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RESEARCH ARTICLE

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After successful hepatitis C virus antiviral therapy: It looks that normal alanine aminotransferase level is not the normal

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Background: Normal serum alanine aminotransferase (ALT) levels differ with age, gender, and body mass index. Adjusting the upper limits of normal (ULN) for ALT needs further research in different populations. Aim of this work was to monitor the effect of successful chronic hepatitis C (CHC) treatment on the ALT levels in patients with normal pretreatment ALT.

Methods: Data of 1160 CHC patients with persistent pretreatment normal liver enzymes were retrospectively analyzed. Treatment response to direct acting antiviral agents (DAAs) therapy was recorded. Changes in ALT levels before and after treatment were analyzed by patients' demographic, laboratory, and radiologic characteristics. Areas under the receiver operating characteristic curve (AUROC) of ALT after treatment were used to generate a new ALT ULN.

Results: Males were 568 (49%) and females 592 (51%) with a mean age of 50.7 years. After treatment, mean (±SD) of ALT levels significantly decreased from (26.3±7.8) to (19.1±10.9). This reduction was more significant in interferon-free than interferon-based regimens. ROC curve analyses suggested a new ALT ULN cut off (26.4 IU/L) in the treated patients (sensitivity=78.6%, specificity=83.8%, AUROC=0.89. This cutoff dropped to 14.7 IU/L in cirrhotic patients (sensitivity=77.4%, specificity=44.7%, AUROC=0.612). The identified cutoffs were 16.3 IU/L (sensitivity=66.7%, specificity=47.5%, AUROC=0.499) and 15.5 IU/L (sensitivity=76.5%, specificity=51.3%, AUROC=0.576) in males and females, respectively.

Conclusion: The current ALT ULN needs readjustment to identify new normal cutoffs in CHC patients. Posttreatment cutoffs differ according to gender, pretreatment liver affection, and treatment regimen.

KEYWORDS

alanine aminotransferase, direct acting antiviral agents, hepatitis C virus, interferon, liver enzymes

1 | INTRODUCTION

Many biomarkers have always been implicated in the diagnosis of hepatic injury especially liver enzymes]alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGT)[. These markers are always documented to vary and fluctuate in different liver diseases.[1] When liver injury occurs, ALT is released from injured liver cells and causes a significant elevation in serum ALT activity. ALT also exists in muscles, adipose tissues, intestines, colon, prostate, and brain;[2,3] however, the concentration of ALT in these organs is much lower than the liver.[4-6] Serum ALT testing is readily available, inexpensive, and routinely used to assess

liver function in clinical practice.[4] There are many causes that can lead to increased serum ALT activity. Viral hepatitis like hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are among the leading causes of ALT elevation[7,8] whether in cases of acute or chronic viral hepatitis.[9] Other common causes include: steatohepatitis,[10] autoimmune hepatitis,[11] hemochromatosis,[12] excessive alcohol intake[13,14] besides long list of drugs and chemicals[15,16] and metabolic covariates.[17,18] Chronic ALT elevation was also found in several metabolic and non-hepatic disorders, such as glucose intolerance, central obesity, dyslipidaemia, hypertension, celiac disease, and muscle injury.[19,20] Average upper limits of normal (ULN) for ALT were defined in blood donors with non-B, non-C hepatitis, and ranged from 40 to 50 Unit/L.[21] These definitions did not consider that metabolic covariates can cause liver injury with slight-to-moderate ALT elevation.[22] Because of the increasing prevalence of patients with metabolic disorders, the clinical significance of ALT values has been recognized and metabolic covariates are more considered when evaluating the ULN of ALT activity by many scholars in recent decades. Some authors raised the concept that subjects with metabolic abnormalities should be excluded in evaluating the ULN of ALT level for the potential risk to the general health and new definitions for ULN were suggested accordingly with gender differences (30 IU/L for males and 19 IU/L for females).[23] In agreement with this, many scientists have reevaluated the ULN of ALT values in specific populations, including adults and adolescents. As an example, and in a study on healthy Korean individuals, the upper limits of normal for ALT were 33 IU/L for men and 25 IU/L for women.[24] In another study from Japan, the upper limits of normal ALT were 29 IU/L in men and 23 IU/L in women when considering lifestyle factors.[25] Regarding HCV patients, and previously where the interferon was the mainstay therapy, rapid normalization of ALT was used together with rapid virological response (RVR), as predictors for treatment outcomes,[26] current practice guidelines for managing HCV, do not consider liver enzymes as a prerequisite for treatment, but still use them as markers of hepatic cell injury.[27] We aim at studying changes in ALT levels during and after treatment of chronic HCV patients using DAAs in a group of patients with baseline enzymes falling below the current upper limit of normal according to currently used cutoff values, and try to explore whether a new upper limit of normal for ALT is to be considered in HCV patients based on the decline in ALT values in those patients as a response to successful treatment.

