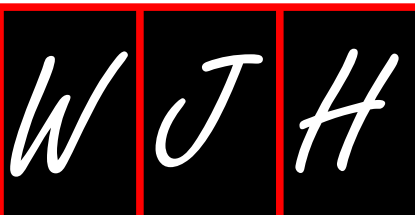


# World Journal of *Hepatology*

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## Living-donor vs deceased-donor liver transplantation for patients with hepatocellular carcinoma

Nobuhisa Akamatsu, Yasuhiko Sugawara, Norihiro Kokudo

Nobuhisa Akamatsu, Yasuhiko Sugawara, Norihiro Kokudo, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan

Author contributions: All authors contributed equally to this work.

Correspondence to: Yasuhiko Sugawara, MD, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. [yasusuga-ky@umin.ac.jp](mailto:yasusuga-ky@umin.ac.jp)

Telephone: +81-3-38155411 Fax: +81-3-56843989

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**Core tip:** The current opinions and clinical reports regarding differences in the recurrence of hepatocellular carcinoma (HCC) between living donor liver transplantation (LDLT) and deceased donor liver transplantation (DDLT) were reviewed. In the absence of a prospective study regarding the use of LDLT vs DDLT for HCC patients, only with some retrospective studies with conflicting results, there is no evidence to support the higher HCC recurrence after LDLT than DDLT, and LDLT remains a reasonable treatment option for HCC patients with cirrhosis.

### Abstract

With the increasing prevalence of living-donor liver transplantation (LDLT) for patients with hepatocellular carcinoma (HCC), some authors have reported a potential increase in the HCC recurrence rates among LDLT recipients compared to deceased-donor liver transplantation (DDLT) recipients. The aim of this review is to encompass current opinions and clinical reports regarding differences in the outcome, especially the recurrence of HCC, between LDLT and DDLT. While some studies report impaired recurrence - free survival and increased recurrence rates among LDLT recipients, others, including large database studies, report comparable recurrence - free survival and recurrence rates between LDLT and DDLT. Studies supporting the increased recurrence in LDLT have linked graft regeneration to tumor progression, but we found no association between graft regeneration/initial graft volume and tumor recurrence among our 125 consecutive LDLTs for HCC cases. In the absence of a prospective study regarding the use of LDLT vs DDLT for HCC patients, there is no evidence to support the higher HCC recurrence after LDLT than DDLT, and LDLT remains a reasonable treatment option for HCC patients with cirrhosis.

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### INTRODUCTION

Hepatocellular carcinoma (HCC) is the 7<sup>th</sup> most common cancer overall and the 3<sup>rd</sup> most common cause of cancer-related death worldwide<sup>[1,2]</sup>. Since the landmark report of the Milan criteria by Mazzaferro *et al*<sup>[3]</sup>, which demonstrated comparable outcomes of patients with HCC having a single tumor smaller than 5 cm in diameter or up to 3 tumors smaller than 3 cm in diameter with no vascular invasion or extra-hepatic disease determined by preoperative imaging studies, deceased - donor liver transplantation (DDLT) has become an established treatment for cirrhotic patients with HCC<sup>[4,5]</sup>. Similarly, in Asian countries where living-donor liver transplantation (LDLT) comprises the majority of liver transplantation procedures, LDLT has become an established treatment



**Table 1** Studies comparing living - donor liver transplantation and deceased - donor liver transplantation for hepatocellular carcinoma

Ref.	Country	Year	Study period	Type of LT	Case number	Recurrence - free survival			P	% Recurrence rate	P	Criteria used	% Outside Milan	Difference in tumor characteristics	Median follow-up period (mo)
						1-yr	3-yr	5-yr							
Impaired results in LDLT															
Park <i>et al</i> <sup>[10]</sup>	South Korea	2014	1999-2010	LDLT	166	89		81	0.045	19	0.045	UCSF	NA	none	35
Vakili <i>et al</i> <sup>[13]</sup>	United States	2009	1999-2007	DDLT	50	96		94		6		UNOS	25	none	41
				LDLT	28				29	< 0.05					
Kulik <i>et al</i> <sup>[12]</sup>	United States Multi-center	2012	1998-2010	LDLT	100	80	66	56	0.05	38	0.0004	UNOS	59	More aggressive in LDLT	60
				DDLT	97	90	81	73		11		30			
Lo <i>et al</i> <sup>[14]</sup>	Hong Kong	2007	1995-2004	LDLT	43	93	71	71	0.029	29	0.029	UCSF	26	More aggressive in LDLT	33
				DDLT	17	100	100	100		0		29			
Comparable results															
Sandhu <i>et al</i> <sup>[15]</sup>	Canada	2013	1996-2009	LDLT	58	88	75	70	NS	17	NS	Toronto criteria	28	none	38
				DDLT	287	86	75	70		15		32			
Bhangui <i>et al</i> <sup>[16]</sup>	France	2011	2000-2009	LDLT	36	100	89	88	NS	13	NS	UCSF	27	none	58
				DDLT	120	93	89	86		13		21			
Li <i>et al</i> <sup>[36]</sup>	China	2010	2005-2009	LDLT	38	71	42		NS	50	NS	UCSF	79	none	25
				DDLT	101	76	41			55		68			
Di Sandro <i>et al</i> <sup>[35]</sup>	Italy	2009	2000-2007	LDLT	25		96	96	NS	4	NS	Milan	20	none	NA
				DDLT	154		91	89		11		31			
Sotiropoulos <i>et al</i> <sup>[20]</sup>	Germany	2007	1998-2006	LDLT	45	88	75		NS	12	NS	UCSF	44	none	NA
				DDLT	55		81			14					
Hwang <i>et al</i> <sup>[8]</sup>	South Korea Multi-center	2005	1992-2002	LDLT	237	83	80		NS	18	NS		27	none	26
				DDLT	75	88	82			16		29			
Gondolesi <i>et al</i> <sup>[17]</sup>	United States	2004	1988-2002	LDLT	36	82	74		NS	19	NS	UNOS	53	none	15
				DDLT	165	90	83			19					

DDLT: Deceased - donor liver transplantation; HCC: Hepatocellular carcinoma; LDLT: Living - donor liver transplantation; LT: Liver transplantation; UCSF: University of California, San Francisco; UNOS: United Network for Organ Sharing; NA: Not applicable; NS: Not significant.

for HCC patients with end-stage liver disease<sup>[6,7]</sup>. LDLT is now considered a promising treatment for HCC patients in Western countries, not only to compensate for the shortage of donor organs but also to reduce the dropout rate on the waiting list<sup>[8]</sup>.

With the accumulation of LDLTs for HCC patients, the impact of LDLT on recipient outcome compared with DDLT, especially the recurrence of HCC after liver transplantation, has become an important topic of debate<sup>[9]</sup>. The aim of this review was to encompass the current opinions and clinical reports regarding the differences in outcome, especially the recurrence of HCC, between LDLT and whole liver DDLT.

## STUDIES COMPARING LDLT AND DDLT FOR HCC PATIENTS

Studies comparing LDLT and DDLT for HCC patients are summarized in Table 1. All DDLTs reviewed here were done with the whole liver graft.

### Studies reporting a poorer outcome in the LDLT setting

Park *et al*<sup>[10]</sup> recently reported poorer recurrence-free survival among 166 LDLT recipients (81% at 5 years) com-

pared to 50 DDLT recipients (94% at 5 years;  $P = 0.045$ ). The noteworthy finding of this study was that the smaller the LDLT graft, the poorer the recurrence - free survival. Based on this finding, Park *et al*<sup>[10]</sup> suggested that the physiology of the small graft may stimulate tumor recurrence.

The results of the A2ALL cohort in United States also demonstrated an impaired outcome in LDLT recipients. In their initial report<sup>[11]</sup>, they found a higher rate of recurrence within 3 years in LDLT than in DDLT (29% *vs* 0%,  $P = 0.002$ ), but there was a clear tendency toward more aggressive tumor characteristics in the LDLT group. The same group recently published an updated report<sup>[12]</sup>, in which HCC recurrence remained significantly different between LDLT and DDLT after adjustment for tumor characteristics. They concluded that the higher recurrence observed after LDLT was likely due to differences in the tumor characteristics, pretransplant HCC management, and waiting time.

Vakili *et al*<sup>[13]</sup> reporting the Lahey Clinic experience, demonstrated that the HCC recurrence rate of LDLT (29%) was significantly higher than that of DDLT (12%) ( $P < 0.05$ ), but survival after LDLT was significantly better than that following DDLT for HCC during the same

period ( $P = 0.02$ ).

Lo *et al*<sup>[14]</sup> from Hong Kong also reported a significantly higher incidence of HCC recurrence, 29% in LDLT and 0% in DDLT ( $P = 0.029$ ). While the tumor characteristics were comparable between groups, the authors speculated that LDLT as a salvage transplantation, microscopic vascular invasion, and liver regeneration led to the difference in the recurrence rate.

### Studies reporting a comparable outcome

Sandhu and colleagues of the Toronto group<sup>[15]</sup> reported that LDLT and DDLT both provide similarly low recurrence rates and high survival rates. They compared the results of 58 LDLT cases with those of 287 DDLT cases having comparable tumor characteristics, in which the 1-, 3-, and 5-year recurrence-free survival rates were 88%, 75%, and 70%, and 86%, 75%, and 70%, respectively.

In a well-designed study by Bhangui *et al*<sup>[16]</sup>, an intention-to-treat analysis was conducted with recurrence rate representing the primary endpoint, comparing 36 LDLT cases and 147 DDLT cases. The authors demonstrated that both LDLT and DDLT provided similar recurrence - free survival rates (88% *vs* 86% at 5 years) for patients with HCC. The dropout rate and waiting time were significantly lower in the LDLT group than in the DDLT group, and there was also a trend toward a longer time to recurrence in the LDLT group, which may guarantee additional advantages with LDLT.

The Mount Sinai group<sup>[17,18]</sup> reported comparable recurrence - free survival between LDLT ( $n = 36$ ) and DDLT ( $n = 165$ ; 74% *vs* 83% at 2 years,  $P = 0.3$ ). When stratified by tumor size (5 cm diameter) and the existence of microvascular invasion, there was still no difference between groups.

Sotiropoulos and colleagues of Essen, Germany<sup>[19,20]</sup>, also supported the comparable recurrence - free survival rates between LDLT and DDLT for HCC (75% *vs* 81% at 3 years).

Hwang *et al*<sup>[21]</sup> of South Korea performed a nationwide survey regarding this issue. Among 237 LDLTs and 75 DDLTs for HCC, the 1 - and 3 - year recurrence - free survival rates were 83% and 80%, and 88% and 82%, respectively, with no significant difference between them.

A comparison of outcomes after liver transplantation obtained from database studies revealed comparable patient survival rates between LDLT and DDLT. According to a report from the Japanese Liver Transplantation Society Registry<sup>[22]</sup>, a total of 6097 LDLTs were performed in Japan by the end of 2010, and 1225 (32%) were indicated for HCC, which was the most common indication in adult patients. The 1-, 3-, 5-, and 10-year cumulative survival rates of LDLT for HCC were 85%, 74%, 69%, and 60%, respectively. Todo and colleagues<sup>[23]</sup> performed a detailed survey using the same database (up to the end of 2005), comprising 653 patients who had undergone LDLT for HCC in Japan. At 1, 3, and 5 years, overall patient survival was 83%, 73%, and 69%, and disease-free survival was 77%, 65%, and 61%, respectively. Based on

preoperative imaging studies, 62% were within the Milan criteria and 38% were beyond the Milan criteria, with 5-year recurrence-free survival rates of 90% and 61%, respectively ( $P < 0.001$ ). These findings do not differ much from those obtained in the DDLT database of the United States and Europe<sup>[24-27]</sup>, and may validate the use of LDLT for HCC patients.

## CURRENT OPINIONS REGARDING THE DIFFERENCE BETWEEN LDLT AND DDLT

A randomized clinical study would be best to settle the controversy regarding the use of LDLT *vs* DDLT for HCC patients, but this is indeed difficult, if not impossible, to realize given the complicated decision-making process involved in LDLT. No prospective study has been conducted to date.

The Toronto group<sup>[28]</sup> recently performed a meta-analysis on 12 retrospective studies comparing the recurrence rates and recurrence - free survival between LDLT and DDLT recipients. A total of 633 LDLTs and 1232 DDLTs were enrolled, and the study provided evidence of lower disease - free survival after LDLT compared with DDLT for HCC (HR = 1.59, 95%CI: 1.02-2.49;  $P = 0.041$ ). In contrast, there was no difference in overall survival between LDLT and DDLT (HR = 0.97, 95%CI: 0.73-1.27;  $P = 0.808$ ). As mentioned by the authors of the paper, however, all involved studies were retrospective, had a low data quality score with poor reporting of baseline patient characteristics and an inadequate statistical approach, and were heterogeneous in critical aspects such as indication criteria and basal tumor characteristics, which warrant further well-designed studies to determine whether differences in HCC recurrence are due to study biases or biologic differences.

A recent review article by experts<sup>[29]</sup> concluded as follows: Although there is no strong evidence to support the higher HCC recurrence rates in LDLT than DDLT, the higher recurrence rates in LDLT recipients reported by several authors cannot be ignored. Actually, there are critical differences among societies such as: (1) differences in the allocation system for DDLT and LDLT; (2) differences in the availability of deceased donors; (3) differences in the potential waiting time; and (4) the differences in regional and national organ transplant law. In addition to taking into account these differences, liver transplant candidates with HCC and their potential live donors should be informed following risks and benefits; the waiting time for DDLT may lead to the dropout due to HCC progression which could be avoided by the prompt LDLT, however, the prompt LDLT may mask the aggressive tumor characteristics which may lead to a higher HCC recurrence rates. Although the currently available literatures can provide a low evidence for the difference of HCC recurrence between DDLT and LDLT, the tumor characteristics and biology seem to significantly influence on the recurrence, while the graft type and waiting time are less likely important as a possible risk factor.

**Table 2** Graft characteristics and hepatocellular carcinoma recurrence

	Patients with recurrence ( <i>n</i> = 11)	Patients without recurrence ( <i>n</i> = 114)	<i>P</i>
Regeneration rate at 3 mo (%)	90 ± 24	93 ± 34	0.732
Graft type: right/left	4/7	36/78	0.702
Initial graft volume ratio to standard liver volume (%)	46 ± 9	47 ± 9	0.842

## POSTULATED THEORIES FOR DIFFERENCES BETWEEN LDLT AND DDLT

LDLT provides several advantages compared with DDLT, such as a shorter waiting time, good quality graft with normal liver function and shorter ischemic time, and pretransplant treatment optimization, which might contribute to improved survival in LDLT recipients. Some of these characteristics, on the other hand, may lead to a favorable milieu for tumor progression<sup>[9]</sup>.

There are several hypotheses other than tumor characteristics to explain the inferior outcome of LDLT. One explanation for the higher recurrence rates in LDLT is fast-tracking patients into liver transplantation, the so-called fast-track effect<sup>[11,30]</sup>. Some patients with more biologically aggressive HCC might drop off the waiting list due to tumor progression beyond the criteria during the wait-time in the DDLT setting. In contrast, due to the shortened wait time for LDLT candidates, progression of HCC with an aggressive tumor biology might not be recognized during such a short wait-time. This scenario might account for the higher HCC recurrence in the LDLT setting.

Another hypothesized mechanism for the higher recurrence rates in LDLT is that growth factors and cytokines released during rapid regeneration of the partial grafts from living donors might contribute to tumor progression and recurrence<sup>[31-34]</sup>. A rapidly regenerating liver parenchyma and ischemic-reperfusion injury facilitated by a small-for-size graft in LDLT setting might be a more favorable environment for tumor progression and HCC recurrence.

Additionally, some authors<sup>[11,35,36]</sup> insist that the technique of LDLT per se foregoes the principles of oncologic surgery. During LDLT, the meticulous dissection and mobilization of the liver might increase the possibility of tumor capsule violation or tumor embolization through the hepatic veins, thus promoting tumor dissemination. Preserving the native vena cava and the bile duct/hepatic artery/portal vein in the hepatic hilum might increase the risk of leaving the residual tumors.

As opposed with the above-mentioned anecdotal explanations, the advanced tumor characteristics of LDLT recipients can reasonably explain the higher recurrence rate in the LDLT setting. Grafts from living donors are

not limited by restrictions imposed by the organ allocation system, meaning that the relation of the graft and recipient is usually one-on-one. Consequently, selection criteria based on the tumor burden, such as the tumor size and number, can be considered relative on a case-by-case basis, taking into account the presence of risk factors for recurrence and the chance of survival, as well as the wishes of the donor<sup>[37]</sup>. Consequently, the majority of Asian transplant centers have adopted extended criteria beyond those of Milan or the University of California, San Francisco (UCSF)<sup>[38]</sup>. Based on some studies, differences in patient tumor characteristics between LDLT and DDLT remain a main reason for the higher recurrence rate in LDLT. Additionally, in the majority of the aforementioned studies comparing LDLT and DDLT for HCC patients, tumor burdens such as the size, number, vascular invasion, and poor differentiation have proved to be independent risk factors for HCC recurrence after liver transplantation, all of which may lead to a rational explanation for the impaired recurrence-free survival of LDLT compared to DDLT.

## OUR EXPERIENCE

At our institution, the University of Tokyo Hospital, a total of 423 adult recipients underwent LDLT by the end of 2012. Among them, 125 (30%) patients had HCC. The principle criterion for LDLT for HCC at our center is “up to 5 nodules with a maximum tumor diameter within 5 cm”, which we call the “5-5 rule”<sup>[39]</sup>. Of the 125 patients, 118 (94%) were within the 5-5 rule criteria and 109 (87%) were within the Milan criteria. Overall survival of the 125 recipients at 1, 3, and 5 years was 88%, 82%, and 76%, respectively, with a median follow-up period of 8 years. A total of 11 (9%) patients developed HCC recurrence with a cumulative recurrence rate at 1, 3, and 5 years of 6%, 9%, and 11%, respectively.

