 (0.2–3.9)
Mean INR	1.08 (0.88–2.23)
Mean albumin (g/dL)	3.9 (2.3–5.2)
Mean platelets ($10^3/\mu L$)	171 (32–467)
Mean creatinine (mg/dL)	1.16 (0.3–8)

Abbreviation: BMI, body mass index; Rx, treatment; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; AIH, autoimmune hepatitis; AFP, α fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio.

Table 2 Mean and frequency (%) of baseline AFP elevation and ALT/AST in patients with cirrhosis vs. without cirrhosis

Baseline	Overall, *n* (%)	With cirrhosis, *n* (%)	Without cirrhosis, *n* (%)	*P*-value
Total patients	141	67 (47.5)	74 (52.5)	
AFP mean and range (ng/mL)	13.3 (1.1–197)	20.4 (2–197)	5.2 (1.1–17.1)	0.0029
AFP > 5.5	68 (48.2)	47 (70)	21 (28.4)	0.0001
AFP > 10	35 (24.8)	28 (41.7)	7 (9.5)	0.0001
AFP > 25	9 (6.3)	9 (13.4)	0 (0)	0.003
AFP > 50	5 (3.5)	5 (7.4)	0 (0)	0.042
ALT mean and range (IU/L)	71.7 (8–496)	82 (12–496)	56.1 (8–365)	0.056
AST mean and range (IU/L)	63.5 (10–244)	77 (19–239)	46.5 (10–244)	0.001

Abbreviation: AFP, α fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

ng/mL, 82 (12–496) IU/L, and 77 (19–239) IU/L compared with 5.2 (1.1–17.1) ng/mL ($P = 0.0029$), 56.1 (8–365) IU/L ($P = 0.056$), and 46.5 (10–244) IU/L ($P = 0.001$) in patients with non-cirrhotic disease, respectively. A total of 9 patients with cirrhosis (13.4%) exhibited serum AFP levels of > 25 ng/mL, which was higher compared with zero in patient with non-cirrhotic disease. A total of 5 patients with cirrhosis (7.4%) showed baseline serum AFP levels of > 50 ng/mL.

As summarized in Table 3, univariate and multivariate analyses were performed to evaluate 13 different clinical, virological, and biochemical parameters associated with

Table 3 Clinical variables associated with baseline AFP elevation and post-treatment week 24 AFP normalization

Variables	Baseline analysis			Post-Rx wk 24 analysis		
	Baseline AFP elevation > 5.5 ng/mL	Univariate *P*-value	Multivariate *P*-value	Post-Rx wk 24 AFP $\leqslant 5.5$ ng/mL	Univariate *P*-value	Multivariate *P*-value
Gender	141			63		
Male	39/73 (53.4)	0.236	–	23/33 (69)	0.599	–
Female	27/68 (39.7)			21/30 (70)		
Age	121			63		
$\geqslant 50$ years	58/102 (56.8)	0.175	–	37/55 (67.3)	0.234	–
< 50 years	8/19 (42.1)			7/8 (87.5)		
BMI	121			63		
$\geqslant 30$ kg/m^2	21/35 (60)	0.286	–	11/16 (68.8)	0.573	–
< 30 kg/m^2	45/86 (53.2)			33/47 (70.2)		
Genotype	141			63		
1	66/112 (58.8)	0.05	0.029	32/48 (66.7)	0.26	–
2–6	11/29 (37.5)			12/15 (80)		
Histologic grade	73			41		
1–2	14/33 (45.2)	0.016	–	18/20 (90)	0.13	–
3–4	28/40 (70)			14/21 (66.6)		
Hepatic fibrosis	75			41		
0–2	15/36 (41.7)	0.008	–	20/23 (87)	0.216	–
3–4	28/39 (71.8)			13/18 (72.2)		
NASH	121			63		
Yes	9/10 (90)	0.018	0.035	5/8 (62.5)	0.455	–
No	57/111 (51.4)			39/55 (70.9)		
Cirrhosis	121			63		
Yes	45/63 (71.4)	0.001	0.001	18/33 (54.5)	0.005	0.003
No	21/58 (36.2)			26/30 (86.7)		
Baseline ALT	121			63		
$\geqslant 40$	49/81 (60.5)	0.047	–	29/45 (64.4)	0.119	–
< 40	17/40 (42.5)			15/18 (83.3)		
Baseline AST	121			63		
$\geqslant 40$	47/76 (61.8)	0.028	–	25/41 (61)	0.032	–
< 40	19/45 (42.2)			19/22 (86.4)		
Baseline ALT-AST	121			63		
$\geqslant 40$	53/90 (58.9)	0.077	–	31/49 (63.3)	0.029	–
< 40	13/31 (41.9)			13/14 (92.3)		
Child–Pugh score	63			34		
$\geqslant 6$	16/23 (69.5)	0.512	–	4/13 (30.8)	0.024	0.015
< 6	29/40 (72.5)			15/21 (71.4)		
Baseline AFP	141			56		
$\geqslant 10$	35 (24.8)	–	–	7/20 (35)	0.001	0.015
< 10	106 (75.2)			30/36 (83)		

