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ORIGINAL ARTICLE

Role of Liver Stiffness Measurement in Predicting HCC Occurrence in Direct-Acting Antivirals Setting: A Real-Life Experience

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Abstract

Purpose The aim of this study was to evaluate the relationship between the liver stiffness measurement and the risk of developing hepatocellular carcinoma (HCC) in HCV cirrhotic patients undergoing new direct-acting antivirals.

Methods From April 2015 to April 2017, all consecutive HCV cirrhotic patients treated by direct-acting antivirals were enrolled. A liver stiffness measurement was computed at baseline, and an ultrasound evaluation was provided for all patients at baseline and every 6 months until 1 year after the stopping of the antiviral therapy. The diagnosis of HCC was performed according to international guidelines by imaging technique workup.

Results Two hundred and fifty-eight HCV patients with a diagnosis of cirrhosis were identified. The median liver stiffness was 25.5 kPa. Thirty-five patients developed HCC. Patients were divided into three groups, based on their liver stiffness: < 20 kPa ($n = 72$), between 20 and 30 kPa ($n = 92$) and > 30 kPa ($n = 94$). Compared to the < 20 kPa and 20–30 kPa groups, the > 30 kPa group showed a statistically significant increased risk of HCC ($p = 0.019$; HR 0.329; 95% CI 0.131–0.830). A ROC curve analysis to assess the overall predictive performance of liver stiffness measurement on the HCC risk was performed. The results allow us to identify a cutoff value of liver stiffness measurement equal to 27.8 kPa, which guarantees the highest sensitivity and specificity (respectively, 72% and 65%).

Conclusions The data underline that the baseline liver stiffness measurement and ultrasound surveillance is a valuable tool for assessing the risk of HCC in cirrhotic patients undergoing the direct-acting antivirals treatment.

Keywords Transient elastography · Direct-acting antiviral · HCC · HCV cirrhosis · Liver stiffness

Introduction

Treatment of hepatitis C virus (HCV) infection has experienced a major advancement with the advent of the new direct-acting antivirals (DAA), with a rate of sustained viral response (SVR) higher than 90% [1]. Among patient with HCV infection and cirrhosis, the risk of hepatocellular carcinoma (HCC) is estimated to be 3–7% per year [2]. So far, several studies showed that cirrhotic patients achieving a SVR, with regimens containing IFN, had a lower risk of HCC development, with an annual incidence rate of 1.2–1.4% [3]. Nevertheless, the risk of HCC remains because advanced fibrosis or cirrhosis, which is the most important risk factor for liver cancer, is not completely resolved by antiviral treatment. As a matter of fact, the degree of liver fibrosis seems to be a strong predictor of the risk of HCC development [4].

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The historical gold standard for quantifying fibrosis is liver biopsy, but its invasive nature and potential complications make it unpopular among patients. For these reasons, in the last few years, liver stiffness measurement (LSM) using transient elastography (FibroScan®; Echosens SA, Paris, France) (TE) a noninvasive method, has been introduced and validated to assess hepatic fibrosis in patients with chronic liver disease and is now widely used in clinical practice [5]. As a matter of fact, the utility of LSM in a risk evaluation for HCC has been investigated [6, 7]. Therefore, assessing the degree of liver fibrosis may enable us to optimize surveillance and management strategies by predicting the development of HCC in patients with HCV cirrhosis, especially after HCV eradication, although very few reports examined the relationship between LSM and HCC development in the setting of HCV infection and its antiviral approach [8–10], in particular with new DAAs IFN-free regimens [11]. However, its performance to predict outcome of HCV cirrhotic patients at risk of liver disease progression is a matter of debate, especially when patients obtain a SVR. The aim of the present multicenter, prospective study was to investigate the relationship between a single LSM and the risk of developing HCC in cirrhotic patients with chronic HCV infection receiving new DAAs IFN-free therapies.

Materials and Methods

Patients

This is a multicenter, observational, prospective, real-life study conducted by five community and academic regional centers of Southern Italy (Campania region). Globally, 318 patients with a chronic HCV infection treated with the new DAAs IFN-free therapies were recruited in a period of 2 years (April 2015–April 2017).