We compared the graft regeneration rate between patients with HCC recurrence (*n* = 11) and those without recurrence (*n* = 114) to confirm the association of liver regeneration with HCC recurrence. The regeneration rate was calculated as follows: (graft volume at 3 mo after LDLT - initial graft volume)/initial graft volume × 100 (%). As shown in Table 2, there was no difference in the regeneration rate between those with HCC recurrence and those without recurrence. At the same time, the graft type (right vs left) and the initial graft volume ratio to the recipient's standard liver volume were also compared between groups, revealing no difference. A similar result was reported by the Asan group of South Korea<sup>[40]</sup>, in which the graft-recipient weight ratio had no impact on HCC recurrence after LDLT among 181 LDLT recipients with HCC. Our result as well as the report of the Asan group clearly demonstrated that graft regeneration of the partial liver graft has no impact on HCC recurrence, at least in a clinical setting. The independent predictors for HCC recurrence in our series were tumors not within the 5-5 rule (Tokyo criteria), AFP level over 400 ng/mL, and des-



gamma-carboxy prothrombin levels over 200 mAU/mL.

## CONCLUSION

In conclusion, there is no strong evidence to support higher HCC recurrence after LDLT than DDLT, and it may be reasonable to use different indication criteria for LDLT and DDLT, while there could be a potential bias in choosing the articles in the present study. LDLT should always be considered as a treatment option for HCC patients with advanced cirrhosis in areas where deceased donors are scarce or for patients whose tumor status interrupts access to DDLT.

## REFERENCES

- 1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]
- 2 Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 3 Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
- 4 Bruix J, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850]
- 5 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 6 de Villa V, Lo CM. Liver transplantation for hepatocellular carcinoma in Asia. *Oncologist* 2007; **12**: 1321-1331 [PMID: 18055852 DOI: 10.1634/theoncologist.12-11-1321]
- 7 Lee Cheah Y, K H Chow P. Liver transplantation for hepatocellular carcinoma: an appraisal of current controversies. *Liver Cancer* 2012; **1**: 183-189 [PMID: 24159583 DOI: 10.1159/000343832]
- 8 Hwang S, Lee SG, Belghiti J. Liver transplantation for HCC: its role: Eastern and Western perspectives. *J Hepatobiliary Pancreat Sci* 2010; **17**: 443-448 [PMID: 19885638 DOI: 10.1007/s00534-009-0241-0]
- 9 Quintini C, Hashimoto K, Uso TD, Miller C. Is there an advantage of living over deceased donation in liver transplantation? *Transpl Int* 2013; **26**: 11-19 [PMID: 22937787]
- 10 Park MS, Lee KW, Suh SW, You T, Choi Y, Kim H, Hong G, Yi NJ, Kwon CH, Joh JW, Lee SK, Suh KS. Living-donor liver transplantation associated with higher incidence of hepatocellular carcinoma recurrence than deceased-donor liver transplantation. *Transplantation* 2014; **97**: 71-77 [PMID: 24056623 DOI: 10.1097/TP.0b013e3182a68953]
- 11 Fisher RA, Kulik LM, Freise CE, Lok AS, Shearon TH, Brown RS, Ghobrial RM, Fair JH, Olthoff KM, Kam I, Berg CL. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am J Transplant* 2007; **7**: 1601-1608 [PMID: 17511683 DOI: 10.1111/j.1600-6143.2007.01802.x]
- 12 Kulik LM, Fisher RA, Rodrigo DR, Brown RS, Freise CE, Shaked A, Everhart JE, Everson GT, Hong JC, Hayashi PH, Berg CL, Lok AS. Outcomes of living and deceased donor liver transplant recipients with hepatocellular carcinoma: results of the A2ALL cohort. *Am J Transplant* 2012; **12**: 2997-3007 [PMID: 22994906 DOI: 10.1111/j.1600-6143.2012.04272.x]
- 13 Vakili K, Pomposelli JJ, Cheah YL, Akoad M, Lewis WD, Khettry U, Gordon F, Khwaja K, Jenkins R, Pomfret EA. Living donor liver transplantation for hepatocellular carcinoma: Increased recurrence but improved survival. *Liver Transpl* 2009; **15**: 1861-1866 [PMID: 19938113 DOI: 10.1002/lt.21940]
- 14 Lo CM, Fan ST, Liu CL, Chan SC, Ng IO, Wong J. Living donor versus deceased donor liver transplantation for early irresectable hepatocellular carcinoma. *Br J Surg* 2007; **94**: 78-86 [PMID: 17016793 DOI: 10.1002/bjs.5528]
- 15 Sandhu L, Sandroussi C, Guba M, Selzner M, Ghanekar A, Cattral MS, McGilvray ID, Levy G, Greig PD, Renner EL, Grant DR. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: comparable survival and recurrence. *Liver Transpl* 2012; **18**: 315-322 [PMID: 22140013 DOI: 10.1002/lt.22477]
- 16 Bhangui P, Vibert E, Majno P, Salloum C, Andreani P, Zocrato J, Ichai P, Saliba F, Adam R, Castaing D, Azoulay D. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. *Hepatology* 2011; **53**: 1570-1579 [PMID: 21520172 DOI: 10.1002/hep.24231]
- 17 Gondolesi GE, Roayaie S, Muñoz L, Kim-Schluger L, Schiano T, Fishbein TM, Emre S, Miller CM, Schwartz ME. Adult living donor liver transplantation for patients with hepatocellular carcinoma: extending UNOS priority criteria. *Ann Surg* 2004; **239**: 142-149 [PMID: 14745320 DOI: 10.1097/01.sla.0000109022.32391.eb]
- 18 Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797 DOI: 10.1002/lt.20128]
- 19 Malagó M, Sotiropoulos GC, Nadalin S, Valentin-Gamazo C, Paul A, Lang H, Radtke A, Saner F, Molmenti E, Beckebaum S, Gerken G, Frilling A, Broelsch CE. Living donor liver transplantation for hepatocellular carcinoma: a single-center preliminary report. *Liver Transpl* 2006; **12**: 934-940 [PMID: 16528715 DOI: 10.1002/lt.20677]
- 20 Sotiropoulos GC, Lang H, Nadalin S, Neuhaus M, Molmenti EP, Baba HA, Paul A, Saner FH, Weber F, Hilgard P, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma: University Hospital Essen experience and meta-analysis of prognostic factors. *J Am Coll Surg* 2007; **205**: 661-675 [PMID: 17964442 DOI: 10.1016/j.jamcollsurg.2007.05.023]
- 21 Hwang S, Lee SG, Joh JW, Suh KS, Kim DG. Liver transplantation for adult patients with hepatocellular carcinoma in Korea: comparison between cadaveric donor and living donor liver transplantations. *Liver Transpl* 2005; **11**: 1265-1272 [PMID: 16184545 DOI: 10.1002/lt.20549]
- 22 Liver transplantation in Japan- registry by the Japanese Liver Transplantation Society. *Ishoku* 2012; **46**: 524-536
- 23 Todo S, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069]
- 24 Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773]
- 25 Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, Castaing D, Neuhaus P, Jamieson N, Salizzoni M, Pollard S, Lerut J, Paul A, Garcia-Valdecasas JC, Rodríguez FS, Burroughs A. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012; **57**: 675-688 [PMID: 22609307 DOI: 10.1016/j.jhep.2012.04.015]
- 26 Singal AK, Guturu P, Hmoud B, Kuo YF, Salameh H, Wiesner RH. Evolving frequency and outcomes of liver transplantation based on etiology of liver disease. *Trans-*

- plantation 2013; **95**: 755-760 [PMID: 23370710 DOI: 10.1097/TP.0b013e31827afb3a]
- 27 **Taniguchi M.** Liver transplantation in the MELD era--analysis of the OPTN/UNOS registry. *Clin Transpl* 2012; **2012**: 41-65 [PMID: 23721009]
  - 28 **Grant RC, Sandhu L, Dixon PR, Greig PD, Grant DR, McGilvray ID.** Living vs. deceased donor liver transplantation for hepatocellular carcinoma: a systematic review and meta-analysis. *Clin Transplant* 2013; **27**: 140-147 [PMID: 23157398 DOI: 10.1111/ctr.12031]
  - 29 **Grant D, Fisher RA, Abecassis M, McCaughan G, Wright L, Fan ST.** Should the liver transplant criteria for hepatocellular carcinoma be different for deceased donation and living donation? *Liver Transpl* 2011; **17** Suppl 2: S133-S138 [PMID: 21634006]
  - 30 **Kulik L, Abecassis M.** Living donor liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S277-S282 [PMID: 15508095]
  - 31 **Man K, Lo CM, Xiao JW, Ng KT, Sun BS, Ng IO, Cheng Q, Sun CK, Fan ST.** The significance of acute phase small-for-size graft injury on tumor growth and invasiveness after liver transplantation. *Ann Surg* 2008; **247**: 1049-1057 [PMID: 18520234 DOI: 10.1097/SLA.0b013e31816ffab6XXX]
  - 32 **Shi JH, Huitfeldt HS, Suo ZH, Line PD.** Growth of hepatocellular carcinoma in the regenerating liver. *Liver Transpl* 2011; **17**: 866-874 [PMID: 21542129 DOI: 10.1002/lt.22325]
  - 33 **Efimova EA, Glanemann M, Liu L, Schumacher G, Settmacher U, Jonas S, Langrehr JM, Neuhaus P, Nüssler AK.** Effects of human hepatocyte growth factor on the proliferation of human hepatocytes and hepatocellular carcinoma cell lines. *Eur Surg Res* 2004; **36**: 300-307 [PMID: 15359093 DOI: 10.1159/000079915]
  - 34 **Man K, Fan ST, Lo CM, Liu CL, Fung PC, Liang TB, Lee TK, Tsui SH, Ng IO, Zhang ZW, Wong J.** Graft injury in relation to graft size in right lobe live donor liver transplantation: a study of hepatic sinusoidal injury in correlation with portal hemodynamics and intra-graft gene expression. *Ann Surg* 2003; **237**: 256-264 [PMID: 12560784 DOI: 10.1097/01.SLA.0000048976.11824.67]
  - 35 **Di Sandro S, Slim AO, Giacomoni A, Lauterio A, Mangoni I, Aseni P, Pirotta V, Aldumour A, Mihaylov P, De Carlis L.** Living donor liver transplantation for hepatocellular carcinoma: long-term results compared with deceased donor liver transplantation. *Transplant Proc* 2009; **41**: 1283-1285 [PMID: 19460539 DOI: 10.1016/j.transproceed.2009.03.022]
  - 36 **Li C, Wen TF, Yan LN, Li B, Yang JY, Wang WT, Xu MQ, Wei YG.** Outcome of hepatocellular carcinoma treated by liver transplantation: comparison of living donor and deceased donor transplantation. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 366-369 [PMID: 20688599]
  - 37 **Tamura S, Sugawara Y, Kokudo N.** Living donor liver transplantation for hepatocellular carcinoma: the Japanese experience. *Oncology* 2011; **81** Suppl 1: 111-115 [PMID: 22212944]
  - 38 **Chan SC.** Liver transplantation for hepatocellular carcinoma. *Liver Cancer* 2013; **2**: 338-344 [PMID: 24400221 DOI: 10.1159/000343849]
  - 39 **Sugawara Y, Tamura S, Makuuchi M.** Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25**: 310-312 [PMID: 17960065 DOI: 10.1159/000106910]
  - 40 **Hwang S, Lee SG, Ahn CS, Kim KH, Moon DB, Ha TY, Park KM, Song GW, Jung DH, Kim BS, Moon KM.** Small-sized liver graft does not increase the risk of hepatocellular carcinoma recurrence after living donor liver transplantation. *Transplant Proc* 2007; **39**: 1526-1529 [PMID: 17580180 DOI: 10.1016/j.transproceed.2007.03.066]

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## Liver involvement in systemic infection

Masami Minemura, Kazuto Tajiri, Yukihiro Shimizu

Masami Minemura, Kazuto Tajiri, The Third Department of Internal Medicine, Faculty of Medicine, University of Toyama, Toyama 930-0194, Japan

Yukihiro Shimizu, Gastroenterology Unit, Nanto Municipal Hospital, Toyama 932-0211, Japan

**Author contributions:** Minemura M wrote most parts of the manuscript; Tajiri K wrote a part of the manuscript; Shimizu Y organized the whole manuscript and wrote a part of the manuscript.

**Correspondence to:** Yukihiro Shimizu, MD, PhD, Gastroenterology Unit, Nanto Municipal Hospital, 938 Inami, Toyama 932-0211, Japan. [rsf14240@nifty.com](mailto:rsf14240@nifty.com)

Telephone: +81-763-821475

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### Abstract

The liver is often involved in systemic infections, resulting in various types of abnormal liver function test results. In particular, hyperbilirubinemia in the range of 2-10 mg/dL is often seen in patients with sepsis, and several mechanisms for this phenomenon have been proposed. In this review, we summarize how the liver is involved in various systemic infections that are not considered to be primarily hepatotropic. In most patients with systemic infections, treatment for the invading microbes is enough to normalize the liver function tests. However, some patients may show severe liver injury or fulminant hepatic failure, requiring intensive treatment of the liver.

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**Key words:** Liver dysfunction; Liver function test; Systemic infection; Immunology; Liver failure

**Core tip:** The liver is frequently involved in systemic infections, resulting in various types of abnormal liver function test results. It is very important to know the frequency and the patterns of abnormal liver function test results in each infection for the appropriate man-

agement of the patients. However, there have been few reports focusing on this issue. Here, we gather information from previous reports on this topic to provide a comprehensive summary that will help clinicians interpret abnormal liver function test results according to the associated infection.

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### INTRODUCTION

It is well known that the classical hepatotropic viruses, hepatitis A through E, can infect the liver and cause hepatic injury. Other systemic infections by non-hepatotropic viruses or bacteria can also cause hepatic injury, either by direct invasion or indirectly through toxins and cytokines, but there are few reports of the correlations between liver function abnormalities and these infections. This review will describe features of liver injury caused by various systemic infections. It will discuss, in order, bacterial infections, infection by specific pathogens, non-hepatitis viral infections, fungal infections, and liver involvement of parasitic diseases.

### BACTERIAL INFECTIONS

#### Sepsis

Sepsis is a clinical syndrome that complicates severe infection and accompanies systemic abnormalities such as tachycardia, tachypnea and/or hypotension. It is thought to be associated with vasodilation and increased microvascular permeability caused by bacterial products and cytokines. Liver function test abnormalities and jaundice frequently accompany a variety of bacterial infections, especially sepsis<sup>[1]</sup>. Various sites of infection can cause jaundice, which include intra-abdominal infection, urinary

**Table 1 Mechanism of Jaundice in Sepsis**

Increased bilirubin load
Hemolysis
Red blood cells lysed by bacterial products ( <i>e.g.</i> , exotoxin)
Red blood cells lysed by immunological mechanisms
Hepatic dysfunction
Hepatocellular injury (hepatitis and/or necrosis)
Hepatic ischemia
Decreased bilirubin uptake; dysfunction of basolateral transport ( <i>e.g.</i> , NTCP)
Decreased transport of conjugated bilirubin; dysfunction of canalicular transport ( <i>e.g.</i> , BSEP, MRP2)
Decreased bile flow

NTCP: Na<sup>+</sup>/taurocholate cotransporting polypeptide; BSEP: Bile salt export pump; MRP2: Multidrug-resistance-associated protein 2.

tract infection, pneumonia, endocarditis, and meningitis. Although several retrospective studies have reported incidences of jaundice ranging from 0.6% to 54% in adults with sepsis<sup>[2,3]</sup>, the precise incidence remains unclear because of the absence of a large-scale prospective study. In patients with sepsis, jaundice can be caused by several organisms including aerobic and anaerobic gram-negative (*Escherichia coli* and *Klebsiella*) and gram-positive bacteria (*Staphylococcus aureus*). Kanai *et al*<sup>[4]</sup> isolated microorganism species in patients with bacteremia and reported *S. aureus*, *E. coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* as comprising 29.3%, 14.4%, 6.0%, and 6.0% of all isolates, respectively. Uslan *et al*<sup>[5]</sup> also reported that the most common organisms identified in the blood of patients with bacteremia were *E. coli* (25.1%) and *S. aureus* (16.6%). Of the bloodstream infections, 44.5% were community acquired, 36.5% were health care associated, and 19.1% were nosocomial<sup>[5]</sup>.

Hyperbilirubinemia in the range of 2-10 mg/dL is often seen in patients with sepsis, but on rare occasions, much higher levels (30 to 50 mg/dL) have been reported<sup>[6]</sup>. Serum alkaline phosphatase (ALP) is usually elevated in range of 1 to 3 times the upper limit of normal (ULN), but serum ALT is only modestly elevated. Infected patients with bacteremia had significantly higher serum levels of gamma-glutamyl transpeptidase ( $\gamma$ -GTP) and ALP and significantly lower serum concentrations of albumin, cholesterol and cholinesterase as compared with those without bacteremia. If septic shock and hypoperfusion complicate, a striking elevation of aminotransferases may occur.

The pathogenesis of jaundice in systemic infections is multifactorial. Jaundice is mainly associated with cholestasis in patients with sepsis<sup>[1]</sup>, but isolated jaundice without cholestasis can occur through increased bilirubin load from hemolysis in some cases<sup>[7,8]</sup>. Several bacterial infections, especially *Clostridium perfringens*, may cause hemolysis. Phospholipase C produced by *C. perfringens* may be associated with the release of lysolecithin and the lysing of red blood cell membranes. It also can produce proteolytic exotoxins, which may lead to hemolysis<sup>[9,10]</sup>.