Abbreviation: AFP, α fetoprotein; BMI, body mass index; NASH, nonalcoholic steatohepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Rx wk: treatment week.

elevated baseline AFP elevation and post-treatment week 24 AFP normalization. Univariate analysis revealed that elevated baseline serum AFP elevation was remarkably associated with GT 1 infection, histologic inflammation grades 3–4, hepatic fibrosis 3–4, co-existing NASH, clinical diagnosis of cirrhosis, baseline ALT ⩾ 40, and AST ⩾ 40. Elevated baseline serum AFP was not associated with gender, age ⩾ 50 years old, and BMI. Multivariate analysis showed that the presence of cirrhosis (P = 0.001), co-existing NASH (P = 0.035), and GT 1 infection (P = 0.029) were independently associated with baseline AFP elevation.

AFP dynamic changes during and post-DAA treatment, and variables associated with post-treatment week 24 AFP normalization

In those with baseline AFP elevation, AFP normalization occurred as early as treatment week 2 in 28.6% (4/14) of patients and was further increased to 35.5% (11/31) at treatment week 4. By the EOT, 52% (26/50) showed normalized AFP. Even after the completion of DAA therapy, numerous patients continued to exhibit serum AFP level normalization, that is, 64.2% (43/67) at post treatment week 12. By the EOF, 73.1% (98/134) of the patients achieved AFP normalization. The mean AFP trend for patients with baseline AFP elevation is shown in Fig. 1. The serum AFP trend showed a steady decline during DAA treatment and reduction continued to EOF.

In our cohort, 4 patients developed HCC after DAA treatment. The average time for HCC diagnosis was 8.7 months from the initiation of DAA treatment (post-treatment week 25), including 2 men; 3 with GT-1, 1 with GT-2 infection, and 2 treatment naïve. All of them had baseline ALT and AST > 40 IU/L. All 4 patients exhibited baseline cirrhosis with mean CP score of 6 and mean MELD score of 9; all of them had SVR 12 and ALT and AST normalization at EOF. All 4 cases presented pre-treatment AFP elevation to 7–51 ng/mL. Three out of four patients exhibited AFP reduction, but no one experienced AFP normalization during and after DAA treatment. Two out of four patients showed recurrent AFP elevation.

As shown in Table 3, univariate analysis revealed that post-treatment week 24 AFP normalization was considerably associated with the absence of cirrhosis, CP score < 6, baseline AFP < 10 ng/mL, baseline AST and ALT < 40, and baseline AST < 40, but this was not associated with gender, age ⩾ 50 years old, BMI, histologic grade, hepatic fibrosis, HCV GT, NASH, and baseline AST < 40. Multivariate analysis showed that the absence of cirrhosis (P = 0.003), CP score < 6 (P = 0.015), and baseline AFP < 10 ng/mL (P = 0.015) were independently associated with post-treatment week 24 AFP normalization.

We also studied 3 other cases with HCV cirrhosis. These patients had pre-treatment uncontrolled HCC and underwent DAA treatment. All these 3 cases had baseline elevated AFP (125–850). During DAA treatment and post-treatment, all had continued AFP increase, although all achieved SVR. All three patients had HCC progression after DAA treatment.