Patients with the following features were excluded: absence of cirrhosis, active HCC on imaging or history of previous treated HCC, HIV and/or HBV co-infection, and liver-transplant recipients. The treatment regimen was chosen individually by the prescribing clinician, in accordance with national and international guidelines [12, 13]. An ultrasound evaluation and the liver stiffness measurement using transient elastography were provided for all patients before starting the antiviral therapy. The patients not fulfilling these criteria were excluded from the final analysis, which was conducted on a definite population of 258 subjects.

Demographic characteristics and clinical parameters at baseline, including age, sex, body mass index (BMI), presence of comorbidities, presence of portal hypertension and biochemistry parameters, were recorded. The diagnosis of cirrhosis was based on clinical, biochemical, ultrasonographic and, if not contraindicated, histological features. Liver function was graded according to the Child–Turcotte–Pugh (CTP) score system. Portal hypertension was defined by the presence of gastroesophageal varices at endoscopy and/or detection of collateral veins at ultrasound.

The established study follow-up was based on ultrasound examination every 6 months until 12 months after the stopping of the antiviral therapy. Thus, at least two ultrasound examinations were performed for every enrolled patient during this period. The follow-up was stopped in case of HCC detection. Any detected liver lesion during the ultrasound surveillance, was evaluated by a dynamic computer tomography [CT], or contrast-enhanced ultrasonography [CEUS], or a dynamic magnetic resonance imaging [MRI]. The diagnosis of HCC was conducted according to the European guidelines of the European Association for the Study of Liver (EASL) [14] by imaging technique workup or by histology where possible.

The present study conforms to the ethical guidelines of the Declaration of Helsinki (revision of Edinburg, 2000), and it was approved by the Institutional Review Board (Ethic Committee Università della Campania—Ospedale dei Colli,). All the enrolled patients provided informed consent.

Liver Stiffness Evaluation

TE by FibroScan was carried out using the M probe by experienced operators (> 1000 examinations for each), according to the manufacturer's instructions. The LSM, expressed in Kilopascal (kPa), range 2.5–75, was assessed for reliability by the interquartile range (IQR)/median ratios (IQR/M). IQR represents an index of the intrinsic variability of the LSM. We considered only "very reliable" or "reliable" examinations according to Boursier et al. [15]. Moreover, the operators were blinded to the clinical and biochemical data of the patients. The IQR corresponds to the interval of LSM results containing 50% of the valid measurement between the 25th and 75th percentiles. Cirrhosis was defined by a FibroScan ≥ 13.5 kPa, according to Castera's cutoffs [16] in combination with clinical, laboratory and ultrasound parameters.

Statistical Analysis

Data are shown as either median or range, in the case of continuous variables or number and percentage, for categorical variables. Differences between groups have been analyzed by Fisher's exact test or Chi-square test for categorical variables. Mann–Whitney U test or Kruskal–Wallis test has instead been performed to compare continuous variables. As multivariate analysis, a logistic regression with the stepwise Wald statistic input was performed.

Kaplan–Meier survival analysis and Cox Proportional Hazard Model were used to identify variables independently associated with the outcome of interest and assess the

correlation between LSM and the risk of HCC development. Finally, a ROC curve analysis was built in order to measure the real risk of development of HCC based on liver stiffness values (kPa) and identify a cutoff value of LSM.

p values below 0.05 were considered statistically significant. All analyses were performed with the SPSS software (IBM, Armonk, New York), version 24.

Results

Two hundred and fifty-eight HCV cirrhotic patients ($n=258$) completed the follow-up according to the inclusion criteria (Fig. 1). There were 143 men and 115 women, with a median age of 68 years [61–74]. Population demographics are shown in Table 1. In particular, the median BMI was 25.8, and the percentage of diabetes was 21.3%. The median liver stiffness was 25.5 kPa.

Among the 258 cirrhotic patients with chronic HCV infection under DAAs interferon-free therapy, 35 developed hepatocellular carcinoma (HCC). Two HCC cases were recorded at the end of treatment, 16 cases 6 months after treatment, and 17 cases 12 months after treatment. The HCC occurrence pattern was heterogeneous: thirty-two patients had a nodular profile, and three patients developed infiltrative HCC (two of them with macrovascular invasion as portal vein thrombosis). The median diameter of lesions was 33 mm (range 18–57 mm). No patient showed extrahepatic metastases. All patients with HCC occurrence achieved a SVR.