Cholestasis is mainly thought to be caused through the inhibition of the canalicular excretion of conjugated bilirubin by proinflammatory cytokines, including tumor

necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1,6 (IL-1,6), which are mainly released by macrophages in response to endotoxins<sup>[11]</sup>. Interestingly the serum concentrations of ALP and bilirubin are often discordant, because jaundice in sepsis is associated with various factors including increased bilirubin load, decreased bilirubin uptake, intra-hepatic processing, and canalicular excretion (Table 1)<sup>[12,13]</sup>.

The major histological finding in sepsis is bland intra-hepatic cholestasis with bile in the bile canaliculi and hepatocytes. Minimal degenerated changes of hepatocytes with Kupffer cell hyperplasia and lymphocyte infiltration may also be seen.

### Pneumonia

Lobar pneumonia is a common disease usually caused by any one of a variety of bacteria (*e.g.*, *S. pneumonia*, *Haemophilus influenza*, *S. aureus*, or *P. aeruginosa*). Patients with pneumococcal pneumonia sometimes show elevated concentrations of serum aminotransferases and bilirubin. Jaundice was reportedly observed in 3%-25% of such patients<sup>[14]</sup>. Pneumonia-associated jaundice is mostly thought to be a result of hepatocellular damage, because hepatic necrosis is often seen in liver biopsies of patients with pneumonia<sup>[15]</sup>. In *Mycoplasma pneumonia* infection, an adult case with acute hepatitis without pulmonary manifestations was also reported by Lee *et al*<sup>[16]</sup>. They also summarized five other cases (5 to 22 years of age) with similar clinical characteristics to those of *M. pneumonia* infection. *Legionella* is an important species of bacteria which causes pneumonia, often accompanied by laboratory abnormalities indicating hepatic dysfunction, renal dysfunction, thrombocytopenia, and hyponatremia<sup>[17]</sup>.

### Microbial foodborne disease

Microbial foodborne illness is very common and mainly causes gastrointestinal symptoms including nausea, vomiting, abdominal pain, diarrhea, and fever. These patients may have other complications such as hepatitis, renal failure, and neurogenic symptoms (Table 2).

**Salmonella typhi infection:** *Salmonella typhi* can cause an acute systemic illness known as typhoid fever, while being nontyphoidal *Salmonella* (most commonly *S. enteritidis* and *S. typhimurium*) primarily induces gastroenteritis. The majority of patients with typhoid fever present with fever, malaise, abdominal discomfort, and hepatosplenomegaly. Typhoid fever may also cause liver injury with elevated aminotransferases and jaundice<sup>[18]</sup>. Hepatomegaly and jaundice were reportedly observed in 44% and in 33% of patients with typhoid fever, respectively. Although severe elevation of aminotransferases is rare in patients with typhoid fever, typhoid fever and viral hepatitis A sometimes need to be discriminated because clinical features of typhoid fever are similar to those of acute viral hepatitis A infection (Table 3). The ALT/LDH ratio may be useful to distinguish these diseases; the ALT/LDH ratio has been shown to be significantly lower (< 4.0) in typhoid fever compared with the ratio (> 5.0) in acute viral hepatitis A<sup>[19]</sup>. The hepatic histology shows

**Table 2 Foodborne pathogens and manifestations**

Pathogens	Manifestations
<b>Bacteria</b>	
<i>Staphylococcus aureus</i>	Vomiting (exotoxin), toxic shock syndrome
<i>Clostridium spp</i>	
<i>C. botulinum</i>	Neurogenic finding (paralysis)
<i>C. perfringens</i>	Diarrhea, gas gangrene, intravascular hemolysis, jaundice, liver abscess, gas in the portal vein
<i>Campylobacter spp</i>	
<i>C. jejuni</i>	Inflammatory diarrhea, liver injury (possible)
<i>C. fetus</i>	Systemic, liver injury (possible)
<i>Escherichia coli</i>	
Enterotoxigenic <i>E. coli</i>	Inflammatory diarrhea
Enterohemorrhagic <i>E. coli</i>	Inflammatory diarrhea, hemolytic uremic syndrome
<i>Listeria monocytogens</i>	Systemic (Listeriosis), elevated aminotransferases
<i>Salmonella spp</i>	
Non-typhoidal	Inflammatory diarrhea
<i>S. typhi</i>	Systemic (Typhoid fever), acute hepatitis (Salmonella hepatitis)
<i>S. paratyphi</i>	
<i>Shigella spp</i>	Inflammatory diarrhea, cholestatic hepatitis
<i>Vibrios spp</i>	
<i>V. cholera</i>	Watery diarrhea
<i>V. parahaemolyticus</i>	Inflammatory diarrhea
<i>V. vulnificus</i>	Systemic (sepsis, DIC)
<i>Yersinia enterocolitica</i>	Inflammatory diarrhea, multiple liver abscesses
<b>Virus</b>	
Hepatitis A	Acute hepatitis, jaundice
Hepatitis E	Acute hepatitis, jaundice
Norovirus	Vomiting, watery diarrhea
Rotavirus	Vomiting, watery diarrhea

minimal parenchymal changes with focal infiltration of mononuclear cells or focal hepatocyte necrosis known as “typhoid nodules”<sup>[20,21]</sup>.

**Campylobacter infection:** *Campylobacter* enteritis is an important cause of acute diarrhea, and several complications are known in patients with *Campylobacter* infection, which include cholecystitis, reactive arthritis and Guillain-Barré syndrome. Mild to severe hepatocellular dysfunction is rarely observed in these patients, and liver biopsy shows nonspecific reactive hepatitis<sup>[22]</sup>. The symptoms and liver dysfunction are commonly improved after antimicrobial therapy.

**Clostridium perfringens infection:** *Clostridium perfringens* is an important cause of watery diarrhea and also a toxin-mediated disease including hemolysis, jaundice, hypotension, and renal failure. *C. perfringens* is well known to cause clostridium myonecrosis (gas gangrene), which is a life-threatening muscle infection spreading directly from the area of trauma or hematogenously from gastrointestinal tract infection<sup>[23]</sup>. Jaundice may develop in up to 20% of patients with gas gangrene. On rare occasions, it can cause necrotizing massive gas gangrene in the liver leading to fulminant hepatic failure<sup>[24]</sup>.

### Pelvic inflammatory disease

Pelvic inflammatory disease (PID) refers to infection of the uterus, fallopian tubes, and adjacent pelvic structures, and the most important causative organisms are *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Occasionally, patients with these infections develop perihepatitis (Fitz-Hugh-Curtis syndrome), an inflammation of the liver capsule and adjacent peritoneal surfaces<sup>[25,26]</sup>. The clinical presentations include right-upper quadrant (RUQ) pain or pleuritic pain, and it may be confused with acute cholecystitis or pleurisy. The levels of aminotransferases are usually normal because of the minimal stromal hepatic involvement.

It has been reported the use of intrauterine devices (IUDs) causes a slight increase in the risk for PID<sup>[27-29]</sup>. Gelfand *et al*<sup>[30]</sup> reported a case of *Streptococcus milleri* bacteremia and multiple hepatic abscesses secondary to a tuboovarian abscess associated with IUD. Long-term indwelling IUDs was also reported to cause pelvic actinomycosis, which is a slowly progressive infection of *Actinomyces* species<sup>[31]</sup>. Moreover, a case of hepatic actinomycotic abscess associated with IUD was reported<sup>[32]</sup>.

### Toxic shock syndrome

Toxic shock syndrome is caused by the staphylococcal toxic shock syndrome toxin (TSST-1) and is commonly associated with *S. aureus* infections<sup>[33]</sup>. The clinical findings include fever, a scarlatiniform rash, mucosal hyperemia, vomiting, and diarrhea. It may cause liver dysfunction with severe jaundice and high levels of aminotransferases by hypoperfusion and circulating toxins.

Moreover, it should be noted that *Aeromonas* bacteremia, mostly *Aeromonas hydrophila*, causes significantly severe soft tissue infection such as necrotizing fasciitis with high mortality in patients with liver cirrhosis in contrast to self-limiting recovery in healthy subjects<sup>[34]</sup>.

### Hepatic encephalopathy and systemic infection

Systemic infection such as sepsis has been associated with the development of hepatic encephalopathy (HE). In patients with acute liver failure, rapidly progressing and severe HE is found more frequently in those with infection and inflammation<sup>[35,36]</sup>. It has also been reported that infection and inflammation exacerbate HE in patients with cirrhosis<sup>[37]</sup>. Systemic inflammation might have synergistic effects with HE. In a bile duct-ligated rat model, lipopolysaccharide (LPS)-treated rats showed severe HE with cytotoxic brain edema and increased nitrotyrosine in the frontal cortex despite preservation of the blood-brain barrier, whereas those without LPS developed precoma status only<sup>[38]</sup>. In systemic inflammation, increased cerebral blood flow, activated neutrophils or produced cytokines such as TNF- $\alpha$ , IL-1 $\beta$  or IL-6 contribute to the pathogenesis of HE<sup>[39,40]</sup>.

## INFECTION BY SPECIFIC PATHOGENS

### Mycobacterium infection

Although the lungs are the major site for *Mycobacterium*

**Table 3** Frequency of symptoms and signs in salmonella and acute viral hepatitis A

	Nausea/ vomiting	Abdominal discomfort	Jaundice <sup>b</sup>	Diarrhea	Relative bradycardia <sup>d</sup>	Fever > 104° F <sup>b</sup>	Hepatomegaly	Splenomegaly
Salmonella hepatitis	70%	33%	33%	48%	37%	41%	44%	7%
Acute viral hepatitis A	89%	63%	89%	30%	4%	0%	66%	11%

Modified from El-Newihi *et al*<sup>[19]</sup>. Salmonella hepatitis *vs* acute viral hepatitis A: <sup>b</sup>*P* < 0.0001; <sup>d</sup>*P* < 0.002.

*tuberculosis* infection, liver involvement has been also reported in patients with mycobacterial infection<sup>[41]</sup>. Miliary tuberculosis is defined as hematogenous dissemination of *Mycobacterium tuberculosis*, and the liver is frequently involved. Hepatic tuberculosis can be classified into various types such as miliary, granulomatous, and localized hepatic tuberculosis. The clinical presentations include fever, abdominal pain, and hepatomegaly. Liver function abnormalities have been observed in patients with hepatic tuberculosis, including elevated ALP and aminotransferases in 83% and 42% of these patients, respectively<sup>[42]</sup>. Cholestatic jaundice has also been reported in miliary tuberculosis, and fulminant hepatic failure can occur, if only rarely<sup>[43]</sup>. Importantly, hepatic tuberculosis can occur in the absence of apparent pulmonary tuberculosis, and tuberculous liver abscess without lung involvement has also been reported<sup>[44]</sup>. Histologically, the presence of caseating granulomas is suggestive of hepatic tuberculosis, but the yields of acid-fast bacillus smears and cultures are low. Detection using tissue PCR for *Mycobacterium tuberculosis* has a higher sensitivity and specificity.

Atypical mycobacteremia caused by *M. avium intracellulare* or *M. genavense*, can also cause granulomatous hepatitis with an elevation of ALP and low-grade fever in immunocompromised hosts such as those with AIDS syndrome<sup>[45]</sup>.

### Syphilis

Hepatic involvement in patients with syphilis is not uncommon. Schlossberg *et al*<sup>[46]</sup> reported that 39% of early syphilis patients had liver enzyme abnormalities at the time of diagnosis and that 2.7% of syphilis patients were diagnosed with syphilitic hepatitis. Other reports also show that liver enzyme abnormalities have been observed in about 10% to 50% of patients with secondary syphilis<sup>[46,47]</sup>. Syphilitic hepatitis is characterized by a high serum ALP level and normal to mild elevation of aminotransferases. Clinical hepatitis is rare, but acute cholestatic syphilitic hepatitis has been reported<sup>[48]</sup>. Hepatic gummas consisting of a caseous mass with a fibrous capsule may present in patients with tertiary syphilis. After starting therapy using penicillin, jaundice may occur with chills, fever, and a rash (erythema of Milan), as part of the Jarisch-Herxheimer reaction.

It is well known that syphilis continues to occur at high rates among human immunodeficiency virus (HIV)-infected patients. Crum-Cianflone *et al*<sup>[49]</sup> reported that syphilitic hepatitis is common, occurring in 38% of HIV-positive patients with early stages of syphilis infection, and

that syphilis should be included in the differential diagnosis of HIV patients with liver dysfunction.

### Leptospirosis

Leptospirosis caused by *Leptospira interrogans* is one of the most common zoonoses, and it may occur as one of two different clinical courses: anicteric leptospirosis (> 90% of cases) or icterohemorrhagic (Weil's) disease (5%-10% of cases)<sup>[50]</sup>. The former is characterized by self-limited viral infection-like symptoms with fever and conjunctival suffusion. The latter presents severe jaundice (approaching 30 mg/dL of total bilirubin) and several complications such as renal failure. Mild elevation of serum aminotransferases and thrombocytopenia can be seen<sup>[51]</sup>. Importantly, it is difficult to distinguish leptospirosis from other febrile infectious diseases such as *Salmonella typhi* or influenza because of similar clinical manifestations in the early phase.

In spite of severe functional impairment of the liver and kidneys, histopathological changes are usually slight, consisting of minimal focal hepatocyte necrosis. In severely jaundiced cases, leukocyte infiltration and bile thrombi can be observed.

### Lyme disease

Lyme disease is a spirochetal infection by *Borrelia burgdorferi*, and it can involve multiple organs including skin, muscle, liver, heart, and nervous system. Hepatic involvement can be seen in 20% of patients with Lyme disease; elevations of aminotransferases and  $\gamma$ -GTP are commonly observed<sup>[52,53]</sup>.

### Q fever

Q fever is one of the zoonotic infections caused by *Coxiella burnetii*; it is characterized by relapsing fevers, headache, and myalgias, and can involve several organs including the lungs, heart and liver. Nearly 50% of patients with Q fever may have liver function abnormalities, and the clinical features may mimic anicteric viral hepatitis<sup>[54]</sup>.

### Rocky Mountain spotted fever

Rocky Mountain spotted fever (RMSF) is the most common manifestation of *Rickettsia rickettsii* infection in the United States. The clinical spectrum of human infection ranges from mild to fulminant, and hepatic involvement is frequent, predominantly in the form of jaundice<sup>[55,56]</sup>. RMSF is commonly mistaken for other viral or bacterial infection, because the symptoms are commonly non-specific during the first few days of illness.



**Table 4** Comparison of clinical features of hepatitis caused by various viruses

	<i>n</i>	Median age (range)	ALT (U/L)	ALP (U/L)	Bilirubin (μmol/L)	Lymphocyte count (× 10 <sup>9</sup> /L)	Lymphocytosis <i>n</i> (%)
EBV	17	40 (18-68)	395 (87-1362)	345 (160-756)	74 (13-165)	6.91 (3.77-24.82)	17 (100)
HAV	11	44 (20-61)	1056 (595-4122)	231 (91-342)	154 (42-214)	2.16 (1.23-4.1)	1 (9)
HBV	16	39 (20-60)	1858 (499-3856)	230 (93-406)	122 (36-355)	2.00 (1.26-3.52)	2 (12.5)
HEV	20	63 (54-81)	1387 (318-6357)	192 (139-464)	61 (8-297)	1.89 (0.96-10.25)	5 (25)

Modified from Vine *et al*<sup>[60]</sup>. EBV: Epstein-Barr virus; HAV: Hepatitis A virus; HBV: Hepatitis B virus; HEV: Hepatitis E virus.

### Hepatic actinomycosis

Actinomycosis is a chronic granulomatous disease caused by filamentous, gram-positive, anaerobic bacteria, mainly *Actinomyces Israelii*. Hepatic involvement may occur through intestinal actinomycosis in the appendix and ileocecal region. The clinical presentation includes fever, anemia, body weight loss, and hepatosplenomegaly, which is not characteristic as actinomycosis; therefore, it is difficult to diagnose as actinomycosis preoperatively<sup>[57]</sup>. Percutaneous liver biopsy is useful, as sulfur granules can typically be observed.

## NON-HEPATITIS VIRAL INFECTION

Although the hepatotropic viruses, hepatitis A though E, are the most common viral cause of acute liver injury (acute hepatitis), other viruses such as Epstein-Barr virus (EBV) or cytomegalovirus (CMV) can also cause acute liver injury. Serological tests and direct-detection by PCR/ISH/IHC are useful to diagnose these viruses, but it is not easy to distinguish between hepatitis viral infections and these non-hepatotropic viral infections at the time of the first medical examination.

### EBV

EBV is a member of the herpes virus family. Infection commonly occurs in childhood and is asymptomatic. On the other hand, symptomatic disease develops mostly in young adults with high fever, sore throat and lymphadenopathy, known as infectious mononucleosis. Liver injury with a mild elevation of aminotransferases often occurs in patients with infectious mononucleosis, but acute hepatitis and jaundice has been observed in some patients with EBV infection without clinical features of infectious mononucleosis<sup>[58]</sup>. Manifestations of liver involvement range from mild hepatitis to hepatosplenomegaly with jaundice, and on rare occasion, acute fulminant hepatitis<sup>[59]</sup>. Vine *et al*<sup>[60]</sup> reported the clinical features of EBV hepatitis compared with those of acute viral hepatitis caused by hepatitis A, B, and E viruses (Table 4). Patients with EBV hepatitis rarely present with more than 1000 IU/L of serum ALT, and usually have lymphocytosis ( $> 5 \times 10^9$ /L). Splenomegaly has been shown to be present in 88% of these patients. In this context, EBV hepatitis may be suggested by the presence of lymphocytosis and splenomegaly. Interestingly, the median age of patients with EBV hepatitis is older than that of patients with typical infectious mononucleosis, with nearly half the patients more than 60 years old<sup>[60]</sup>.