Dynamic changes of serum AFP in association with ALT/AST and HCV RNA during and post DAA treatment

Figs. 2 and 3 show the dynamic changes in serum AFP in association with those in serum HCV RNA, ALT, and AST

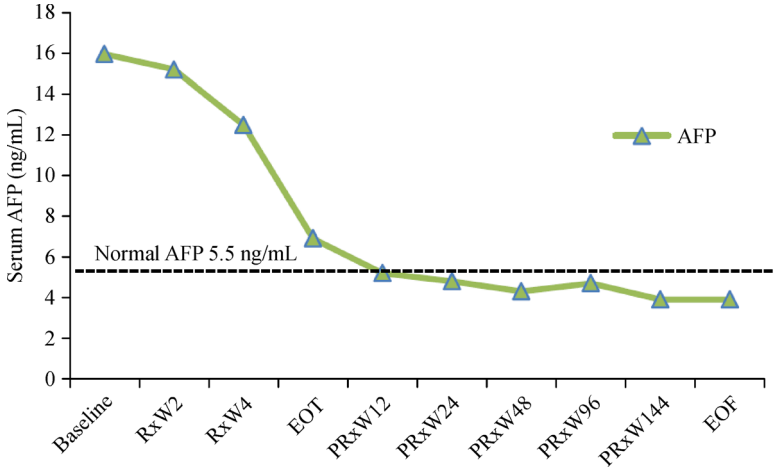

Fig. 1 Dynamic changes in mean serum α fetoprotein (AFP) during direct acting antiviral (DAA) and following DAA Treatment. The mean serum AFP levels (ng/mL) are shown on the *y*-axis. The baseline and timeline of the DAA treatment and post treatment follow-up are shown on the *x*-axis with treatment week (RxW), end of treatment (EOT), post treatment week (PRxW), and end of follow-up (EOF). An AFP level of 5.5 ng/mL is the upper normal limit. A steady decline of serum AFP was observed during DAA treatment, and continued AFP reduction was obvious up to the end of follow-up.

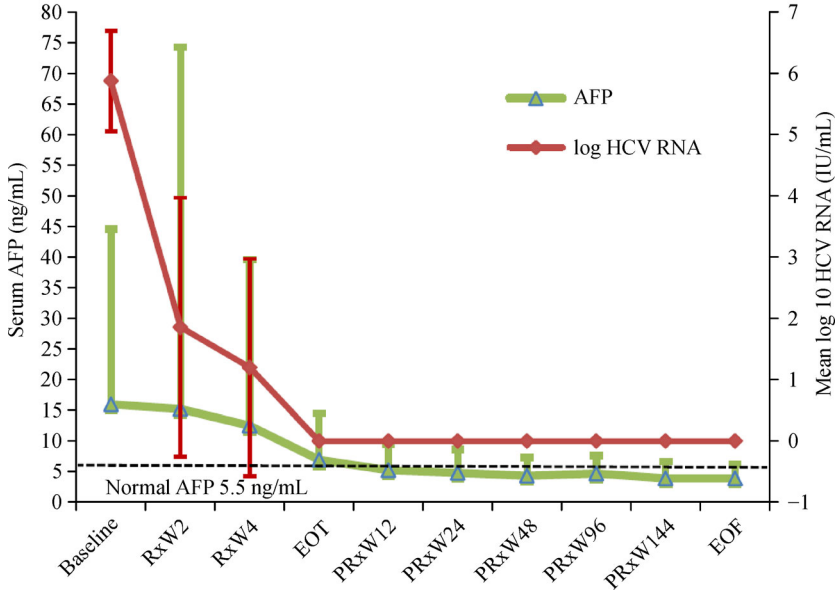

Fig. 2 Dynamic changes in mean hepatitis C virus (HCV) RNA and serum α fetoprotein (AFP) during and following direct acting antiviral (DAA) treatment. The dynamic changes of serum AFP (ng/mL) and mean log 10 HCV RNA (IU/mL) with standard deviations are shown on the *y*-axis. Baseline and timeline of the DAA treatment and post treatment follow-up are shown on the *x*-axis with treatment week (RxW), end of treatment (EOT), post treatment week (PRxW), and end of follow-up (EOF). A rapid decline in HCV RNA concentration was observed as early as treatment week 2 and continued to decline to undetectable at EOF. The AFP decline was gradual and continued even after the DAA treatment.