At univariate analysis, HCC development was associated with higher LSM (median 37.2 vs. 23.9 kPa; $p=0.000$) and duration of therapy ($p=0.000$), as seen in Table 2. Moreover, platelets were significantly lower among patients with HCC at both baseline and SVR ($p=0.000$ and $p=0.002$, respectively). Finally, CTP score was significantly higher (Class B) among HCC patients (14.3 vs. 5% than in the control group; $p=0.035$).

At the multivariate analysis, conducted through a Wald stepwise logistic regression analysis, age, liver stiffness and platelet count at baseline were revealed as independent predictors of HCC development. Data are shown in Table 2.

Patients were divided into three groups, based on their liver stiffness: < 20 kPa ($n=72$), between 20 and 30 kPa ($n=92$) and > 30 kPa ($n=94$). Group cutoffs were decided to allow similar sized populations in each group. Kaplan–Meier survival analysis compared the groups to assess the cumulative risk of HCC. Data are shown in Fig. 2. Overall differences in HCC incidence were statistically significant (log-rank $p=0.007$). Moreover, also Cox proportional hazard model, adjusted for multiple comparisons, was conducted. Cox proportional hazard regression univariate analysis revealed age and high liver stiffness as significant risk factor for HCC. In particular, compared to the < 20 kPa and 20–30 kPa groups, higher liver stiffness groups had an increased risk of HCC,

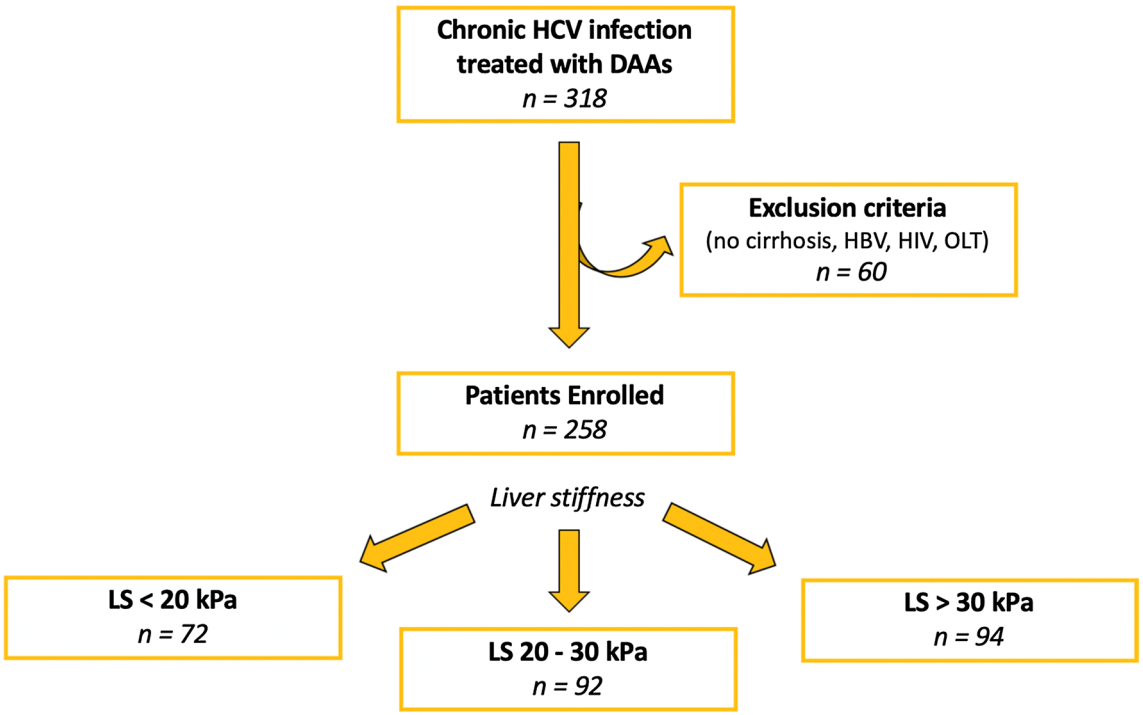

Fig. 1 Study flow

Table 1 Baseline characteristics of the entire cohort of study ($n=258$)