It is well known that EBV primarily replicates nasopharyngeal epithelial cells and B lymphocytes, and expression of EBV-encoded small RNA is also observed in liver specimens from transplant recipients. Histologically, various findings can be seen, including sinusoidal infiltration of mononuclear cells and mildly ballooning hepatocytes with vacuolization.

Chronic active EBV infection (CAEBV) is well known as a rare disorder occurring in immunocompetent as well as immunocompromised hosts, and may cause EBV-associated lymphoproliferative diseases (LPDs). It has been reported that a third of the patients with EBV-driven LPDs have liver involvement<sup>[61]</sup>. The incidence of post-transplant lymphoproliferative diseases has been reported to range from 0.5% to 30% depending on the EBV status and the transplanted organs<sup>[62,63]</sup>.

There are also several reports on chronic hepatitis by EBV infection in immunocompetent adults, which might be caused by the reactivation of EBV and increased viral-specific CTL responses<sup>[64,65]</sup>. The criteria for establishing this diagnosis have been proposed by Drebber *et al*<sup>[66]</sup>: namely, the presence of suggestive histopathological features, a specific serological profile, and detection of EBV genome in the liver tissue. When chronic hepatitis with unknown etiology is diagnosed, EBV infection could be the cause.

### Human cytomegalovirus

Human cytomegalovirus (CMV) infection is commonly subclinical in immunocompetent adults, but it sometimes can cause a disease such as infectious mononucleosis or hepatic injury<sup>[67]</sup>. Liver dysfunction associated with CMV infection usually presents with mild to moderate elevation of serum aminotransferases and ALP, but jaundice is not common. CMV hepatitis rarely includes granulomatous hepatitis, cholestatic hepatitis, or acute hepatic failure with massive necrosis.

Watanabe *et al*<sup>[68]</sup> retrospectively analyzed the clinical features and laboratory data of patients with CMV hepatitis compared with EBV hepatitis. Although common signs and symptoms were similar, epigastralgia was more common in CMV hepatitis than EBV hepatitis ( $P < 0.05$ ), and cervical lymphadenopathy was more frequently observed in EBV hepatitis than CMV hepatitis ( $P < 0.01$ ). Also, the ratio of peripheral blood monocytes in the white blood cells was greater in CMV hepatitis ( $P < 0.01$ ). On the other hand, CMV is one of the most important opportunistic pathogens and can cause severe pulmonary, retinal, gastrointestinal, and hepatic disease in im-



munocompromised hosts. About 10% of recipients of liver transplantation suffer from hepatitis associated with CMV, and the hepatitis may be caused by reactivation rather than primary infection<sup>[69]</sup>.

The typical histological finding of CMV hepatitis is thought to be vital inclusion bodies in hepatocytes in recipients after liver transplantation, but this is not absolute. Microabscesses, lymphocytic infiltration, and parenchymal alterations are also common.

### Other human herpes viruses

Other human herpes viruses besides EBV or CMV, including herpes simplex virus-1 and -2 (HSV-1, HSV-2), Varicella-Zoster virus (VZV), and human herpesvirus-6, -7 and -8 (HHV-6, -7, and -8), can occasionally cause liver injury<sup>[70]</sup>. Although HSV hepatitis is uncommon in immunocompetent patients, mild elevations of aminotransferases can be observed in 14% of patients with acute HSV infection. Severe hepatitis associated with HSV was reported in neonatal infection, pregnancy, and immunocompromised hosts. Rakela *et al.*<sup>[71]</sup> reported that 6% of fulminant hepatitis cases at the Mayo Clinic were associated with HSV infection.

In primary infection by VZV, a typical manifestation is generalized exanthematous rash, which is known as varicella, and mild elevations of aminotransferases can be observed in up to 25% of children with varicella. After organ transplantations, the primary infection and reactivation of varicella can occasionally cause severe hepatitis including fatal fulminant hepatitis<sup>[72]</sup>.

HHV-6 infection, known as Sixth Disease, typically occurs in infants under the age of 2. Härmä *et al.*<sup>[73]</sup> reported HHV-6 was found in 80% of patients with acute liver failure (ALF) of unknown cause, which suggests that HHV-6 might be one of the causes of ALF. Reactivation of HHV-6 is also known to relate to the pathogenesis of drug-induced hypersensitivity syndrome (DIHS), which is characterized by fever, rash, lymphadenopathy, hepatitis, and leukocytosis with eosinophilia after administration of specific drugs. Shiohara *et al.*<sup>[74]</sup> reported that an altered immune response, including changes of functional regulatory T cells, may influence the pathogenesis of DIHS. There are a few reports of hepatitis associated with HHV-7 infection<sup>[75]</sup>. Liver involvement of HHV-8 may occur in patients with HIV infection.

### Other viruses

Parvovirus B19 infection commonly causes the erythema infectiosum in children, and causes liver dysfunction and hematologic disorder in adults. There are several case reports of parvovirus B19-infected patients with fulminant hepatitis and aplastic anemia<sup>[76]</sup>.

It is well known that rubella infection during pregnancy may cause hepatocellular injury in the newborn as a part of the congenital rubella syndrome. Acquired rubella infection also may cause acute hepatitis with mild elevation of aminotransferases in adults<sup>[77,78]</sup>. In these cases, the most characteristic finding of the laboratory data

is a marked increase of LDH level, whereas cholestasis is rarely observed. It has been reported that the increase of LDH consisted of both LDH isozyme-3 derived from lymphocytes/platelets and LDH isozyme-5 derived from the liver<sup>[79,80]</sup>. The reported hepatic histological findings were compatible with acute hepatitis, including the ballooning of hepatocytes, spotty necrosis, and infiltration of inflammatory cells<sup>[79,80]</sup>.

Hepatic involvement in measles has also been reported<sup>[81]</sup>, with the prevalence of hepatitis in measles patients ranging from 71% to 89%<sup>[82-85]</sup>. Therefore, hepatitis should be considered as a common finding in patients with measles. Although the liver enzymes are elevated to more than 5 times ULN in 22% of patients with measles, clinical jaundice is rare<sup>[86]</sup>. It should be noted that rubella or measles could be a cause of liver dysfunction in patients with skin rash or fever, which may be misdiagnosed as drug-induced liver injury under medication.

Moreover, adenovirus commonly causes acute infections of the respiratory system and gastrointestinal tracts, and a few cases of ALF associated with adenovirus have been reported<sup>[87]</sup>.

### The mechanism of liver injury associated with non-hepatitis viral infection

Although the mechanism of liver injury caused by non-hepatitis viral infection remains unclear, several factors may be concerned. Hepatocellular injury may be caused by both host immune responses with activated CD8<sup>+</sup> T cells and direct viral cytopathy. On the other hand, pro-inflammatory cytokines induced by virus infection may influence the function of sinusoidal and canalicular transporting systems, which may lead to cholestasis<sup>[88,89]</sup>.

EBV-infected T or NK cells could cause chronic active EBV infection (CAEBV) in some cases; therefore, the possibility exists that EBV-infected T cells affect the pathogenesis of hepatitis.

An animal model study showed that activated CD8<sup>+</sup> T cells are recruited to and trapped in the liver through interaction with intracellular adhesion molecule 1, which is expressed on sinusoidal endothelial cells and Kupffer cells<sup>[90]</sup>. A number of experiments have shown that soluble factors of the immune responses, especially interferon- $\gamma$ , the Fas ligand and TNF- $\alpha$ , induce hepatitis<sup>[91-93]</sup>.

## FUNGAL INFECTION

Deep fungal infections can usually occur in immunocompromised hosts, including patients with HIV infection, neutropenia after chemotherapy, and organ-transplanted recipients. The liver is often involved in deep fungal infections, together with other organs.

### Hepatosplenic candidiasis

Hepatosplenic candidiasis may be caused by *Candida* species, including *C. albicans* and *C. tropicalis*, through candidemia or the portal vasculature from the gut with degenerated barriers of gastrointestinal mucosa<sup>[94]</sup>. Dis-

**Table 5** Parasitic infection of the liver

Disease (organism)	Organs/status	Clinical presentation
Malaria ( <i>P. falciparum</i> , <i>malariae</i> , <i>vivax</i> , <i>ovale</i> )	Pre-erythrocytic phase Erythrocytic phase	Asymptomatic Anemia, jaundice, mild elevation of aminotransferases, tender hepatomegaly, splenomegaly
Amebiasis ( <i>Entamoeba histolytica</i> )	Intestine Amebic liver abscess	Right upper quadrant pain, fever, hepatomegaly (50%), jaundice (< 10%)
Cystic echinococcosis ( <i>Echinococcus granulosus</i> )	Single cyst (> 70%), < 10 cm and no complication Size up (1-5 cm/year), > 10 cm Rupture	Asymptomatic Abdominal pain, mass effect (possible) Peritonitis, hypersensitivity reactions
Alveolar echinococcosis ( <i>E. multilocularis</i> )		Malaise, tender hepatomegaly, eosinophilia, obstructive jaundice, portal hypertension
Schistosomiasis ( <i>S. mansoni</i> , <i>japonicum</i> )	Acute phase Chronic phase	Eosinophilic infiltrate Presinusoidal portal hypertension, splenomegaly, gastroesophageal varices
Fascioliasis ( <i>F. hepatica</i> )	Acute phase Chronic phase	Abdominal pain, fever, hemobilia, hepatomegaly Biliary colic, cholangitis, cholelithiasis, obstructive jaundice
Ascariasis ( <i>A. lumbricoides</i> )		Abdominal pain, fever, obstructive jaundice

seminated candidemia is usually seen among patients with hepatologic malignancies with prolonged severe neutropenia. The incidence of hepatosplenic candidiasis has been reported to be 3% to 7% in these high-risk patients, but it may have decreased recently due to the use of anti-fungal prophylaxis.

The clinical presentation of hepatosplenic candidiasis consists of persistent fever with spikes and right upper quadrant discomfort, nausea, and anorexia. Laboratory testing commonly shows elevated serum concentrations of ALP and  $\gamma$ -GTP, associated with small liver abscesses or granulomas<sup>[95]</sup>. Multiple hypoechoic lesions and non-enhanced low-attenuation lesions can be detected by ultrasound and CT scan, respectively<sup>[96]</sup>.

### Other fungal infections

Liver involvement in other fungal infections, including disseminated aspergillosis, cryptococcosis, mucormycosis, trichosporonosis, and histoplasma capsulatum occurs on rare occasion in immunocompromised hosts<sup>[97]</sup>. The incidence of liver involvement with other fungal infections is very low, because these are acquired exogenously. Park<sup>[97]</sup> reported that up to 90% of these patients could have liver involvement in spite of the very low incidence of disseminated histoplasmosis. Hepatic histological findings could be variable, including granulomatous changes and sinusoidal Kupffer cell hyperplasia.

## LIVER INVOLVEMENT OF PARASITIC DISEASES

Parasitic liver involvement is common in highly endemic areas, and it should be considered in an individual who has visited such areas. Parasitic involvement is dominated by *Plasmodium spp.*, but *Entamoeba histolytica*, *Schistosoma spp.* and *Echinococcus spp.* infections are also important in clinical practice<sup>[98]</sup>. Hepatobiliary involvement is also caused by *Ascaris lumbricoides*, *Fasciola hepatica* and *Liver flukes* (Table 5).

### Malaria

Malaria is one of the most important public health problems worldwide, as an estimated 300 to 500 million persons suffer from malaria annually. The malarial life cycle consists of the pre-erythrocytic and the erythrocytic phases. Usually malarial schizogony takes place in the liver without obvious liver injury in the pre-erythrocytic phase. In the erythrocytic phase, symptoms such as cyclical fever, malaise, nausea, vomiting, diarrhea, tender hepatomegaly and splenomegaly develop<sup>[99]</sup>. Jaundice associated with hemolysis can be observed in severe malarial infection, and hepatic failure can occasionally be seen in patients with severe *P. falciparum* infection<sup>[100]</sup>. Jaundice in malaria consists of both unconjugated and conjugated bilirubin, which could be caused by intravascular hemolysis of parasitized red blood cells, and hepatocellular dysfunction. Hepatic histological findings may show Kupffer cell hyperplasia with pigment deposition, hepatocyte necrosis, and cholestasis.

### Schistosomiasis

Schistosomiasis is caused by trematodes of the genus *Schistosoma*, including *S. mansoni* and *S. japonicum*. Mesenteric infection may cause deposition of the eggs in the liver, which may lead to presinusoidal occlusion and periportal fibrosis associated with granulomatous response<sup>[101]</sup>. Chronic hepatic schistosomiasis presents with portal hypertension with splenomegaly and gastroesophageal varices. Laboratory test abnormalities include eosinophilia and increased serum IgE levels. Because hepatic schistosomiasis is one of the most common causes of noncirrhotic portal hypertension, it should be considered in differential diagnosis for that symptom.

### Amebiasis

Amebiasis is caused by the *Entamoeba histolytica*, and about 40 to 50 million persons are infected annually. Amebiasis includes amebic dysentery and extraintestinal disease such as amebic liver abscess. Patients with amebic liver abscess

usually present with RUQ pain and fever. Although hepatomegaly can be seen in about 50% of cases, jaundice can be seen in less than 10%<sup>[102]</sup>. In the liver, *E. histolytica* lyses host's tissue and infiltrating neutrophils with its proteolytic enzymes<sup>[103]</sup>. Amebic liver abscess grows inexorably, and the retardation of making diagnosis leads to perforation in about 2% to 7% of these patients<sup>[104-106]</sup> with the mortality rate being 23% to 42% in the perforated patients<sup>[105,107]</sup>. Therefore, prompt diagnosis and treatment are very important for successful treatment in patients with hepatic amebiasis<sup>[108]</sup>.

### Hydatid disease

Hydatid disease is caused by infection with the metacystode stage of the tapeworm *Echinococcus*. Liver involvement may occur in about two-third of patients with *Echinococcus granulosus* infection, and commonly can form single cyst. Although a patient has no symptom when the cyst is small (< 10 cm in diameter) and without complication, intra-peritoneal rupture may be frequent and cause abdominal pain. Rupture into the biliary tract may cause biliary colic, obstructive jaundice, or cholangitis.

*Echinococcus multilocularis* can cause alveolar echinococcosis, which commonly presents obvious complaints such as tender hepatomegaly, malaise, and weight loss. Because *E. multilocularis* can invade the biliary tract, hepatic vein, inferior vena cava, and/or diaphragm, a high mortality rate has been reported in untreated patients<sup>[109]</sup>.

### Liver flukes (Fascioliasis)

Fascioliasis is a trematode infection caused by *Fasciola hepatica* or *Fasciola gigantica*. Fascioliasis commonly consists of two phases, the acute/invasive and chronic obstructive phase. In the acute phase, symptoms usually include fever, RUQ pain, hepatomegaly and eosinophilia. The chronic phase usually begins about six months after infection and is characterized by bile duct obstruction associated with bile duct inflammation and hyperplasia due to the presence of adult flukes. Clinical presentations include recurrent biliary colic, cholangitis, cholelithiasis, and obstructive jaundice<sup>[110]</sup>.

### Ascariasis

*Ascaris lumbricoides* is an intestinal nematode, and arrives in the liver through the bile duct by a retrograde manner. Migration of adult worms into the biliary tree can cause biliary colic, cholecystitis, cholangitis, obstructive jaundice, and secondary liver abscess<sup>[111]</sup>.

## CONCLUSION

The liver is exposed to many systemic infectious pathogens including not only hepatotropic but also non-hepatotropic microorganisms through both the systemic and portal circulation. These pathogens may directly or indirectly cause liver injury presenting with various manifestations described in this review, but the mechanisms

of these liver injuries have not been completely clarified. When making correct diagnosis of liver dysfunction in systemic infections, knowledge about non-hepatotropic pathogens and appropriate microbiological examinations are very important.