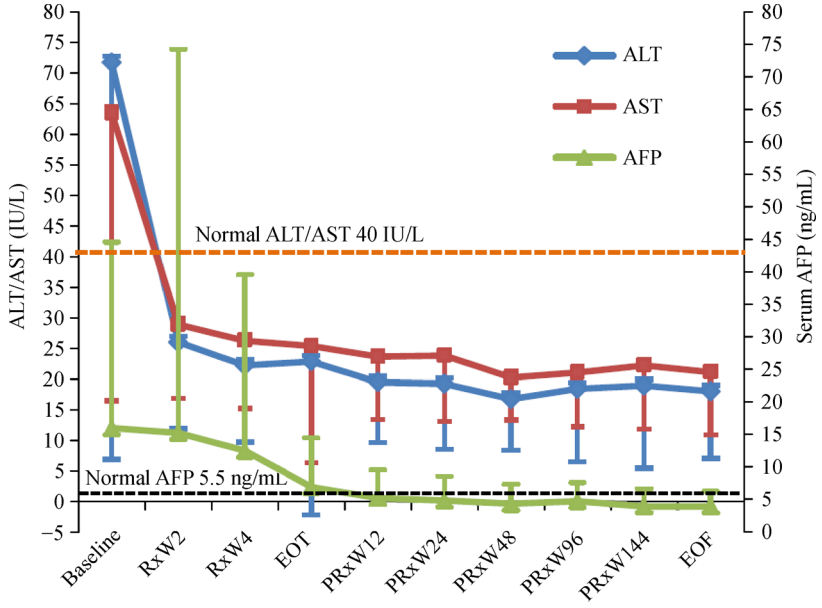

Fig. 3 Dynamic changes in mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum α fetoprotein (AFP) during and following direct acting antiviral (DAA) treatment. The dynamic changes of mean ALT and AST (IU/L) and serum AFP (ng/mL) with standard deviations are shown on the *y*-axis. Baseline and timeline of the DAA treatment and post treatment follow-up are shown on the *x*-axis with treatment week (RxW), end of treatment (EOT), post treatment week (PRxW), and end of follow-up (EOF). A rapid decline in both ALT and AST was observed as early as treatment week 2 of DAA treatment, with stabilization at treatment week 4, and minimal decline after treatment week 4. AFP decline was gradual and continued even after the DAA treatment.

during and after DAA therapy. In Fig. 2, serum AFP reduction was well associated with a rapid decline, which was seen in HCV RNA. In Fig. 3, serum AFP reduction was also well associated with a rapid decline in both ALT/AST. However, AST/ALT and HCV RNA exhibited a sharper initial slope of decline compared with that in serum AFP. Serum AFP decline was gradual and continued even after the completion of the DAA treatment.

Discussion

Serum AFP elevation has been reported in HCV infection even without HCC [10–15]. The incidence of AFP elevation in patients with CHC has ranged from 10% to 43% [11,15–19]. In our study, 48.2% of patients had baseline serum AFP elevation, which to our knowledge is the highest that has ever been reported in patients with CHC without HCC. This result is possibly due to the more advanced age of our patients (mean age 57.8 years old) and their increased rate of cirrhosis. Compared with patients with non-cirrhotic disease, patients with cirrhotic disease exhibited both significantly higher mean AFP levels (20.4 vs. 5.2 ng/mL, $P = 0.0029$) and rate of AFP elevation (70% vs. 28.4%, $P = 0.0001$) and higher mean ALT/AST levels (82/77 vs. 56.1/46.5 IU/L).

Stages 3 and 4 hepatic fibrosis, elevated AST, and elevated INR were associated with AFP elevation [15]. Chen *et al.* found that old age, advanced fibrosis, elevated AST, and low platelet count are associated with baseline AFP elevation [19]. Chu *et al.* reported that GT 1b infection, serum albumin < 4.2 g/dL, and stage 4 fibrosis were associated with AFP elevation [15]. In the present study, elevated baseline AFP was notably associated with histologic grades 3–4, hepatic fibrosis stages 3–4, presence of cirrhosis, co-existing NASH, baseline ALT $\geqslant 40$, and AST $\geqslant 40$, and GT 1 infection. However, multivariate analysis indicated that the presence of cirrhosis ($P = 0.001$), co-existing NASH ($P = 0.035$), and GT 1 infection ($P = 0.029$) were independently associated with baseline AFP elevation. Our results corroborated previous reports' findings that the presence of HCV-cirrhosis is independently associated with baseline AFP elevation. Our finding that co-existing NASH is independently associated with AFP elevation in patients with CHC is consistent with that of a previous study, which indicated a trend toward significance for the presence of hepatic steatosis and baseline serum AFP elevation [15]; however, the sample size for patients with NASH was very small, which may limit the strength of analysis. Our finding that HCV GT 1 infection is independently associated with baseline AFP elevation is consistent with the findings of a previous report by Chu *et al.* [14]. However, these findings must be re-confirmed by further studies with higher sample sizes.