2 | PATIENTS AND METHODS

In January 2017, data of treatment naïve CHC patients (n=1160) with baseline ALT values falling within the current normal laboratory range (40 IU/L) were retrospectively analyzed. Patients were retrospectively selected from New Cairo viral hepatitis treatment center registry, one of the specialized viral hepatitis management facilities affiliated to the National Committee for Control of Viral Hepatitis (NCCVH), Egypt, in the period between September 2014 and September 2016. Pretreatment sociodemographic and clinical data (gender, age, weight, height, body mass index, and treatment status), laboratory investigations done before treatment (hemoglobin, white blood cell count, platelets count, ALT, AST, HCV viral load, blood glucose, albumin, and total bilirubin), and abdominal ultrasound in addition to assessment of hepatic fibrosis using FIB-4 score were collected. Patients with other types of chronic liver diseases, Child-Turcotte-Pugh class B or C, or receiving any concomitant drugs were excluded. ALT levels were followed up during treatment and at time of assessing SVR (12 weeks after treatment cessation). Treatment response to antiviral therapy (interferon and non-interferon containing) was recorded. ALT level changes were monitored during and after therapy in all patients and were analyzed in correlation with other demographic, laboratory, and radiologic characteristics.

2.1 | Statistical analysis

To maintain confidentiality; participants were assigned serial identification numbers. Descriptive analysis of liver enzymes and other variables at baseline was conducted. The 2.5th, 5th, 10th, 15th, 20th, 25th, 50th (median), 75th, 95th, and 97.5th percentiles were calculated for ALT and AST levels at baseline—the percentiles of interest are at the lowest ranges. As the normal mean value of ALT is higher in males than in females, this was further stratified by gender.

Univariate analyses including the Student's *t* test were used to compare the baseline levels of ALT and AST with continuous variables. The chi-square test was used to examine the association of baseline ALT and AST with categorical variables.

The effect of treatment on change in ALT and AST levels from baseline to during and after treatment was analyzed using multivariate analysis by a general linear model for repeated measurements with correction for sphericity.

To explore for a possible new cutoff for ALT, we used the proposed new definitions for ULN enzymes as suggested by Prati et al.[23] to classify patients into four groups according to their ALT levels: group 1a (Males with ALT ≥30), group 1b (Females with ALT ≥19), group 2a (Males with ALT <30), and group 2b (Females with ALT <19). The effect of treatment on changes in ALT and AST in these four groups was studied to determine if there is any difference in enzyme response to treatment in different groups using multivariate analysis by Cochran's statistics in related samples for non-parametric tests. Comparison of the difference in ALT levels in these four patient groups was done according to the diagnosis of cirrhosis either by ultrasound or a FIB-4 score more than 3.25, using chi-square test.

The receiver operating characteristic curves were generated for further exploration of a possible upper limit of normal for ALT at baseline based on response to treatment and diagnosis of cirrhosis (either by ultrasound or FIB-4 score). The sensitivity was plotted against the false-positive rate (1-the value of specificity), then Youden index was calculated to extract the ALT cutoff values. *P*-values <.05 were considered statistically significant. Data were analyzed using the Statistical Package for Social Sciences (SPSS, Version 22; 2013; SPSS; IBM Corp, Armonk, NY, USA).