## REFERENCES

- 1 Szabo G, Romics L, Frenzl G. Liver in sepsis and systemic inflammatory response syndrome. *Clin Liver Dis* 2002; **6**: 1045-1066, x [PMID: 12516206]
- 2 Vermillion SE, Gregg JA, Baggenstoss AH, Bartholomew LG. Jaundice associated with bacteremia. *Arch Intern Med* 1969; **124**: 611-618 [PMID: 4981285 DOI: 10.1001/archinte.196.9.00300210093014]
- 3 Hawker F. Liver dysfunction in critical illness. *Anaesth Intensive Care* 1991; **19**: 165-181 [PMID: 2069235]
- 4 Kanai S, Honda T, Uehara T, Matsumoto T. Liver function tests in patients with bacteremia. *J Clin Lab Anal* 2008; **22**: 66-69 [PMID: 18200569 DOI: 10.1002/jcla.20205]
- 5 Uslan DZ, Crane SJ, Steckelberg JM, Cockerill FR, St Sauver JL, Wilson WR, Baddour LM. Age- and sex-associated trends in bloodstream infection: a population-based study in Olmsted County, Minnesota. *Arch Intern Med* 2007; **167**: 834-839 [PMID: 17452548]
- 6 Thiele DL. Hepatic manifestations of systemic disease and other disorders of the liver. In: Feldman M, Friedman LS, Sleisenger MH, et al, editors. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease*. 7<sup>th</sup> ed. Philadelphia: Elsevier Science, 2002: 1603-1619
- 7 Scharfe M, Fink MP. Red blood cell physiology in critical illness. *Crit Care Med* 2003; **31**: S651-S657 [PMID: 14724462 DOI: 10.1097/01.CCM.0000098036]
- 8 Berkowitz FE. Hemolysis and infection: categories and mechanisms of their interrelationship. *Rev Infect Dis* 1991; **13**: 1151-1162 [PMID: 1775848 DOI: 10.1093/clinids/13.6.1151]
- 9 Bätge B, Filejski W, Kurowski V, Klüter H, Djonlagic H. Clostridial sepsis with massive intravascular hemolysis: rapid diagnosis and successful treatment. *Intensive Care Med* 1992; **18**: 488-490 [PMID: 1289375 DOI: 10.1007/BF01708587]
- 10 Smith LD. Virulence factors of *Clostridium perfringens*. *Rev Infect Dis* 1979; **1**: 254-262 [PMID: 232935 DOI: 10.1093/clinids/1.2.254]
- 11 Ding Y, Zhao L, Mei H, Huang ZH, Zhang SL. Alterations of biliary biochemical constituents and cytokines in infantile hepatitis syndrome. *World J Gastroenterol* 2006; **12**: 7038-7041 [PMID: 17109502]
- 12 Roelofs H, van der Veere CN, Ottenhoff R, Schoemaker B, Jansen PL, Oude Elferink RP. Decreased bilirubin transport in the perfused liver of endotoxemic rats. *Gastroenterology* 1994; **107**: 1075-1084 [PMID: 7926459]
- 13 Bolder U, Ton-Nu HT, Scheingart CD, Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. *Gastroenterology* 1997; **112**: 214-225 [PMID: 8978362 DOI: 10.1016/S0016-5085(97)70238-5]
- 14 Radford AJ, Rhodes FA. The association of jaundice with lobar pneumonia in the territory of Papua and New Guinea. *Med J Aust* 1967; **2**: 678-681 [PMID: 6057201]
- 15 Zimmerman HJ. Jaundice due to bacterial infection. *Gastroenterology* 1978; **77**: 362-374
- 16 Lee SW, Yang SS, Chang CS, Yeh HJ, Chow WK. Mycoplasma pneumonia-associated acute hepatitis in an adult patient without lung infection. *J Chin Med Assoc* 2009; **72**: 204-206 [PMID: 19372077]
- 17 Kirby BD, Snyder KM, Meyer RD, Finegold SM. Legionnaires' disease: clinical features of 24 cases. *Ann Intern Med* 1978; **89**: 297-309 [PMID: 686539 DOI: 10.7326/0003-4819-89-



- 3-297]
- 18 **Gitlin N.** Liver involvement in systemic infection. In: Gitlin N, et al, editors. *The Liver and Systemic Disease*. Hong Kong: Pearson Professional Limited, 1997: 229-236
  - 19 **El-Newihi HM,** Alamy ME, Reynolds TB. Salmonella hepatitis: analysis of 27 cases and comparison with acute viral hepatitis. *Hepatology* 1996; **24**: 516-519 [PMID: 8781316 DOI: 10.1002/hep.510240308]
  - 20 **Khosla SN,** Singh R, Singh GP, Trehan VK. The spectrum of hepatic injury in enteric fever. *Am J Gastroenterol* 1988; **83**: 413-416 [PMID: 3126648]
  - 21 **Ramachandran S,** Godfrey JJ, Perera MV. Typhoid hepatitis. *JAMA* 1974; **230**: 236-240 [PMID: 4213522 DOI: 10.1001/jama.1974.03240020026016]
  - 22 **Reddy KR,** Farnum JB, Thomas E. Acute hepatitis associated with campylobacter colitis. *J Clin Gastroenterol* 1983; **5**: 259-262 [PMID: 6863882 DOI: 10.1097/00004836-198306000-00013]
  - 23 **Awad MM,** Bryant AE, Stevens DL, Rood JI. Virulence studies on chromosomal alpha-toxin and theta-toxin mutants constructed by allelic exchange provide genetic evidence for the essential role of alpha-toxin in Clostridium perfringens-mediated gas gangrene. *Mol Microbiol* 1995; **15**: 191-202 [PMID: 7746141 DOI: 10.1111/j.1365-2958.1995.tb02234.x]
  - 24 **Bergert H,** Illert T, Friedrich K, Ockert D. Fulminant liver failure following infection by Clostridium perfringens. *Surg Infect (Larchmt)* 2004; **5**: 205-209 [PMID: 15353119]
  - 25 **Tajiri T,** Tate G, Iwaku T, Takeyama N, Fusama S, Sato S, Kunimura T, Mitsuya T, Morohoshi T. Right pleural effusion in Fitz-Hugh-Curtis syndrome. *Acta Med Okayama* 2006; **60**: 289-294 [PMID: 17072375]
  - 26 **Cano A,** Fernandez C, Scapa M, Boixeda D, Plaza G. Gonococcal perihepatitis: diagnostic and therapeutic value of laparoscopy. *Am J Gastroenterol* 1984; **79**: 280-282 [PMID: 6231855]
  - 27 **Workowski KA,** Berman SM. Sexually transmitted diseases treatment guidelines, 2006. *MMWR Recomm Rep* 2006; **55**: 1-94 [PMID: 16888612]
  - 28 **Lee NC,** Rubin GL, Borucki R. The intrauterine device and pelvic inflammatory disease revisited: new results from the Women's Health Study. *Obstet Gynecol* 1988; **72**: 1-6 [PMID: 3380496]
  - 29 **Grimes DA.** Intrauterine device and upper-genital-tract infection. *Lancet* 2000; **356**: 1013-1019 [PMID: 11041414]
  - 30 **Gelfand MS,** Hodgkiss T, Simmons BP. Multiple hepatic abscesses caused by Streptococcus milleri in association with an intrauterine device. *Rev Infect Dis* 1989; **11**: 983-987 [PMID: 2602778]
  - 31 **Lee YC,** Min D, Holcomb K, Buhl A, DiMaio T, Abulafia O. Computed tomography guided core needle biopsy diagnosis of pelvic actinomycosis. *Gynecol Oncol* 2000; **79**: 318-323 [PMID: 11063665]
  - 32 **Hochsztein JG,** Koenigsberg M, Green DA. US case of the day. Actinomycotic pelvic abscess secondary to an IUD with involvement of the bladder, sigmoid colon, left ureter, liver, and upper abdominal wall. *Radiographics* 1996; **16**: 713-716 [PMID: 8897636]
  - 33 **DeVries AS,** Leshner L, Schlievert PM, Rogers T, Villaume LG, Danila R, Lynfield R. Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features, and molecular characteristics. *PLoS One* 2011; **6**: e22997 [PMID: 21860665 DOI: 10.1371/journal.pone.0022997]
  - 34 **Lau SM,** Peng MY, Chang FY. Outcomes of Aeromonas bacteremia in patients with different types of underlying disease. *J Microbiol Immunol Infect* 2000; **33**: 241-247 [PMID: 11269369]
  - 35 **Rolando N,** Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. *Hepatology* 2000; **32**: 734-739 [PMID: 11003617 DOI: 10.1053/jhep.2000.17687]
  - 36 **Vaquero J,** Polson J, Chung C, Helenowski I, Schiodt FV, Reisch J, Lee WM, Blei AT. Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology* 2003; **125**: 755-764 [PMID: 12949721 DOI: 10.1016/S0016-5085(03)01051-5]
  - 37 **Shawcross DL,** Sharifi Y, Canavan JB, Yeoman AD, Abeles RD, Taylor NJ, Auzinger G, Bernal W, Wendon JA. Infection and systemic inflammation, not ammonia, are associated with Grade 3/4 hepatic encephalopathy, but not mortality in cirrhosis. *J Hepatol* 2011; **54**: 640-649 [PMID: 21163546 DOI: 10.1016/j.jhep.2010.07.045]
  - 38 **Wright G,** Davies NA, Shawcross DL, Hodges SJ, Zwiggmann C, Brooks HF, Mani AR, Harry D, Stadlbauer V, Zou Z, Williams R, Davies C, Moore KP, Jalan R. Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* 2007; **45**: 1517-1526 [PMID: 17523148 DOI: 10.1002/hep.21599]
  - 39 **Shawcross DL,** Shabbir SS, Taylor NJ, Hughes RD. Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis. *Hepatology* 2010; **51**: 1062-1069 [PMID: 19890967 DOI: 10.1002/hep.23367]
  - 40 **Tranah TH,** Vijay GK, Ryan JM, Shawcross DL. Systemic inflammation and ammonia in hepatic encephalopathy. *Metab Brain Dis* 2013; **28**: 1-5 [PMID: 23224356 DOI: 10.1007/s10111-012-9370-2]
  - 41 **Chong VH,** Lim KS. Hepatobiliary tuberculosis. *Singapore Med J* 2010; **51**: 744-751 [PMID: 20938617]
  - 42 **Maartens G,** Willcox PA, Benatar SR. Miliary tuberculosis: rapid diagnosis, hematologic abnormalities, and outcome in 109 treated adults. *Am J Med* 1990; **89**: 291-296 [PMID: 2393033 DOI: 10.1016/0002-9343(90)90340-J]
  - 43 **Hussain W,** Mutimer D, Harrison R, Hubscher S, Neuberger J. Fulminant hepatic failure caused by tuberculosis. *Gut* 1995; **36**: 792-794 [PMID: 7797133 DOI: 10.1136/gut.36.5.792]
  - 44 **Hayashi M,** Yamawaki I, Okajima K, Tomimatsu M, Ohkawa S. Tuberculous liver abscess not associated with lung involvement. *Intern Med* 2004; **43**: 521-523 [PMID: 15283192 DOI: 10.2169/internalmedicine.43.521]
  - 45 **Gordon SC.** Bacterial and systemic infections. In: Schiff ER, Sorrell MF, Maddrey WC, editors. *Schiff's Diseases of the Liver*. 9<sup>th</sup> ed. Tokyo: Lippincott William & Wilkins, 2003: 1529-1545
  - 46 **Schlossberg D.** Syphilitic hepatitis: a case report and review of the literature. *Am J Gastroenterol* 1987; **82**: 552-553 [PMID: 3578236]
  - 47 **Young MF,** Sanowski RA, Manne RA. Syphilitic hepatitis. *J Clin Gastroenterol* 1992; **15**: 174-176 [PMID: 1401840 DOI: 10.1097/00004836-199209000-00029]
  - 48 **Comer GM,** Mukherjee S, Sachdev RK, Clain DJ. Cardiolipin-fluorescent (M1) antimitochondrial antibody and cholestatic hepatitis in secondary syphilis. *Dig Dis Sci* 1989; **34**: 1298-1302 [PMID: 2666056 DOI: 10.1007/BF01537283]
  - 49 **Crum-Cianflone N,** Weekes J, Bavaro M. Syphilitic hepatitis among HIV-infected patients. *Int J STD AIDS* 2009; **20**: 278-284 [PMID: 19304979]
  - 50 **Farr RW.** Leptospirosis. *Clin Infect Dis* 1995; **21**: 1-6; quiz 7-8 [PMID: 7578715 DOI: 10.1093/clinids/21.1.1]
  - 51 **Chierakul W,** Tientadakul P, Suputtamongkol Y, Wuthiekanun V, Phimda K, Limpaboon R, Opartkiattikul N, White NJ, Peacock SJ, Day NP. Activation of the coagulation cascade in patients with leptospirosis. *Clin Infect Dis* 2008; **46**: 254-260 [PMID: 18171258 DOI: 10.1086/524664]
  - 52 **Kazakoff MA,** Sinusas K, Macchia C. Liver function test abnormalities in early Lyme disease. *Arch Fam Med* 1993; **2**: 409-413 [PMID: 8130920 DOI: 10.1001/archfam.2.4.409]
  - 53 **Horowitz HW,** Dworkin B, Forseter G, Nadelman RB, Connolly C, Luciano BB, Nowakowski J, O'Brien TA, Calmann M, Wormser GP. Liver function in early Lyme disease. *Hepatology* 1996; **23**: 1412-1417 [PMID: 8675158 DOI: 10.1002/hep.510230617]

- 54 **Domingo P**, Muñoz C, Franquet T, Gurguí M, Sancho F, Vazquez G. Acute Q fever in adult patients: report on 63 sporadic cases in an urban area. *Clin Infect Dis* 1999; **29**: 874-879 [PMID: 10589906 DOI: 10.1086/520452]
- 55 **Adams JS**, Walker DH. The liver in Rocky Mountain spotted fever. *Am J Clin Pathol* 1981; **75**: 156-161 [PMID: 6781327]
- 56 **Dantas-Torres F**. Rocky Mountain spotted fever. *Lancet Infect Dis* 2007; **7**: 724-732 [PMID: 17961858 DOI: 10.1016/S1473-3099(07)70261-X]
- 57 **Cintron JR**, Del Pino A, Duarte B, Wood D. Abdominal actinomycosis. *Dis Colon Rectum* 1996; **39**: 105-108 [PMID: 8601346 DOI: 10.1007/BF02048278]
- 58 **Crum NF**. Epstein Barr virus hepatitis: case series and review. *South Med J* 2006; **99**: 544-547 [PMID: 16711324 DOI: 10.1097/01.smj.0000216469.04854.2a]
- 59 **Papatheodoridis GV**, Delladetsima JK, Kavallierou L, Kapranos N, Tassopoulos NC. Fulminant hepatitis due to Epstein-Barr virus infection. *J Hepatol* 1995; **23**: 348-350 [PMID: 8551000]
- 60 **Vine LJ**, Shepherd K, Hunter JG, Madden R, Thornton C, Ellis V, Bendall RP, Dalton HR. Characteristics of Epstein-Barr virus hepatitis among patients with jaundice or acute hepatitis. *Aliment Pharmacol Ther* 2012; **36**: 16-21 [PMID: 22554291 DOI: 10.1111/j.1365-2036.2012.05122.x]
- 61 **Jaffe ES**, Wilson WH. Lymphomatoid granulomatosis: pathogenesis, pathology and clinical implications. *Cancer Surv* 1997; **30**: 233-248 [PMID: 9547995]
- 62 **Randhawa PS**, Markin RS, Starzl TE, Demetris AJ. Epstein-Barr virus-associated syndromes in immunosuppressed liver transplant recipients. Clinical profile and recognition on routine allograft biopsy. *Am J Surg Pathol* 1990; **14**: 538-547 [PMID: 2159731 DOI: 10.1097/00000478-199006000-00004]
- 63 **Holmes RD**, Sokol RJ. Epstein-Barr virus and post-transplant lymphoproliferative disease. *Pediatr Transplant* 2002; **6**: 456-464 [PMID: 12453197 DOI: 10.1034/j.1399-3046.2002.02043.x]
- 64 **Petrova M**, Muhtarova M, Nikolova M, Magaev S, Taskov H, Nikolovska D, Krastev Z. Chronic Epstein-Barr virus-related hepatitis in immunocompetent patients. *World J Gastroenterol* 2006; **12**: 5711-5716 [PMID: 17007027]
- 65 **Petrova M**, Kamburov V. Epstein-Barr virus: silent companion or causative agent of chronic liver disease? *World J Gastroenterol* 2010; **16**: 4130-4134 [PMID: 20806428 DOI: 10.3748/wjg.v16.i33.4130]
- 66 **Drebbler U**, Kasper HU, Krupacz J, Haferkamp K, Kern MA, Steffen HM, Quasdorff M, Zur Hausen A, Odenthal M, Dienes HP. The role of Epstein-Barr virus in acute and chronic hepatitis. *J Hepatol* 2006; **44**: 879-885 [PMID: 16554102 DOI: 10.1016/j.jhep.2006.02.006]
- 67 **Bonkowsky HL**, Lee RV, Klatskin G. Acute granulomatous hepatitis. Occurrence in cytomegalovirus mononucleosis. *JAMA* 1975; **233**: 1284-1288 [PMID: 169402 DOI: 10.1001/jama.1975.03260120046019]
- 68 **Watanabe S**, Arima K, Nishioka M, Yoshino S, Hasui H, Fujikawa M. Comparison between sporadic cytomegalovirus hepatitis and Epstein-Barr virus hepatitis in previously healthy adults. *Liver* 1997; **17**: 63-69 [PMID: 9138274 DOI: 10.1111/j.1600-0676.1997.tb00782.x]
- 69 **Razonable RR**. Cytomegalovirus infection after liver transplantation: current concepts and challenges. *World J Gastroenterol* 2008; **14**: 4849-4860 [PMID: 18756591]
- 70 **Gallegos-Orozco JF**, Rakela-Brödnér J. Hepatitis viruses: not always what it seems to be. *Rev Med Chil* 2010; **138**: 1302-1311 [PMID: 21279280 DOI: 10.4067/S0034-98872010001100016]
- 71 **Rakela J**, Lange SM, Ludwig J, Baldus WP. Fulminant hepatitis: Mayo Clinic experience with 34 cases. *Mayo Clin Proc* 1985; **60**: 289-292 [PMID: 3921780 DOI: 10.1016/S0025-6196(12)60534-5]
- 72 **Dits H**, Frans E, Wilmer A, Van Ranst M, Fevery J, Bobbaers H. Varicella-zoster virus infection associated with acute liver failure. *Clin Infect Dis* 1998; **27**: 209-210 [PMID: 9675478 DOI: 10.1086/514613]
- 73 **Härmä M**, Höckerstedt K, Lautenschlager I. Human herpesvirus-6 and acute liver failure. *Transplantation* 2003; **76**: 536-539 [PMID: 12923440 DOI: 10.1097/01.TP.0000069233.13409.DF]
- 74 **Shiohara T**, Kano Y, Takahashi R, Ishida T, Mizukawa Y. Drug-induced hypersensitivity syndrome: recent advances in the diagnosis, pathogenesis and management. *Chem Immunol Allergy* 2012; **97**: 122-138 [PMID: 22613858 DOI: 10.1159/000335624]
- 75 **Hashida T**, Komura E, Yoshida M, Otsuka T, Hibi S, Imashuku S, Imashuku S, Ishizaki T, Yamada A, Suga S. Hepatitis in association with human herpesvirus-7 infection. *Pediatrics* 1995; **96**: 783-785 [PMID: 7567349]
- 76 **Ho JK**, Tha SP, Coupland R, Dalal BI, Bowie WR, Sreenivasan GM, Krajden M, Yoshida EM. Parvovirus B19 in an immunocompetent adult patient with acute liver failure: an underdiagnosed cause of acute non-A-E viral hepatitis. *Can J Gastroenterol* 2005; **19**: 161-162 [PMID: 15776137]
- 77 **Tameda Y**, Kosaka Y, Shiraki K, Ohashi Y, Hamada M, Miyazaki M, Ito N, Takase K, Nakano T. Hepatitis in an adult with rubella. *Intern Med* 1993; **32**: 580-583 [PMID: 8286839 DOI: 10.2169/internalmedicine.32.580]
- 78 **Arai M**, Wada N, Maruyama K, Nomiyama T, Tanaka S, Okazaki I. Acute hepatitis in an adult with acquired rubella infection. *J Gastroenterol* 1995; **30**: 539-542 [PMID: 7550869 DOI: 10.1007/BF02347575]
- 79 **Sezai Y**, Nagashima T. Significance of LDH isozyme pattern in rubella. *Nihon Hifuka Gakkai Zasshi* 1989; **99**: 811-817 [PMID: 2585778]
- 80 **Kawai K**, Kawai A. [Increased serum lactate dehydrogenase and changes in its isozymes pattern correlated to the atypical lymphocytes in adult rubella]. *Nihon Naika Gakkai Zasshi* 1989; **78**: 500-505 [PMID: 2746080 DOI: 10.2169/naika.78.500]
- 81 **Gavish D**, Kleinman Y, Morag A, Chajek-Shaul T. Hepatitis and jaundice associated with measles in young adults. An analysis of 65 cases. *Arch Intern Med* 1983; **143**: 674-677 [PMID: 6838292 DOI: 10.1016/j.ijid.2013.06.014]
- 82 **Giladi M**, Schulman A, Kedem R, Danon YL. Measles in adults: a prospective study of 291 consecutive cases. *Br Med J (Clin Res Ed)* 1987; **295**: 1314 [PMID: 3120991 DOI: 10.1136/bmj.295.6609.1314]
- 83 **Khatib R**, Siddique M, Abbass M. Measles associated hepatobiliary disease: an overview. *Infection* 1993; **21**: 112-114 [PMID: 8491519 DOI: 10.1007/BF01710744]
- 84 **Biron C**, Beaudoux O, Ponge A, Briend-Godet V, Corne F, Tripodi D, Hazart I, Esbelin J, Biron A, Boutoille D, Raffi F. Measles in the Nantes Teaching Hospital during the 2008-2009 epidemic. *Med Mal Infect* 2011; **41**: 415-423 [PMID: 21703787 DOI: 10.1016/j.medmal.2010.09.002]
- 85 **Monsel G**, Rapp C, Duong TA, Farhi D, Bouaziz JD, Meysonnier V, Mirkamali A, Jaureguiberry S, Caumes E. [Measles in adults: an emerging disease not sparing medical staff]. *Ann Dermatol Venerol* 2011; **138**: 107-110 [PMID: 21333820 DOI: 10.1016/j.annder.2010.12.015]
- 86 **Dinh A**, Fleuret V, Hanslik T. Liver involvement in adults with measles. *Int J Infect Dis* 2013; **17**: e1243-e1244 [PMID: 23938044]
- 87 **Rothenberg M**, Cheung R, Ahmed A. Adenovirus-induced acute liver failure. *Dig Dis Sci* 2009; **54**: 218-221 [PMID: 19034647 DOI: 10.1007/s10620-008-0628-9]
- 88 **Kimura H**, Nagasaka T, Hoshino Y, Hayashi N, Tanaka N, Xu JL, Kuzushima K, Morishima T. Severe hepatitis caused by Epstein-Barr virus without infection of hepatocytes. *Hum Pathol* 2001; **32**: 757-762 [PMID: 11486177 DOI: 10.1053/hupa.2001.25597]
- 89 **Tănăsescu C**. Correlation between cholestasis and infection. *Rom J Gastroenterol* 2004; **13**: 23-27 [PMID: 15054522]
- 90 **Mehal WZ**, Juedes AE, Crispe IN. Selective retention of activated CD8+ T cells by the normal liver. *J Immunol* 1999; **163**: 3202-3210 [PMID: 10477588]