Parameter	
Age (years), median [IQR]	68 [61–74]
Sex, *n* (%)	
Male	143 (55.4)
Female	115 (44.6)
BMI, median [IQR]	25.8 [24–28]
Smoke, *n* (%)	14 (5.4)
Diabetes, *n* (%)	55 (21.3)
Metabolic syndrome, *n* (%)	26 (10.1)
Bright liver, *n* (%)	
0	223 (87.1)
1	30 (11.7)
2	5 (1.2)
Liver stiffness (kPa), median [IQR]	25.5 [18–35.6]
Duration of therapy, median [IQR]	12 [12–14]
Platelets, median [IQR]	
T0	119,000 [81,500–162,000]
SVR	120,000 [82,500–158,500]
Genotype, *n* (%)	
1	195 (76.7)
2	48 (18.6)
3	10 (3.2)
4	5 (1.6)
Child–Pugh score, *n* (%)	
A	242 (93.7)
B	16 (6.3)
HCC, *n* (%)	35 (13.6)
Portal invasion, *n* (%)	4 (1.6)

with the > 30 kPa group reaching statistical significance ($p=0.019$; HR 0.329; 95% CI 0.131–0.830). At multivariate analysis, high liver stiffness confirmed independently associated with HCC risk (Table 3).

Finally, we also computed a ROC curve analysis to assess the overall predictive performance of liver stiffness on the risk of HCC development. The test appeared moderately accurate, as the area under the ROC curve was equal to 0.691 (95% CI 0.594–0.789, $p=0.000$), as shown in Fig. 3. The obtained results allow us to identify a cutoff value of liver stiffness equal to 27.8, which guarantees the highest sensitivity and specificity (respectively, 72% and 65%).

Discussion

The main goal of the antiviral therapy is the viral clearance and eventually to stop the progression of liver disease and its complications. The DAAs allow a high rate of SVR in patients with liver cirrhosis, but the HCC occurrence rate should be carefully evaluated. The identification of patients at increased risk of developing HCC represents the main point for the long-term prognosis. In this study, the TE showed that the basal LSM was significantly higher in patients who developed HCC (35 vs. 25 kPa). The division into groups according to the entity of the LSM, identified a fourfold increase in risk in the population with LSM > 30 kPa. The cutoff value identified for the increase in risk was 27.8 kPa. The ROC curve identified a discrete significant sensitivity and specificity of LSM discriminant power on the risk of developing HCC in cirrhotic patients [AUROC score = 0.691; $p=0.000$]. This result suggests that specificity and sensitivity could be higher with a larger sample size.

The results confirm the literature data, which showed a high LSM significantly associated with the HCC appearance. Masuzaki et al. [17] showed a 45.5 times higher HCC risk in patients with LSM > 25 kPa when compared to cases with TE < 10 kPa; moreover, Alder et al. [6] showed a significant increase in the risk of HCC in cirrhotic patients with LSM > 30 kPa. At univariate analysis, HCC was significantly associated with LSM ($p=0.000$), CTP B ($p=0.035$) and platelets count ($p=0.000$). Metabolic syndrome did not reach statistical significance, though, according to several studies concerning the non-alcoholic steatohepatitis, revealed a pivot role in HCC development [18–20]. CTP B and platelets count are commonly associated with a long-standing liver disease.

At multivariate analysis, the factors independently associated with HCC were age ($p=0.020$), LSM ($p=0.012$) and platelets count ($p=0.008$). It is well known the association between thrombocytopenia and HCC risk and its outcome. Recently, two meta-analysis provided evidence that the presence of thrombocytopenia significantly predicts shorter survival in patients with HCC in terms of both overall survival and disease-free survival [21, 22]. These data could suggest a closer ultrasound surveillance of patients with higher LSM at baseline, also in case of viral clearance, even if the six-monthly ultrasound monitoring should be mandatory for all the cirrhotic patients. Furthermore, according to literature data, the cirrhosis stage is not subject to regression [23]; therefore, liver, although deprived of the pro-inflammatory viral trigger, showed a potentially carcinogenic persistence. These features are confirmed by our findings in the association between the advanced liver disease and significantly higher LSM and may be related to the increased risk of HCC in patients with longer disease duration [24]. FibroScan is confirmed to be a reliable noninvasive tool for both the non-invasive evaluation of liver fibrosis and the identification of a state of inveterate cirrhosis. Previous studies have shown a correlation between the increase in portal hypertension with LSM in cirrhotic or in predicting a composite outcome including death, decompensation and HCC [25, 26]. Our