In an era of DAA regimens with very high SVR rates, AFP changes during and after DAA treatment have been rarely reported [29,31–33]. Recently, we demonstrated that ALT/AST normalization occurs as early as 2 weeks of DAA treatment [34]. The present study included a large sample size and long-term detailed post-treatment observation during and post-treatment dynamics of serum AFP in association with HCV RNA, ALT/AST dynamics, and other clinical variables. AFP normalization occurred as early as DAA treatment week 2 in 28.6% of the patients and continued with DAA treatment. By the end of the DAA treatment, 52% of patients experienced AFP normalization. The present study also demonstrated that DAA treatment resulted in steady improvement in serum AFP levels during treatment. Moreover, serum AFP levels continued to decline even after treatment completion. By the EOF, 73.1% of patients exhibited AFP normalization. By contrast, in 3 additional cases with baseline HCV cirrhosis and HCC, all showed markedly elevated baseline AFP (125–850 ng/mL) and continuous AFP increase during DAA treatment and post-treatment follow-up, despite achieving SVR. Thus, DAA-mediated AFP reduction or normalization does not occur in cases with baseline HCC. The present study corroborated the previous findings that DAA treatment results in serum AFP reduction [19–29,31,32] and provided details of the dynamic changes of AFP during and after DAA treatment.

We then analyzed 13 host and viral factors that were associated with post-treatment week 24 AFP normalization. Post-treatment week 24 AFP normalization was notably associated with the absence of cirrhosis, CP score < 6, baseline AFP < 10 ng/mL, baseline ALT and AST < 40 IU/L, and baseline AST < 40 IU/L by univariable analysis. However, only the absence of cirrhosis ($P = 0.003$), CP score < 6 ($P = 0.015$), and baseline AFP < 10 ng/mL ($P = 0.015$) were independently associated with week 24 AFP normalization by multivariate analysis.

The clinical relevance of DAA-mediated AFP normalization remained uncertain. DAA treatment resulted in rapid AFP normalization in association with HCV RNA clearance and ALT/AST normalization. This finding occurred only in patients with HCV without HCC. The rapid AFP normalization might be due to DAAs' potent anti-HCV efficacy that resulted in the termination of HCV-mediated hepatocytic injury. A prolonged course of AFP normalization could improve hepatic fibrosis. Thus, serum AFP could be a biomarker for HCV-mediated hepatic injury and fibrosis.

In the HALT-C trial, six patients developed HCC within 20 weeks of EOT. Four of the six patients had AFP elevation, and only three of the six had cirrhosis at the start of the study [35]. Post-treatment AFP is associated with HCC development as shown with PEG-IFN [21,24,26,30]. HCC developed in four patients in the present study after DAA treatment completion; three out of the four patients

showed AFP reduction. These results indicated that DAA-mediated AFP reduction may not provide protection from HCC risk in these patients. In two out of four cases, recurrent AFP elevation occurred during HCC diagnosis, indicating the clinical value of continuous AFP monitoring as part of HCC screening. Large and long-term studies must be performed to determine the clinical relevance of DAA-mediated AFP normalization in these patients.

Our study provided important details on the dynamic changes of serum AFP in patients with HCV during and after DAA treatment. These changes correlated with various biochemical, virological, and clinical variables. Some limitations must be noted in the present study. First, this study was a single center and retrospective study. Although this study had the largest sample size and longest follow-up period thus far among studies that determined AFP trends in patients with CHC treated with DAAs, an even larger sample size might improve statistical analysis. Thus, studies with large sample sizes and subgroup analysis can be conducted in the future to evaluate additional factors that may affect AFP reduction, and long-term outcomes are needed.

In conclusion, AFP elevation is commonly seen in patients with CHC. DAA treatment results in a rapid normalization of serum AFP in as early as treatment week 2. This normalization further increases during and after DAA treatment in association with the absence of cirrhosis, CP score < 6, and baseline AFP < 10 ng/mL.

Acknowledgements

The authors want to thank Drs. Johnathan Zhang and Mohit Mittal for their contribution on data collection.

Compliance with ethics guidelines

Tung Huynh and Ke-Qin Hu declare that they have no conflict of interest related to this study. This retrospective study included human subjects and was conducted after approval by our IRB. This study was an investigator-initiated study, and no external funding in any source has been obtained. Tung Huynh has nothing to disclose. Dr. Hu is on speaker bureau for Gilead Sciences.

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