- 91 **Bradham CA**, Plümpe J, Manns MP, Brenner DA, Trautwein C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. *Am J Physiol* 1998; **275**: G387-G392 [PMID: 9724248]
- 92 **Kondo T**, Suda T, Fukuyama H, Adachi M, Nagata S. Essential roles of the Fas ligand in the development of hepatitis. *Nat Med* 1997; **3**: 409-413 [PMID: 9095174 DOI: 10.1038/nm0497-409]
- 93 **Küsters S**, Gantner F, Künstle G, Tiegs G. Interferon gamma plays a critical role in T cell-dependent liver injury in mice initiated by concanavalin A. *Gastroenterology* 1996; **111**: 462-471 [PMID: 8690213 DOI: 10.1053/gast.1996.v111.pm8690213]
- 94 **Hay RJ**. Fungal infections affecting the liver. In: Rodés J, Benhaumou JP, Blei AT, Reichen J, Rizzetto M, editors. Textbook of Hepatology. 3<sup>rd</sup> ed. Oxford: Blackwell Publishing, 2007: 1011-1019 [DOI: 10.1002/9780470691861.ch10b]
- 95 **Lewis JH**, Patel HR, Zimmerman HJ. The spectrum of hepatic candidiasis. *Hepatology* 1982; **2**: 479-487 [PMID: 7095748 DOI: 10.1002/hep.1840020415]
- 96 **Pastakia B**, Shawker TH, Thaler M, O'Leary T, Pizzo PA. Hepatosplenic candidiasis: wheels within wheels. *Radiology* 1988; **166**: 417-421 [PMID: 3275982]
- 97 **Park GR**, Drummond GB, Lamb D, Durie TB, Milne LJ, Lambie AT, Cameron EW. Disseminated aspergillosis occurring in patients with respiratory, renal, and hepatic failure. *Lancet* 1982; **2**: 179-183 [PMID: 6123887 DOI: 10.1016/S0140-6736(82)91029-7]
- 98 **Cook GC**. Liver involvement in systemic infection. *Eur J Gastroenterol Hepatol* 1997; **9**: 1239-1247 [PMID: 9471032]
- 99 **Cook GC**. Malaria in the liver. *Postgrad Med J* 1994; **70**: 780-784 [PMID: 7824409 DOI: 10.1136/pgmj.70.829.780]
- 100 **Thakur N**, Sodani R, Chandra J, Mahto D. A Rare Case Report of Fatal Fulminant Hepatic Failure in a Child due to Mixed vivax and falciparum Infection. *Case Rep Pediatr* 2011; **2011**: 614054 [PMID: 22606519]
- 101 **Gryseels B**, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; **368**: 1106-1118 [PMID: 16997665 DOI: 10.1016/S0140-6736(06)69440-3]
- 102 **Maltz G**, Knauer CM. Amebic liver abscess: a 15-year experience. *Am J Gastroenterol* 1991; **86**: 704-710 [PMID: 2038992]
- 103 **Stanley SL**. Amoebiasis. *Lancet* 2003; **361**: 1025-1034 [PMID: 12660071]
- 104 **Hoffner RJ**, Kilagbayan T, Esekogwu VI, Henderson SO. Common presentations of amebic liver abscess. *Ann Emerg Med* 1999; **34**: 351-355 [PMID: 10459092]
- 105 **Meng XY**, Wu JX. Perforated amebic liver abscess: clinical analysis of 110 cases. *South Med J* 1994; **87**: 985-990 [PMID: 7939926]
- 106 **Salles JM**, Moraes LA, Salles MC. Hepatic amebiasis. *Braz J Infect Dis* 2003; **7**: 96-110 [PMID: 12959680]
- 107 **Eggleston FC**, Handa AK, Verghese M. Amebic peritonitis secondary to amebic liver abscess. *Surgery* 1982; **91**: 46-48 [PMID: 7054906]
- 108 **Papavramidis TS**, Sapalidis K, Pappas D, Karagianopoulou G, Trikoupi A, Souleimanis CH, Papavramidis ST. Gigantic hepatic amebic abscess presenting as acute abdomen: a case report. *J Med Case Rep* 2008; **2**: 325 [PMID: 18847505]
- 109 **Dhar P**, Chaudhary A, Desai R, Agarwal A, Sachdev A. Current trends in the diagnosis and management of cystic hydatid disease of the liver. *J Commun Dis* 1996; **28**: 221-230 [PMID: 9057445]
- 110 **Mairiang E**, Haswell-Elkins MR, Mairiang P, Sithithaworn P, Elkins DB. Reversal of biliary tract abnormalities associated with *Opisthorchis viverrini* infection following praziquantel treatment. *Trans R Soc Trop Med Hyg* 1993; **87**: 194-197 [PMID: 8337727 DOI: 10.1016/0035-9203(93)90489-D]
- 111 **Sandouk F**, Haffar S, Zada MM, Graham DY, Anand BS. Pancreatic-biliary ascariasis: experience of 300 cases. *Am J Gastroenterol* 1997; **92**: 2264-2267 [PMID: 9399767]

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## Vertical transmission of hepatitis C virus: Current knowledge and perspectives

Chun-Yan Yeung, Hung-Chang Lee, Wai-Tao Chan, Chun-Bin Jiang, Szu-Wen Chang, Chih-Kuang Chuang

Chun-Yan Yeung, Department of Medicine, Mackay Medical College, New Taipei City 252, Taiwan

Chun-Yan Yeung, Hung-Chang Lee, Wai-Tao Chan, Chun-Bin Jiang, Szu-Wen Chang, Division of Gastroenterology and Nutrition, Department of Pediatrics, Mackay Memorial Hospital, Taipei 10449, Taiwan

Chun-Yan Yeung, Wai-Tao Chan, Institute of Biotechnology and Department of Chemical Engineering, National Taipei University of Technology, Taipei 10608, Taiwan

Chun-Yan Yeung, Chun-Bin Jiang, Department of Nursing, Mackay Junior College of Medicine, Nursing and Management, Taipei 10449, Taiwan

Hung-Chang Lee, Department of Pediatrics, Taipei Medical University, Taipei 10449, Taiwan

Chih-Kuang Chuang, Department of Medical Research, Mackay Memorial Hospital, Taipei 10449, Taiwan

Chih-Kuang Chuang, College of Medicine, Fu-Jen Catholic University, New Taipei City 24205, Taiwan

**Author contributions:** Yeung CY contributed to conception, drafting of the article, literature review and approved the final version of the article; Lee HC, Chan WT, Jiang CB and Chang SW contributed to drafting of the article and literature review; Chuang CK contributed to conception, drafted the article, critically reviewed the manuscript and approved the final version of this article.

**Correspondence to:** Chih-Kuang Chuang, MSc, PhD, Department of Medical Research, Mackay Memorial Hospital, No.92, Sec.2, Chung Shan North Road, Taipei 10449, Taiwan. [cyyeung@mmh.org.tw](mailto:cyyeung@mmh.org.tw)

Telephone: +886-2-25433535 Fax: +886-2-25433642

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and the worldwide prevalence is between 1% and 8% in pregnant women and between 0.05% and 5% in children. Following the introduction of blood product screening, vertical transmission becomes the leading cause of childhood HCV infection. The prevalence of pediatric HCV infection varies from 0.05% to 0.36% in developed countries and between 1.8% and 5% in the developing world. All children born to women with anti-HCV antibodies should be checked for HCV infection. Though universal screening is controversial, selective antenatal HCV screening on high-risk populations is highly recommended and should be tested probably. Multiple risk factors were shown to increase the possibility of HCV vertical transmission, including coinfections with human immunodeficiency virus, intravenous drug use and elevated maternal HCV viral load, while breastfeeding and HCV genotypes have been studied to have little impact. At present, no clinical intervention has been clearly studied and proved to reduce the HCV vertical transmission risk. Cesarean section should not be recommended as a procedure to prevent vertical transmission, however, breastfeeding is generally not forbidden. The high prevalence of global HCV infection necessitates renewed efforts in primary prevention, including vaccine development, as well as new approaches to reduce the burden of chronic liver disease. Future researches should focus on the interruption of vertical transmission, developments of HCV vaccine and direct-acting antivirals in infancy and early childhood.

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**Key words:** Hepatitis C virus; Vertical transmission; Perinatal infection; Chronic liver disease

### Abstract

Hepatitis C virus (HCV) infection is a major global health issue. Infection by the HCV can cause acute and chronic liver diseases and may lead to cirrhosis, hepatocellular carcinoma or liver failure. The World Health Organization estimates that approximately 3% of the world population have been infected with HCV

**Core tip:** Hepatitis C virus (HCV) infection is a major global health issue. World Health Organization estimates that the worldwide prevalence is 1%-8% in pregnant women and 0.05%-5% in children. Vertical transmission becomes the leading cause of childhood

HCV infection. Current understanding of the epidemiology of mother-to-child transmission of HCV is limited. At present, no clinical intervention has been clearly studied and proved to reduce the vertical transmission risk. Though universal screening is controversial, selective antenatal HCV screening on high-risk populations is highly recommended and should be tested probably. This review provides the current knowledge and perspectives of HCV vertical transmission and summarizes the updated follow up guidelines for clinical practice.

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## GLOBAL EPIDEMIOLOGY OF HEPATITIS C INFECTION

Hepatitis C virus (HCV) infection is a major global health issue<sup>[1]</sup>. Infection by the HCV can cause acute and chronic liver diseases and may lead to cirrhosis, hepatocellular carcinoma or liver failure<sup>[2]</sup>. The World Health Organization estimates that approximately 3% of the world population have been infected with HCV<sup>[3]</sup>. There are approximately 170 million HCV patients worldwide, and three to four million cases are newly diagnosed every year<sup>[4,5]</sup>. It is estimated that about 0.2% to 26% of the general population in different countries are chronically infected by HCV<sup>[6]</sup>. The prevalence of HCV infection in the United States between 1999 and 2002 was found to be 1.6%<sup>[7]</sup>. In China, approximately 40 million people are infected with HCV, and 50% to 85% of them may develop chronic hepatitis; of these patients, 20% to 30% progress to liver cirrhosis and/or hepatocellular carcinoma<sup>[8,9]</sup>.

Before blood product screening for HCV was introduced, transfusion represented an important route of HCV transmission for infants and children<sup>[10]</sup>. Following the introduction of blood product screening, vertical transmission becomes the leading cause of childhood HCV infection and approximately 4000 new cases are diagnosed each year in the United States<sup>[11]</sup>. It is estimated that the prevalence varies from 0.05% to 0.36% in developed countries and 1.8% to 5% in the developing world<sup>[12,13]</sup>.

## PREVALENCE OF HCV INFECTION IN PREGNANT WOMEN

The worldwide prevalence of HCV infection is between 1% and 8% in pregnant women and between 0.05% and 5% in children<sup>[14]</sup>. Antenatal HCV infection rates vary worldwide, from 1% to 2.5% in the United States and Europe to more than 10% in some sub-Saharan countries<sup>[15-17]</sup>. Studies have shown the prevalence to be as high as 40% in some parts of Egypt<sup>[18]</sup>. According to the

result of the maternal HCV screening project conducted in Tottori Prefecture, Japan, the prevalent rate of HCV carrier mothers who were both anti-HCV and HCV RNA positive was 0.39%, while the rate of vertical transmission was found to be 8%<sup>[19]</sup>. However, the above maternal HCV prevalent rates may be underestimated since the current practice of HCV screening among high-risk pregnant women might miss a large number of HCV-infected patients, and besides there are no large scale HCV serosurvey studies available at present<sup>[20]</sup>.

In a recent study performed in Taiwan, a total of 7355 healthy asymptomatic pregnant women were screened for anti-HCV during a 6-year study period, 44 (0.6%) were found to be HCV-infected and 22 mothers were enrolled<sup>[21]</sup>. Half of the anti-HCV positive mothers were found to be positive for HCV RNA. All the mothers were negative for anti-HIV, 9 had invasive obstetric procedures such as amniocentesis. Of the 22 mother and baby pairs who were successfully followed up, two (9.1%) had eventually confirmed infected with HCV. Both of them were born to mothers with high viral load (HCV RNA > 10<sup>5</sup> copies/mL).

However, a methadone program in Australia showed that more than 70% of the pregnant women in this program are HCV positive, but less than 20% of their offsprings are examined for HCV status<sup>[22]</sup>. As a result of the lack of awareness of HCV in this high-risk population, many of these children are lost to follow-up and not diagnosed<sup>[23,24]</sup>.

## PATHOGENESIS OF HCV INFECTION DURING PREGNANCY

The pathogenesis of HCV infection during pregnancy remains poorly understood<sup>[14]</sup>. Recent studies have demonstrated there is a decrease of levels of serum alanine aminotransferase (ALT) during the second and third trimesters of pregnancy. However, the HCV viral load increases and reaches a peak during the third trimester<sup>[25-26]</sup>. Postpartum exacerbation of clinical HCV manifestations were found<sup>[27]</sup>. Conversely, seroconversion in pregnancy has been demonstrated and pregnancy may improve the natural course of HCV infection in some studies<sup>[26,28]</sup>.

Besides, recent researches suggests that HCV infection during pregnancy may increase the risks for preterm delivery, low Apgar scores, low birth weight, gestational diabetes, congenital malformations and overall perinatal mortality<sup>[29-31]</sup>. Other risk factors, such as limited prenatal care and intravenous drug use, are also found to be more prevalent in HCV patients<sup>[32]</sup> which could influence maternal and fetal morbidities and outcomes<sup>[26]</sup>. Conversely, increased risks for these obstetric complications were not shown in other studies<sup>[27,33]</sup>.