3 | RESULTS

Our study included 1160 CHC patients with liver enzymes falling within the normal laboratory range (≤40 IU/L). They were 568 males (49%) and 592 females (51%) with mean age of 50.7 years. General characteristics of the studied patients are presented in Table 1. Mean values of ALT and AST at baseline (before treatment) were significantly higher in males than females. They were also significantly higher in patients with cirrhosis or treatment experience. Obesity had no effect on ALT and AST at baseline (Table S1). The percentiles of ALT and AST at baseline are presented by gender in Table 2. Although ALT and AST levels decreased with treatment, there was only significant effect of treatment regimen and treatment response on enzyme lowering. Patients treated with interferon-free regimens had more significant lowering of liver enzymes than those treated with interferon containing regimens. Also, patients who achieved sustained virologic response (SVR) had more significant lowering of liver enzymes than those who did not respond to treatment (Table S2). Mean (±SD) ALT level significantly decreased from (26.3±7.8) before treatment to (19.1±10.9) after treatment (Table 3). Absence of advanced fibrosis/cirrhosis (as identified by FIB-4 score <3.25) was associated with more significant lowering of ALT levels. Gender, body mass index, cirrhosis (as detected by ultrasound), treatment status (naïve vs experienced), treatment response (responders vs non-responders), and treatment regimen (interferon containing

TABLE 1 Baseline characteristics of study participants

Baseline variables	Total N=1160 Mean±SD or N (%)	Male N=568 Mean±SD or N (%)	Female N=592 Mean±SD or N (%)	*P*-value[b]
ALT, IU/L	26.5±9.5	28.1±9.7	24.9±9.1	**<.001**
AST, IU/L	26.5±8.1	27.1±7.9	25.9±8.3	**.019**
Age, y	50.7±11.5	50.2±11.9	51.1±11.2	.186
Quantitative PCR, log^{10}[a]	5.7±0.6	5.8±0.9	5.6±0.8	**<.001**
Blood glucose, mg/dL[a]	100.6±25.7	99.7±25.5	101.4±25.9	.286
Albumin, g/dL	4.1±0.5	4.1±0.5	3.9±0.4	**<.001**
Platelets, $\times10^3/\mu L$	217.2±74.4	206.8±67.5	227.2±79.3	**<.001**
Total Bilirubin, mg/dL	0.7±0.3	0.8±0.3	0.7±0.3	**<.001**
Hemoglobin, g/dL	13.4±1.7	14.4±1.5	12.5±1.2	**<.001**
WBC, $\times10^3/\mu L$	6.4±2.2	6.3±2.1	6.5±2.3	.198
Body mass index, kg/m^2[a]				
Normal (<25)	133 (11.5)	89 (26.9)	44 (13.1)	**<.001**
Overweight (25-<30)	224 (19.3)	142 (42.9)	82 (24.5)	
Obese (≤30)	309 (26.6)	100 (30.2)	209 (62.4)	
Fib4 score				
<1.45	642 (55.3)	296 (52.1)	346 (58.4)	.094
1.45-3.25	435 (37.5)	229 (403)	206 (34.8)	
>3.25	83 (7.2)	43 (7.6)	40 (6.8)	
Liver ultrasound				
Non-cirrhotic	1013 (87.3)	487 (85.7)	526 (88.9)	.111
Cirrhotic	147 (12.7)	81 (14.3)	66 (11.1)	
Treatment regimen				
Non-interferon based	916 (78.9)	422 (74.3)	494 (834)	**<.001**
Interferon based	244 (21.1)	146 (25.7)	98 (16.6)	
Treatment status[a]				
Naive	990 (86.1)	443 (79.2)	547 (92.6)	**<.001**
Experienced	160 (13.9)	116 (20.8)	44 (7.4)	
End of treatment response				
Responder	1132 (97.6)	544 (95.8)	588 (99.3)	**<.001**
Non-responder	28 (2.4)	24 (4.2)	4 (0.7)	

P-value <.001 is highly significant.

[a]Variables with missing data (Body mass index=494, Quantitative PCR=7, Blood glucose=67, treatment status=10).

[b]Univariate analysis for Quantitative variables compared with *t* test, qualitative variables compared with chi-square test.