Table 2 Baseline characteristics of cirrhotic patients according to HCC development: univariate and multivariate analysis ($n=258$)

Parameter	Univariate analysis			Multivariate analysis	
	HCC		*p*	O.R. [95% CI]	*p*
	Yes ($n=35$)	No ($n=223$)			
Age (years), median [IQR]	66 [70–77]	68 [61–73.5]	0.075	1.067 [1.010–1.127]	0.020
Sex, *n* (%)			0.380		
M/F	17 (48.6)/18 (51.4)	126 (56.5)/97 (43.5)			
BMI, median [IQR]	25 [23–27]	26 [24–28.3]	0.070		
Smoke, *n* (%)	4 (11.4)	10 (4.5)	0.093		
Diabetes, *n* (%)	10 (28.6)	45 (20.2)	0.260		
Metabolic syndrome, *n* (%)	6 (17.1)	20 (9)	0.135		
Liver stiffness (kPa), median [IQR]	37.2 [24.1–44.2]	23.9 [17.6–34]	0.000	1.113 [1.024–1.210]	0.012
Duration of therapy (weeks), median [IQR]	24 [12–24]	12 [12]	0.000	1.039 [1.010–1.068]	0.008
Platelets, median [IQR]					
T0	81,000 [50,000–108,000]	131,000 [86,500–168,250]	0.000		
SVR	98,000 [69,000–122,000]	130,000 [84,000–180,250]	0.002		
Genotype, *n* (%)			0.868		
1	25 (77.4)	171 (76.6)			
2	6 (16.1)	42 (18.9)			
3	2 (3.2)	7 (3.2)			
4	2 (3.2)	2 (1.4)			
Bright liver, *n* (%)			0.420		
0	29 (82.4)	196 (87.8)			
1	6 (17.6)	24 (10.8)			
2	0 (–)	3 (1.4)			
Child–Pugh score, *n* (%) A/B	30 (85.7)/5 (14.3)	212 (95)/11 (5)	0.035		
Portal invasion, *n* (%)	4 (11.4)	0 (–)	0.000		

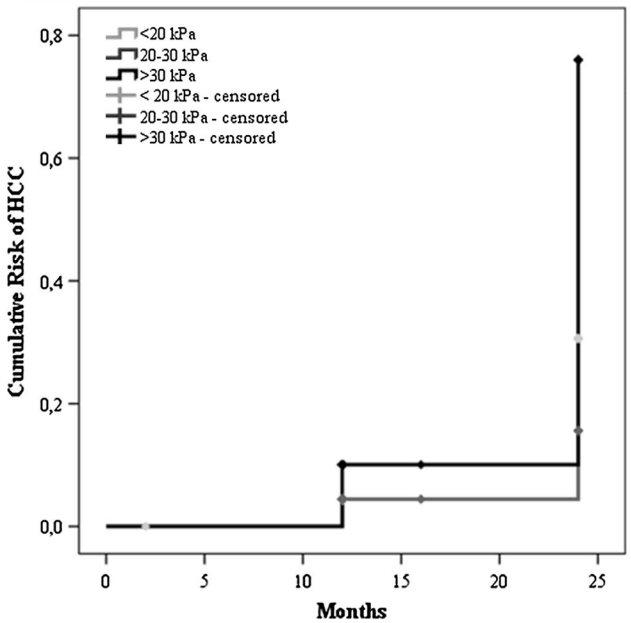

Fig. 2 Cumulative incidence of HCC stratified by liver stiffness (kPa)

Table 3 Risk factors for HCC (Cox's proportional hazard model; $n=258$)

Variable	Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age	1.020 (1.002–1.123)	0.046		
Sex	0.714 (0.365–1.396)	0.325		
BMI	0.973 (0.918–1.031)	0.358		
Child–Pugh	0.776 (0.288–2.095)	0.617		
Liver stiffness				
< 20 kPa	–	0.040	–	0.109
20–30 kPa	0.504 (0.200–1.271)	0.147	0.518 (0.205–1.306)	0.163
> 30 kPa	0.329 (0.131–0.830)	0.019	0.412 (0.163–1.040)	0.042