## INCIDENCE OF VERTICAL TRANSMISSION

As mentioned previously, vertical transmission becomes

a leading cause of pediatric HCV infection after blood product screening for hepatitis C was introduced, and it is also the leading cause of pediatric chronic liver disease in developed countries<sup>[34]</sup>. Although vertical transmission leading to chronic infection is reported in 4%-8%, transient HCV perinatal infection also occurs, with an incidence of about 14%-17%<sup>[35,36]</sup>. Incidence of HCV vertical transmission has been documented to be 3%-10%<sup>[14,33,37,38]</sup> and are higher in infants born to mothers coinfectd with human immunodeficiency virus (HIV).

## RISK FACTORS OF VERTICAL TRANSMISSION

Multiple risk factors were studied to increase the risk of HCV vertical transmission, including coinfections with HIV, intravenous drug use, high maternal HCV viral load, mode of delivery, preterm labor, prolonged rupture of membranes and amniocentesis, while breastfeeding and HCV genotypes have little impact on vertical transmission<sup>[14,30,39-41]</sup>. However, most of the reports are still controversial.

### HIV

Multiple researches have demonstrated that HCV vertical transmission rate increases 2-4-fold if coinfectd with HIV<sup>[10,42,43]</sup>. Vertical transmission in the group of infants in which the mother was HIV coinfectd antenatally was 5.9%, and thus supports the current recommendations for cesarean delivery in HIV and HCV coinfectd mothers<sup>[13]</sup>. It has been demonstrated that coinfections with HCV and HIV during pregnancy increase the vertical transmission odds by 90% according to a meta analysis of 10 studies<sup>[43]</sup>.

### Viral load

Another main risk factor identified for vertical transmission was maternal hepatitis C viremia. For mothers who tested positive for HCV RNA, vertical transmission was significantly higher at 7.1% when compared with 0% transmission for those who tested HCV RNA negative antenatally<sup>[10]</sup>. This has been reported previously in the literature and reflects that viremia holds a higher risk of vertical transmission<sup>[44,45]</sup>. Many studies have demonstrated that the risk of HCV vertical transmission increases if the maternal serum HCV viral load is above 10<sup>6</sup> copies HCV-RNA/mL, however there are many uninfected infants even though their mothers have a higher HCV viral load<sup>[46-48]</sup>. Since the maternal serum HCV-RNA viral load may fluctuate during pregnancy, it is recommended to repeat the HCV-RNA load in the third trimester<sup>[38]</sup>.

Although there are a few reports of vertical transmission in which the mother did not have viremia detected antenatally<sup>[49,50]</sup>. Maternal HCV RNA status can be of benefit in the patients counseling, patients can be reassured and advised that the risk of vertical transmission is minimal if hepatitis C RNA is not detected antenatally.

### Mode of delivery

More controversial is the effect of mode of delivery on vertical transmission. Whereas some studies have shown a protective benefit from cesarean section (CS) delivery<sup>[51,52]</sup>, many have not<sup>[25,53-55]</sup>. Few studies in the past recommend the use of elective CS to prevent the possible obstetric risks in order to lower the incidence of HCV vertical transmission<sup>[56]</sup>. Okamoto *et al.*<sup>[19]</sup> previously reported that children born to mothers with high viral loads had a significantly higher incidence of vertical transmission when delivered transvaginally. However, other studies, including a large-scale multicenter research project conducted in Europe, have failed to show significant evidence to prove its protective effect<sup>[25,47,57]</sup>. Some questioned the results since probably because most of the studies did not analyze high viral loads incidence along with elective CS.

Several conditions must be elucidated before the recommendation of elective cesarean section to prevent HCV vertical transmission<sup>[58]</sup>. Though studies show that the HCV vertical transmission rate is low in the infants born to mothers with high viral load<sup>[38]</sup>, however, taking into consideration the risks involved in CS and the natural course of HCV in infants, most research studies do not recommend elective CS for vertical transmission prevention at present<sup>[38,50,59,60]</sup>. Thus, the majority of the published literature would suggest that mode of delivery is not a key factor influencing HCV vertical transmission.

In 2008, the Japanese Society of Obstetrics and Gynecology's guideline recommended informing high viral loads women that the risk of vertical transmission might be significantly reduced by elective CS<sup>[61]</sup>. However, emergency CS should be considered separately from elective CS because emergency CS may allow conditions such as maternal blood contamination of the fetus and other complications<sup>[58,62]</sup>. However, cesarean delivery has been recommended for HCV-positive women coinfectd with HIV as mentioned before<sup>[63]</sup>.

### Breast-feeding

Breast-feeding does not increase the vertical transmission rate<sup>[38,60,64]</sup>. Avoidance of breast feeding is not an effective way for preventing HCV vertical transmission<sup>[65]</sup>. It is true that HCV RNA has been detected in breast milk and colostrum<sup>[66]</sup>, however breast-feeding does not shown to be a route of maternal to infant transmission<sup>[45,67,68]</sup>. HCV infected mothers are encouraged to breast-feed if there are no other contraindications, such as HIV co-infection<sup>[69]</sup>. The Centers for Disease Control and Prevention (CDC) suggests mothers should interrupt breast-feeding temporarily if there are bleeding or traumatized nipples, which could increase infants' HCV exposure<sup>[70]</sup>.

### Premature rupture of the membranes

Premature rupture of the membranes is considered a risk factor for HCV vertical transmission by exposing the fetus to maternal HCV in the birth canal<sup>[45,54]</sup>. The duration of rupture has been found to be significantly longer in



infected children<sup>[45,54,60]</sup>. These parameters are potentially related to contamination of the fetus with infected maternal blood in the birth canal.

### Other factors

Besides, the European Paediatric HCV Network described the significance of infantile sex as a risk factor for HCV vertical transmission, girls are twice as likely as boys to be infected<sup>[60]</sup>, however, no significant difference is found in other study<sup>[46]</sup>. In addition, some experts recommend avoiding invasive procedures that promote fetal exposure to maternal blood, such as fetal scalp monitoring<sup>[45,68]</sup>. As described previously, other parameters, such as birthweight, Apgar score, gestational age and bleeding volume during delivery were not significant risks for HCV vertical transmission<sup>[45,46,55,71,72]</sup>. Besides, HCV genotype was not associated with vertical transmission of HCV<sup>[73]</sup>. Despite an increased understanding of the risk factors involved, its transmission mechanisms and timing are still unknown and recommendations regarding prevention are limited<sup>[41,53,74]</sup>.

## NATURAL COURSE OF HCV-INFECTED INFANTS

HCV infection may lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma in adult populations. However researches about the natural history of hepatitis C in children is little. Studies showed that perinatally-acquired HCV infection becomes chronic in approximately 80% of cases<sup>[45,75,76]</sup>, similar to that observed in adults<sup>[77]</sup>, but higher than that reported in children who were infected through contaminated blood products<sup>[78]</sup>. Most studies show that HCV infected children are mostly asymptomatic<sup>[75,79]</sup>. Spontaneous clearance have reported rates ranging from 21% to 75%<sup>[76,78,80,81]</sup>. The European Paediatric HCV Network evaluated 266 children with vertically-acquired HCV infection and found clearance occurred in 21%-25%. Among cases of neonatal infection, 25% demonstrated spontaneous clearance by 7.3 years<sup>[80]</sup>.

However it has not been studied clearly whether the virus is completely eliminated, and there is possibility the infants will become HCV-RNA positive again later in their life. In a study performed in United Kingdom, the overall rate of spontaneous viral clearance was 17.5% with higher clearance (27%) in the transfusion group compared to the vertically acquired group (9%). Most children are asymptomatic with mildly abnormal hepatic transaminases<sup>[82]</sup>. An infected infant becomes HCV-RNA positive between 0 and 3 mo after birth<sup>[38]</sup>. Fortunately there is no case of fulminant hepatitis reported among infected infants to date.

Long-term outcomes for young HCV infected children in general are good<sup>[83-85]</sup>. Studies following patients for 10 to 20 years after perinatal acquisition of HCV show that 5% to 12% of them has significant fibrosis and 5% has cirrhosis<sup>[79,86]</sup>. No studies have yet studied the incidence of cirrhosis and hepatocellular carcinoma in

adults who acquired vertical HCV infection.

## DIAGNOSIS OF PERINATAL TRANSMISSION

A practical and widely acceptable recommendation by most studies is to consider children born to anti-HCV positive mothers infected with HCV when: (1) HCV RNA is detected in at least two serum samples and at least three months apart during the first year of life; and (2) HCV antibody is positive after 18 mo of age<sup>[73]</sup>. There is agreement on delaying PCR testing until 3 mo of age and to repeat it, if positive, at 6 mo of age. Testing of HCV antibody is of limited value before 18 mo of age due to passive transfer of maternal antibodies<sup>[60,73,87]</sup>.

## TREATMENT AND PREVENTION

Interferon and other treatments for women with high viral load who are of child-bearing age are useful for decreasing HCV levels, both for women as carriers and to decrease the risk of possible vertical transmission in future deliveries<sup>[58]</sup>. However, the available pharmacological therapies are contraindicated in pregnancy: ribavirin for its teratogenic effects and pegylated interferon alfa for its possible effects on fetal growth<sup>[88]</sup>. Thus, these treatments of HCV are contraindicated during pregnancy and there are no antiviral treatment recommendations for HCV-infected women at present<sup>[89]</sup>. Finally, whether CS is effective in preventing vertical transmission of HCV is still unclear as stated previously<sup>[42,60,90,91]</sup>.

Generally, children who are younger than 3 years should not be treated, and treatment is not approved in this age group. There are no published studies or reports of treatment in children who are younger than 3 years<sup>[92]</sup>. At present, treatment modalities that were initially restricted to adult subjects are now recommended for the treatment of HCV in children 3-17 years of age<sup>[93,94]</sup>. Treatment should consider several aspects including age, severity of disease, its adverse effects and compliance to treatment<sup>[92]</sup>.

## PRENATAL SCREENING

According to the recent recommendations published by the American College of Obstetricians and Gynecologists and CDC, routine prenatal HCV screening is not recommended in the general population<sup>[20,95,96]</sup>. However, women with significant risk factors should be offered screening. Generally, selective antenatal HCV screening is used on the basis of risk factors for exposure to the virus, such as a history of intravenous drug abuse<sup>[10]</sup>. However, there are currently no official recommendations addressing how often high-risk populations should be tested probably due to a lack of available data<sup>[37]</sup>.

In clinical practice, HCV screening in pregnancy has proven difficult, and it is likely that most HCV infected pregnant women are not identified<sup>[97-99]</sup>. Forty to 70% of



HCV-infected pregnant women do not initially report major risk factors<sup>[25,100]</sup>. In fact, a study in the United Kingdom showed that only one-third were identified through selective antenatal screening, suggesting that there may be many unidentified perinatally infected children in the absence of routine maternal antenatal screening<sup>[81]</sup>. A recent report by Delgado-Borrego *et al.*<sup>[101]</sup> estimated that about 85% to 95% of HCV-infected children in the United States have not been identified. Given the inherent inadequacies of risk factor-based screening, researches have investigated whether universal HCV screening in pregnant women would be a worthy approach.

In 2012, the CDC added recommendations for universal screening of all United States “baby boomers” regardless of reported risk factors<sup>[102]</sup>. This new recommendation was prompted by a recognition of the increasing rate of HCV complications in the United States and the failure of risk factor-based screening to identify most infected infants. Universal screening would ensure that infants born to HCV-infected women are properly identified and evaluated. However, when Plunkett and Grobman modeled universal screening in a pregnant population with 1% HCV seroprevalence, they found that it was not cost-effective, even when benefits of HCV diagnosis and treatment were considered for both mothers and infants and assuming that CS eliminated perinatal transmission<sup>[103]</sup>.

Prenatal screening itself is expensive, even in developed countries. An effective screening strategy utilizes an inexpensive and sensitive test to identify asymptomatic individuals at risk of a disease that has reasonably high prevalence, serious consequences if left untreated, and an effective treatment available<sup>[58,104]</sup>.

## FOLLOW UP GUIDELINES

Chronic pediatric HCV infection is usually associated with minimal or mild liver disease, however some cases may progress to advanced liver damage<sup>[80,105,106]</sup>. A broad range of ALT levels have been observed during the first year of life, with some infants exhibiting acute hepatitis pictures and others showing normal or mild elevated levels<sup>[75,105,107]</sup>.

In 2008, Shiraki *et al.*<sup>[38]</sup> presented guidelines for doctors in consulting and treating HCV-carrying pregnant women and their infants basing on current knowledge of vertical transmission. For those infants born to mother who is positive for anti-HCV and negative for HCV-RNA, an anti-HCV test should be performed later than 18 mo after birth to confirm that the infant is negative for anti-HCV. If the infant is still anti-HCV positive, the infant is considered to have been infected with HCV, HCV-RNA viral load and ALT level should be examined to determine whether the infection is a past one or whether it has continued up to the present time.

For those infants born to HCV-RNA-positive mother, tests for AST and ALT levels and HCV-RNA load should be performed 3 or 4 mo after birth. When HCV-RNA is positive, tests for AST, ALT, HCV-RNA and anti-HCV should be performed every 6 mo starting from the 6 mo

of birth to determine the persistence of infection. If the infant is negative for HCV-RNA 3 or 4 mo after birth, an HCV-RNA test should be performed at the ages of 6 mo and 12 mo to confirm the infant's negativity<sup>[38]</sup>.

CDC guidelines recommend testing for anti-HCV in children born to HCV infected mothers after 12 mo of age. However, if earlier testing is required, nucleic acid-based testing for HCV RNA is recommended 1 to 2 mo after birth<sup>[96,102]</sup>. If positive for either anti-HCV or HCV RNA, children should be evaluated for liver disease, and those with persistently elevated ALT levels should be referred to a specialist for medical management<sup>[96,108,109]</sup>. To further confirm HCV-RNA negativity, anti-HCV is tested at 18 mo of age if possible, and follow-up tests are no longer required when anti-HCV is also negative.

## CONCLUSION

HCV infection affects a large number of women of reproductive age worldwide, and vertical transmission remains a serious public health problem. The high prevalence of global HCV infection necessitates renewed efforts in primary prevention, including vaccine development, as well as new approaches to reduce the burden of chronic liver diseases.

Based on present knowledge of perinatal transmission of HCV, all children born to women with anti-HCV antibodies need to be tested for HCV infection. Though universal screening is controversial, selective antenatal HCV screening on high-risk populations is highly recommended and should be tested probably. At present, no intervention has been clearly demonstrated to reduce the risk for HCV vertical transmission. Cesarean section should not be recommended as a method to prevent vertical transmission of HCV, however, breastfeeding is generally not forbidden.

Awareness of HCV infection status in those high-risk population is mandatory. Novel approaches need to be considered to improve the knowledge of HCV transmission and hopefully improve HCV-associated health outcomes in high-risk populations. Future researches should focus on the interruption of vertical transmission, developments of HCV vaccine and direct-acting antivirals in infancy and early childhood. To prepare a more comprehensive and concrete standard for the prevention of HCV vertical transmission, a large scale and long-term follow-up study of children should be organized, as this may establish the need for more aggressive measures for prevention and treatment. Eventually, we believe that the number of new patients with HCV vertical transmission can be further decreased in the future.