TABLE 2 Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) percentiles at baseline by gender

Gender	Percentiles	ALT	AST
Male (N=568)	2.5	12.0	14.2
	5	14.1	16.7
	10	16.3	19.0
	15	18.0	21.0
	20	19.4	23.0
	25	21.1	24.4
	50	26.7	29.9
	75	32.8	34.1
	95	38.9	39.0
	97.5	39.8	40.0
Female (N=592)	2.5	10.7	15.2
	5	12.2	17.2
	10	15.0	20.0
	15	17.0	21.0
	20	18.8	23.0
	25	20.0	23.5
	50	26.7	29.4
	75	32.7	35.0
	95	38.2	39.0
	97.5	39.0	40.0

vs interferon free) had no significant effect on ALT lowering during antiviral therapy (Table 3). Applying a new cutoff for upper limit of normal (30 IU/L) for males showed that 88.8% of patients had ALT level falling below the new cutoffs after treatment (Table 4). In females, the used new cutoff for upper limit of normal (19 IU/L) showed that 59% of patients had ALT level falling below the new cutoffs after treatment (Table 3). Receiver operating characteristic (ROC) curves analyses suggested a new cutoff for upper limit of normal for ALT (26.4 IU/L) after treatment in the studied group of patients with sensitivity of 78.6%, specificity of 83.8%, and area under ROC curve (AUROC) of 0.89 (Figure 1). This cutoff dropped to 14.7 IU/L in patients with cirrhosis detected by ultrasound (Sensitivity=77.4%, Specificity=44.7%, and AUROC of 0.612; Figure 2) and to 15.5 IU/L in patients with advanced fibrosis/cirrhosis detected by FIB-4 (Sensitivity=71.1%, Specificity=46.6%, and AURO of 0.538). In males, the identified cutoff was 16.3 IU/L (Sensitivity=66.7%, Specificity=47.5%, and AUROC of 0.499) and in females the identified cutoff was 15.5 IU/L (Sensitivity=76.5%, Specificity=51.3%, and AUROC of 0.576).

4 | DISCUSSION

Liver enzymes are considered biomarkers of hepatocellular injury[28] and in the majority of patients with CHC, serum levels reflects the degree of hepatocellular damage, although many people with significant liver damage show persistently serum ALT levels. Accordingly, different factors affecting the integrity of hepatocytes are supposed to affect the level of the liver enzymes. Although persistently elevated ALT for more than 6 months is used as a criterion for diagnosing chronic hepatitis,[29] the upper limit of normal for liver enzymes was always debatable. Many scientists tried to suggest lower cutoffs for transaminases through various methods. In 2002, Prati et al.[23] suggested new cutoff values for ALT being 30 IU/L for men and 19 IU/L for women. In this study, we tried to add an indirect evidence that the current definition of normal liver enzymes in chronic HCV patients may need revisions to reach new lower levels. This was showed by a new approach through exploring the changes in liver transaminases following successful antiviral therapy in patients who are considered to have normal liver enzymes according to the current standard. Before the era of DAAs, chronic HCV patients with persistent normal ALT were not always prioritized for antiviral therapy. Many of those patients showed marked degrees of fibrosis on continuous follow up, and they were subjected to treatment as those with elevated liver enzymes.[30] In our study, we have studied ALT levels falling within the normal laboratory range before treatment along a course of HCV antiviral therapy by new DAAs in both treatment naïve and experienced patients. We excluded patients with other liver diseases or those under treatment with any other drugs to omit any factors that may cause liver injury. We found significant difference between the levels of ALT in males and females being higher in males. This is supported by the results of Mera et al.[31] who found that ALT and AST levels were significantly higher in male than females with or without hyperbilirubinemia and by different epidemiological studies showing that male gender have a higher risk of elevated ALT.[32] However, this is in controversy to the results of a study published at 2014 by Bakht and Grace which found that females showed higher liver enzymes than males.[33] Although ALT is supposed to be very specific to liver disease and is related to liver fat accumulation,[34] obesity as a factor showed no statistical significance on the level of ALT. This was different from a study carried by Shuang Chen and his colleagues[35] who found that ALT level elevation was independently associated with metabolic syndrome. The same study did show a significant elevation in the level of AST with obesity but not as strong as in ALT. In our study; cirrhosis correlated significantly in an inverse manner with the ALT level. This lies in concordance with a previously published study which observed that advanced fibrosis was accompanied by persistently normal ALT.[33] That is why liver biopsy was considered for some time as a mean for judging the eligibility of treatment in HCV patients with normal liver enzymes.[36] In our study, we found that patients treated with interferon-free regimens had more significant lowering of liver enzymes than those treated with interferon containing regimens. This may be explained by the fact that interferon exerts a more complex actions (antiangiogenic, antifibrotic, and immunomodulating), on the host rather than on the virus, while DAAs have only direct antiviral action. There was statistically significant decrease in the mean of ALT levels after treatment in treatment naïve patients than experienced patients. Also, the mean ALT decreased significantly after treatment in responders than non-responders. This statistical significance was not found when comparing ALT values at baseline,