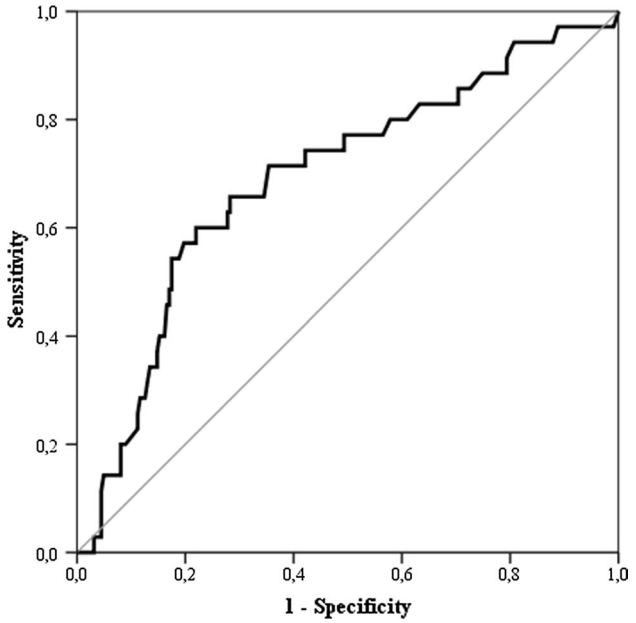

Fig. 3 ROC curve describing the discriminant power of the liver stiffness value (kPa) on the risk of developing HCC in cirrhotic patients ($n = 258$, AUROC score = 0.691). The p value for the significance of liver stiffness on the risk of HCC was 0.000 (Kruskal–Wallis test)

study has collected data from five centers, and therefore, LSM by FibroScan was obtained by several operators, thus determining a possible inter-operator bias. However, the experience in the methodology of all involved operators has minimized this risk. The selection of reliable and very reliable examinations has given a greater strength to elastomeric data, avoiding the bias deriving from the technical limitations related to the anthropometric characteristics of the patients. Furthermore, the study population only included HCV cirrhotic patients, and therefore, the comparative cut-offs between LSM and fibrosis were homogeneous and not influenced by cytolytic flare, typical of the chronic HBV hepatitis [27]. Moreover, TE only was performed, before the treatment with a similar inflammatory activity; thus, there could be no a variable overestimation of LSM.

The future perspectives of the study could suggest a monitoring of LSM over time, in order to evaluate any subsequent change after the SVR. Some studies have already reported an early decline in LSM already at the end of antiviral therapy related to the extinguishing of inflammatory activity and the normalization of aminotransferases [28, 29]. On the other hand, either an increase or a decline in long-term LSM could provide an estimate of the portal pressure and fibrosis in cirrhotic patients and an assessment of the risk of HCC development over time. In these studies, despite the viral clearance, the correlation with a high baseline LSM or a non-decline to follow-up and the HCC is underlined.

The genesis of HCC cases in SVR patients and the possible drug influence has not been fully clarified yet. In some studies in the literature, an unexpected incidence of overlapping HCC occurrence or recurrence is reported after about a year of follow-up [30, 31]. Globally, the de novo incidence rate was between the range of 0–7.4% (maximum follow-up: 18 months) [32]. Based on the opinion of several authors, the HCC appearance could be related to two main hypothesis: first, the presence of small nodules of HCC already before the start of treatment, and second, the induction of carcinogenesis with misknown mechanisms involved immune system related, including a possible role of several cytokines (i.e., TRAIL, IL 21, VEGF) [33–36].

Finally, TE is a noninvasive and reliable method in the clinical practice of patients with chronic HCV hepatitis. The usefulness in the identification of HCV patients with cirrhotic at baseline can be implemented with the possibility of identifying patients with greater risk of complications; HCC is one of the conditions which may worsen the prognosis, even in patients with SVR. TE at baseline and ultrasound surveillance has been shown to be a valuable tool for assessing the risk of HCC and should be routinely considered in the management of patients with HCV undergoing antiviral treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have not any personal or financial conflicts of interest.

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