TABLE 3 Change in alanine aminotransferase (ALT) among baseline, during, and after treatment

	ALT at baseline (N=1160)	ALT during treatment (N=990)	ALT after treatment (N=433)	*F*	*P*-value*
Total sample	26.3±7.8	38.3±75.5	19.1±10.9	17.141	**<.001**
Gender					
Male	26.6±7.6	34.2±69.8	19.5±10.9	1.508	.222
Female	26.1±7.9	42.2±80.4	18.7±11.1		
Body mass index, kg/m^2					
Non-obese (<30)	26.6±7.4	31.3±54.8	19.4±11.2	1.295	.271
Obese (≤30)	26.4±7.8	42.7±91.0	20.2±12.9		
Fib4 score					
≤3.25	26.6±7.8	39.6±77.2	19.1±11.2	2.446	**.045**
>3.25	22.9±7.3	21.1±43.7	19.1±8.8		
Liver ultrasound					
Non-cirrhotic	26.3±7.8	37.7±73.2	18.5±22.8	0.168	.86
Cirrhotic	26.1±7.7	41.9±89.3	10.6±12.7		
Treatment regimen					
Non-interferon based	26.1±7.8	36.3±72.2	18.5±10.2	2.564	.078
Interferon based	27.1±7.6	45.0±85.6	21.1±12.9		
Treatment status					
Naive	26.2±7.8	38.5±76.2	18.3±10.1	0.694	.500
Experienced	26.9±7.6	33.7±51.3	22.2±13.5		
End of treatment response					
Responder	26.9±7.9	39.2±81.9	18.4±9.9	0.836	.434
Non-responder	28.2±8.2	60.5±164.0	42.4±18.9		

*Multivariate analysis using General linear model for repeated measurements with correction for sphericity.

TABLE 4 Comparison of alanine aminotransferase levels by gender using an exploratory new ULN at different treatment points (Male cutoff point=30, Female cutoff point=19)

Male	Before (N=568) N (%)	During (N=489) N (%)	After (N=223) N (%)	*P*-value*
<30	356 (62.7)	383 (78.3)	198 (88.8)	<.001
≥30	212 (37.3)	106 (21.7)	25(11.2)	
Female	**Before (N=592) N (%)**	**During (N=501) N (%)**	**After (N=210) N (%)**	***P*-value***
<19	122 (20.6)	217 (43.3)	124 (59.0)	<.001
≥19	470 (79.4)	284 (56.7)	86 (41.0)	

*Multivariate analysis by Cochran's statistics in K-related samples for non-parametric tests was used in this analysis.

during, and after treatment due to transient elevation of ALT during the course of treatment in some patients. In spite of this, significant decrease in ALT was observed in patients with and without advanced fibrosis (as measured by FIB-4 score) along the course of treatment. This denotes that the already settled normal values of ALT should be revised. Consequently, we tried to find a better cutoff value according to the examined groups to reach a better sensitivity and specificity. This was achieved by a cutoff value of 26.4 IU/L after treatment in the studied group of patients showing a sensitivity of 78.6%, a specificity of 83.8%, and the area under ROC curve (AUROC) of 0.89. Similar results for ALT values were obtained on a cutoff value of 20 IU/L in a study carried out for diagnosing metabolic syndrome with sensitivity of 76.8% and specificity of 81.4%.[35] Then we divided the patients with diseased liver into cirrhosis and advanced fibrosis/cirrhosis. This in return caused the cutoff to drop to 14.7 IU/L in patients with cirrhosis detected by ultrasound and to 15.5 IU/L in patients with advanced fibrosis/cirrhosis detected by FIB-4. This goes in concordance with the previous observations that revealed a significantly higher AST but not ALT in cirrhotics vs non-cirrhotics.[37] Once more, we divided the patients according to

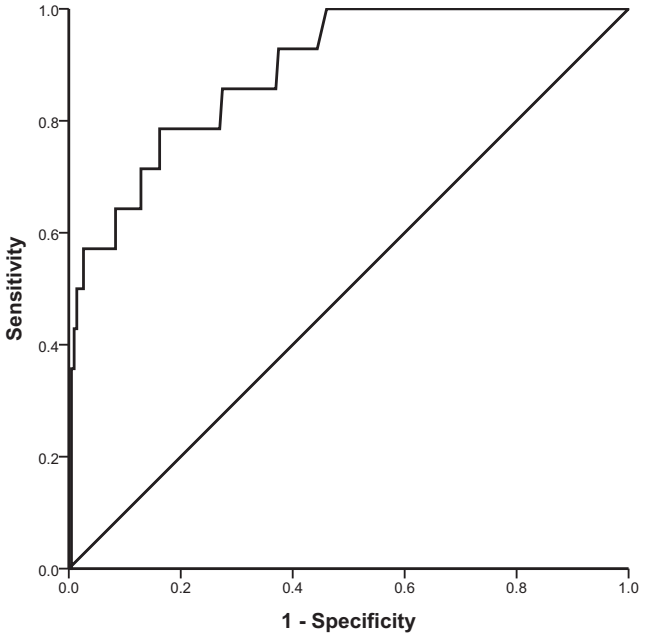

FIGURE 1 Receiver operating characteristic curve for alanine aminotransferase after treatment

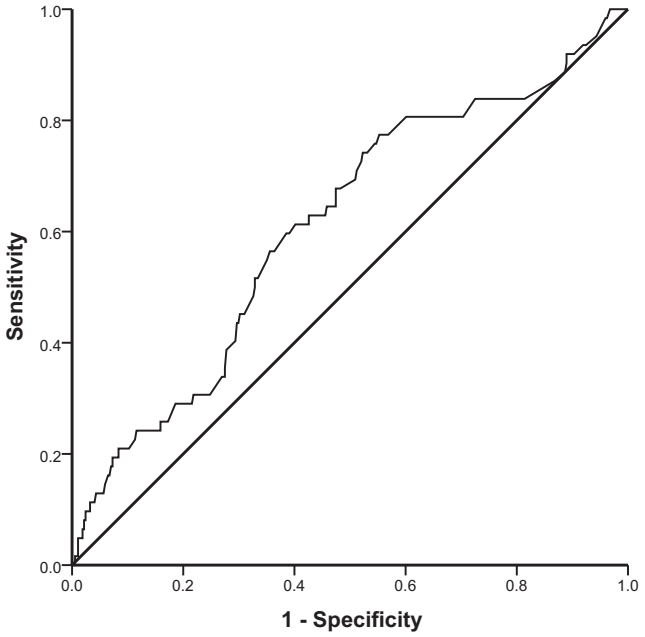

FIGURE 2 Receiver operating characteristic curve for alanine aminotransferase by cirrhosis status

gender. The cutoff value in males was 16.3 IU/L and in females was 15.5 IU/L.

Limitations of our study include:

Despite being a retrospective data analysis study, the large number of the studied population could add to the reliability of results. Our results and cutoff values should be carefully interpreted. We have studied chronic HCV patients only, so our results may not be applicable in other chronic liver diseases. We did not have baseline liver histology before treatment, so careful interpretation should be done also in patients with cirrhosis. Lack of a healthy population cohort to be used as a control group makes these results only exploratory and prospective studies in different chronic liver diseases with healthy control arm is needed to confirm our results.

Our study has many points of strength. First, it is done on large number of patients which makes the results more realistic. Second, various treatment regimens for HCV were used including interferon and non-interferon-based therapies, which added strength to our results as many regimens did not affect our hypothesis of new cutoff values for liver enzymes.

5 | CONCLUSION

The current upper limit of normal for ALT needs readjustment to best identify normal cutoffs in CHC patients after SVR. Posttreatment cutoffs for ALT differ according to gender, pretreatment stage of liver disease, and treatment regimen.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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