## REFERENCES

- 1 Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; 57: 1333-1342 [PMID: 23172780 DOI: 10.1002/hep.26141]
- 2 Pereira LM, Martelli CM, Moreira RC, Merchan-Hamman

- E, Stein AT, Cardoso MR, Figueiredo GM, Montarroyos UR, Braga C, Turchi MD, Coral G, Crespo D, Lima ML, Alencar LC, Costa M, dos Santos AA, Ximenes RA. Prevalence and risk factors of Hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. *BMC Infect Dis* 2013; **13**: 60 [PMID: 23374914 DOI: 10.1186/1471-2334-13-60]
- 3 **Raja NS**, Janjua KA. Epidemiology of hepatitis C virus infection in Pakistan. *J Microbiol Immunol Infect* 2008; **41**: 4-8 [PMID: 18327420]
- 4 **Lo Re V**, Kostman JR. Management of chronic hepatitis C. *Postgrad Med J* 2005; **81**: 376-382 [PMID: 15937203]
- 5 **Ray Kim W**. Global epidemiology and burden of hepatitis C. *Microbes Infect* 2002; **4**: 1219-1225 [PMID: 12467763]
- 6 **Te HS**, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. *Clin Liver Dis* 2010; **14**: 1-21, vii [PMID: 20123436 DOI: 10.1016/j.cld.2009.11.009]
- 7 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhner WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586]
- 8 **Li D**, Long Y, Wang T, Xiao D, Zhang J, Guo Z, Wang B, Yan Y. Epidemiology of hepatitis C virus infection in highly endemic HBV areas in China. *PLoS One* 2013; **8**: e54815 [PMID: 23372775 DOI: 10.1371/journal.pone.0054815]
- 9 **McMenamin MB**, Jackson AD, Lambert J, Hall W, Butler K, Coulter-Smith S, McAuliffe FM. Obstetric management of hepatitis C-positive mothers: analysis of vertical transmission in 559 mother-infant pairs. *Am J Obstet Gynecol* 2008; **199**: 315.e1-315.e5 [PMID: 18771997 DOI: 10.1016/j.jog.2008.05.021]
- 10 **Cottrell EB**, Chou R, Wasson N, Rahman B, Guise JM. Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2013; **158**: 109-113 [PMID: 23437438]
- 11 **Klevens RM**, Hu DJ, Jiles R, Holmberg SD. Evolving epidemiology of hepatitis C virus in the United States. *Clin Infect Dis* 2012; **55** Suppl 1: S3-S9 [PMID: 22715211 DOI: 10.1093/cid/cis393]
- 12 **Schwimmer JB**, Balistreri WF. Transmission, natural history, and treatment of hepatitis C virus infection in the pediatric population. *Semin Liver Dis* 2000; **20**: 37-46 [PMID: 10895430]
- 13 **Jafri W**, Jafri N, Yakoob J, Islam M, Tirmizi SF, Jafar T, Akhtar S, Hamid S, Shah HA, Nizami SQ. Hepatitis B and C: prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. *BMC Infect Dis* 2006; **6**: 101 [PMID: 16792819]
- 14 **Le Campion A**, Larouche A, Fauteux-Daniel S, Soudeyns H. Pathogenesis of hepatitis C during pregnancy and childhood. *Viruses* 2012; **4**: 3531-3550 [PMID: 23223189 DOI: 10.3390/v4123531]
- 15 **World Health Organization**. Global distribution of hepatitis A, B, and C. *Wkly Epidemiol Rec* 2002; **77**: 45-47
- 16 **Boxall E**, Skidmore S, Evans C, Nightingale S. The prevalence of hepatitis B and C in an antenatal population of various ethnic origins. *Epidemiol Infect* 1994; **113**: 523-528 [PMID: 7527780]
- 17 **Goldberg D**, McIntyre PG, Smith R, Appleyard K, Dunlop J, Taylor A, Hutchinson S. Hepatitis C virus among high and low risk pregnant women in Dundee: unlinked anonymous testing. *BJOG* 2001; **108**: 365-370 [PMID: 11305542]
- 18 **Rao MR**, Naficy AB, Darwish MA, Darwish NM, Schisterman E, Clemens JD, Edelman R. Further evidence for association of hepatitis C infection with parenteral schistosomiasis treatment in Egypt. *BMC Infect Dis* 2002; **2**: 29 [PMID: 12464161]
- 19 **Okamoto M**, Nagata I, Murakami J, Kaji S, Iitsuka T, Hoshika T, Matsuda R, Tazawa Y, Shiraki K, Hino S. Prospective reevaluation of risk factors in mother-to-child transmission of hepatitis C virus: high virus load, vaginal delivery, and negative anti-NS4 antibody. *J Infect Dis* 2000; **182**: 1511-1514 [PMID: 11023474]
- 20 **Prasad MR**, Honegger JR. Hepatitis C virus in pregnancy. *Am J Perinatol* 2013; **30**: 149-159 [PMID: 23389935 DOI: 10.1055/s-0033-1334459]
- 21 **Chang ST**, Yeung CY, Lee HC, Fang SB, Chen HW, Chan WT, Chiang CB. Mother-to-child vertical transmission of hepatitis C virus: a prospective study on risk factors and clinical courses – preliminary report. Proceedings of 2009 Annual Meeting of Taiwan Pediatric Association, Taipei, Taiwan
- 22 **Liu AJ**, An EI, Murray HG, Tetstall E, Leroi MJ, Nanan RK. Screening for hepatitis C virus infection in methadone-maintained mothers and their infants. *Med J Aust* 2009; **191**: 535-538 [PMID: 19912084]
- 23 **Hardikar W**, Elliott EJ, Jones CA. The silent infection: should we be testing for perinatal hepatitis C and, if so, how? *Med J Aust* 2006; **184**: 54-55 [PMID: 16411867]
- 24 **Nightingale S**, Stormon MO, Day AS, Webber MT, Ward KA, O'Loughlin EV. Chronic hepatitis B and C infection in children in New South Wales. *Med J Aust* 2009; **190**: 670-673 [PMID: 19527200]
- 25 **Conte D**, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. *Hepatology* 2000; **31**: 751-755 [PMID: 10706568]
- 26 **Gervais A**, Bacq Y, Bernuau J, Martinot M, Auperin A, Boyer N, Kilani A, Erlinger S, Valla D, Marcellin P. Decrease in serum ALT and increase in serum HCV RNA during pregnancy in women with chronic hepatitis C. *J Hepatol* 2000; **32**: 293-299 [PMID: 10707870]
- 27 **Jabeen T**, Cannon B, Hogan J, Crowley M, Devereux C, Fanning L, Kenny-Walsh E, Shanahan F, Whelton MJ. Pregnancy and pregnancy outcome in hepatitis C type 1b. *QJM* 2000; **93**: 597-601 [PMID: 10984554]
- 28 **Zambon MC**, Lockwood DM. Hepatitis C seroconversion in pregnancy. *Br J Obstet Gynaecol* 1994; **101**: 722-724 [PMID: 7524645]
- 29 **Reddick KL**, Jhaveri R, Gandhi M, James AH, Swamy GK. Pregnancy outcomes associated with viral hepatitis. *J Viral Hepat* 2011; **18**: e394-e398 [PMID: 21692952 DOI: 10.1111/j.1365-2893.2011.01436.x]
- 30 **Pergam SA**, Wang CC, Gardella CM, Sandison TG, Phipps WT, Hawes SE. Pregnancy complications associated with hepatitis C: data from a 2003-2005 Washington state birth cohort. *Am J Obstet Gynecol* 2008; **199**: 38.e1-38.e9 [PMID: 18486089 DOI: 10.1016/j.jog.2008.03.052]
- 31 **Buresi MC**, Lee J, Gill S, Kong JM, Money DM, Yoshida EM. The prevalence of gestational diabetes mellitus and glucose abnormalities in pregnant women with hepatitis C virus infection in British Columbia. *J Obstet Gynaecol Can* 2010; **32**: 935-941 [PMID: 21176301]
- 32 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
- 33 **Floreani A**, Paternoster D, Zappala F, Cusinato R, Bombi G, Grella P, Chiaramonte M. Hepatitis C virus infection in pregnancy. *Br J Obstet Gynaecol* 1996; **103**: 325-329 [PMID: 8605128]
- 34 **Ruiz-Extremera A**, Muñoz-Gámez JA, Abril-Molina A, Salmerón-Ruiz MA, Muñoz-de-Rueda P, Pavón-Castillero EJ, Quiles-Pérez R, Carazo A, Gila A, Jimenez-Ruiz SM, Casado J, Martín AB, Sanjuán-Núñez L, Ocete-Hita E, Viota JL, León J, Salmerón J. Variation of transaminases, HCV-RNA levels and Th1/Th2 cytokine production during the post-partum

- period in pregnant women with chronic hepatitis C. *PLoS One* 2013; **8**: e75613 [PMID: 24130726 DOI: 10.1371/journal.pone.0075613]
- 35 **Shebl FM**, El-Kamary SS, Saleh DA, Abdel-Hamid M, Mikhail N, Allam A, El-Arabi H, Elhenawy I, El-Kafrawy S, El-Daly M, Selim S, El-Wahab AA, Mostafa M, Sharaf S, Hashem M, Heyward S, Stine OC, Magder LS, Stoszek S, Strickland GT. Prospective cohort study of mother-to-infant infection and clearance of hepatitis C in rural Egyptian villages. *J Med Virol* 2009; **81**: 1024-1031 [PMID: 19382251 DOI: 10.1002/jmv.21480]
- 36 **Ruiz-Extremuera A**, Muñoz-Gámez JA, Salmerón-Ruiz MA, de Rueda PM, Quiles-Pérez R, Gila-Medina A, Casado J, Belén Martín A, Sanjuan-Núñez L, Carazo A, Pavón EJ, Ocete-Hita E, León J, Salmerón J. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011; **53**: 1830-1838 [PMID: 21413051 DOI: 10.1002/hep.24298]
- 37 **Kanaan T**, Liu A, Leroi M, Nanan R. A multicentre survey of hepatitis C awareness in a high-risk population. *J Paediatr Child Health* 2013; **49**: 649-653 [PMID: 23742262 DOI: 10.1111/jpc.12259]
- 38 **Shiraki K**, Ohto H, Inaba N, Fujisawa T, Tajiri H, Kanzaki S, Matsui A, Morishima T, Goto K, Kimura A, Hino S. Guidelines for care of pregnant women carrying hepatitis C virus and their infants. *Pediatr Int* 2008; **50**: 138-140 [PMID: 18279227 DOI: 10.1111/j.1442-200X.2007.02518.x]
- 39 **Kumar RM**, Shahul S. Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. *J Hepatol* 1998; **29**: 191-197 [PMID: 9722199]
- 40 **Ruiz-Extremuera A**, Salmerón J, Torres C, De Rueda PM, Giménez F, Robles C, Miranda MT. Follow-up of transmission of hepatitis C to babies of human immunodeficiency virus-negative women: the role of breast-feeding in transmission. *Pediatr Infect Dis J* 2000; **19**: 511-516 [PMID: 10877164]
- 41 **Safir A**, Levy A, Sikuler E, Sheiner E. Maternal hepatitis B virus or hepatitis C virus carrier status as an independent risk factor for adverse perinatal outcome. *Liver Int* 2010; **30**: 765-770 [PMID: 20214739 DOI: 10.1111/j.1478-3231.2010.02218.x]
- 42 **Mariné-Barjoan E**, Berrébi A, Giordanengo V, Favre SF, Haas H, Moreigne M, Izopet J, Tricoire J, Tran A, Pradier C, Bongain A. HCV/HIV co-infection, HCV viral load and mode of delivery: risk factors for mother-to-child transmission of hepatitis C virus? *AIDS* 2007; **21**: 1811-1815 [PMID: 17690581]
- 43 **Polis CB**, Shah SN, Johnson KE, Gupta A. Impact of maternal HIV coinfection on the vertical transmission of hepatitis C virus: a meta-analysis. *Clin Infect Dis* 2007; **44**: 1123-1131 [PMID: 17366462]
- 44 **Ferrero S**, Lungaro P, Bruzzzone BM, Gotta C, Bentivoglio G, Ragni N. Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990-2000). *Acta Obstet Gynecol Scand* 2003; **82**: 229-234 [PMID: 12694118]
- 45 **Mast EE**, Hwang LY, Seto DS, Nolte FS, Nainan OV, Wurtzel H, Alter MJ. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. *J Infect Dis* 2005; **192**: 1880-1889 [PMID: 16267758]
- 46 **Hayashida A**, Inaba N, Oshima K, Nishikawa M, Shoda A, Hayashida S, Negishi M, Inaba F, Inaba M, Fukasawa I, Watanabe H, Takamizawa H. Re-evaluation of the true rate of hepatitis C virus mother-to-child transmission and its novel risk factors based on our two prospective studies. *J Obstet Gynaecol Res* 2007; **33**: 417-422 [PMID: 17688606]
- 47 **Ceci O**, Margiotta M, Marelo F, Francavilla R, Loizzi P, Francavilla A, Mautone A, Impedovo L, Ierardi E, Mastroianni M, Bettocchi S, Selvaggi L. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. *J Pediatr Gastroenterol Nutr* 2001; **33**: 570-575 [PMID: 11740231]
- 48 **Chamie G**, Bonacini M, Bangsberg DR, Stapleton JT, Hall C, Overton ET, Scherzer R, Tien PC. Factors associated with seronegative chronic hepatitis C virus infection in HIV infection. *Clin Infect Dis* 2007; **44**: 577-583 [PMID: 17243063]
- 49 **Dore GJ**, Kaldor JM, McCaughan GW. Systematic review of role of polymerase chain reaction in defining infectiousness among people infected with hepatitis C virus. *BMJ* 1997; **315**: 333-337 [PMID: 9270453]
- 50 **Pembrey L**, Newell ML, Tovo PA. The management of HCV infected pregnant women and their children European paediatric HCV network. *J Hepatol* 2005; **43**: 515-525 [PMID: 16144064]
- 51 **Gibb DM**, Goodall RL, Dunn DT, Healy M, Neave P, Cafferkey M, Butler K. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. *Lancet* 2000; **356**: 904-907 [PMID: 11036896]
- 52 **Paccagnini S**, Principi N, Massironi E, Tanzi E, Romanò L, Muggiasca ML, Ragni MC, Salvaggio L. Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis J* 1995; **14**: 195-199 [PMID: 7761184]
- 53 **Resti M**, Azzari C, Mannelli F, Moriondo M, Novembre E, de Martino M, Vierucci A. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. *BMJ* 1998; **317**: 437-441 [PMID: 9703524]
- 54 **Spencer JD**, Latt N, Beeby PJ, Collins E, Saunders JB, McCaughan GW, Cossart YE. Transmission of hepatitis C virus to infants of human immunodeficiency virus-negative intravenous drug-using mothers: rate of infection and assessment of risk factors for transmission. *J Viral Hepat* 1997; **4**: 395-409 [PMID: 9430360]
- 55 **European Paediatric Hepatitis C Virus Network**. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. European Paediatric Hepatitis C Virus Network. *BJOG* 2001; **108**: 371-377 [PMID: 11305543]
- 56 **Lin HH**, Kao JH, Hsu HY, Mizokami M, Hirano K, Chen DS. Least microtransfusion from mother to fetus in elective cesarean delivery. *Obstet Gynecol* 1996; **87**: 244-248 [PMID: 8559532]
- 57 **van Ham MA**, van Dongen PW, Mulder J. Maternal consequences of caesarean section. A retrospective study of intra-operative and postoperative maternal complications of caesarean section during a 10-year period. *Eur J Obstet Gynecol Reprod Biol* 1997; **74**: 1-6 [PMID: 9243191]
- 58 **Murakami J**, Nagata I, Iitsuka T, Okamoto M, Kaji S, Hoshika T, Matsuda R, Kanzaki S, Shiraki K, Suyama A, Hino S. Risk factors for mother-to-child transmission of hepatitis C virus: Maternal high viral load and fetal exposure in the birth canal. *Hepatol Res* 2012; **42**: 648-657 [PMID: 22404371 DOI: 10.1111/j.1872-034X.2012.00968.x]
- 59 **Tajiri H**, Miyoshi Y, Funada S, Etani Y, Abe J, Onodera T, Goto M, Funato M, Ida S, Noda C, Nakayama M, Okada S. Prospective study of mother-to-infant transmission of hepatitis C virus. *Pediatr Infect Dis J* 2001; **20**: 10-14 [PMID: 11176560]
- 60 **European Paediatric Hepatitis C Virus Network**. A significant sex—but not elective cesarean section—effect on mother-to-child transmission of hepatitis C virus infection. *J Infect Dis* 2005; **192**: 1872-1879 [PMID: 16267757]
- 61 **Japan Society of Obstetrics and Gynecology**. Guideline for Obstetrical Practice in Japan 2008. Available from: URL: <http://www.jsog.or.jp/activity/pdf/FUJ-FULL.pdf>
- 62 **Kaneda T**, Shiraki K, Hirano K, Nagata I. Detection of maternal-fetal transmission by placental alkaline phosphatase levels. *J Pediatr* 1997; **130**: 730-735 [PMID: 9152281]
- 63 **Coll O**, Fiore S, Floridia M, Giaquinto C, Grosch-Wörner



- I, Guiliano M, Lindgren S, Lyall H, Mandelbrot L, Newell ML, Peckham C, Rudin C, Semprini AE, Taylor G, Thorne C, Tovo PA. Pregnancy and HIV infection: A european consensus on management. *AIDS* 2002; **16** Suppl 2: S1-18 [PMID: 12479261]
- 64 Kage M, Ogasawara S, Kosai K, Nakashima E, Shimamatsu K, Kojiro M, Kimura A, Fujisawa T, Matsukuma Y, Ito Y, Kondo S, Kawano K, Sata M. Hepatitis C virus RNA present in saliva but absent in breast-milk of the hepatitis C carrier mother. *J Gastroenterol Hepatol* 1997; **12**: 518-521 [PMID: 9257243]
- 65 Bhole K, McGuire W. Does avoidance of breast feeding reduce mother-to-infant transmission of hepatitis C virus infection? *Arch Dis Child* 2007; **92**: 365-366 [PMID: 17376949]
- 66 Ogasawara S, Kage M, Kosai K, Shimamatsu K, Kojiro M. Hepatitis C virus RNA in saliva and breastmilk of hepatitis C carrier mothers. *Lancet* 1993; **341**: 561 [PMID: 8094800]
- 67 Lin HH, Kao JH, Hsu HY, Ni YH, Chang MH, Huang SC, Hwang LH, Chen PJ, Chen DS. Absence of infection in breast-fed infants born to hepatitis C virus-infected mothers. *J Pediatr* 1995; **126**: 589-591 [PMID: 7535353]
- 68 Airolidi J, Berghella V. Hepatitis C and pregnancy. *Obstet Gynecol Surv* 2006; **61**: 666-672 [PMID: 16978426]
- 69 Hepatitis C virus infection. American Academy of Pediatrics. Committee on Infectious Diseases. *Pediatrics* 1998; **101**: 481-485 [PMID: 9499195]
- 70 Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59**: 1-110 [PMID: 21160459]
- 71 Steininger C, Kundi M, Jatzko G, Kiss H, Lischka A, Holzmann H. Increased risk of mother-to-infant transmission of hepatitis C virus by intrapartum infantile exposure to maternal blood. *J Infect Dis* 2003; **187**: 345-351 [PMID: 12552417]
- 72 Dal Molin G, D'Agaro P, Ansaldi F, Ciana G, Fertz C, Alberico S, Campello C. Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti-HCV as risk factors. *J Med Virol* 2002; **67**: 137-142 [PMID: 11992574]
- 73 Indolfi G, Resti M. Perinatal transmission of hepatitis C virus infection. *J Med Virol* 2009; **81**: 836-843 [PMID: 19319981 DOI: 10.1002/jmv.21437]
- 74 Ohto H, Terazawa S, Sasaki N, Sasaki N, Hino K, Ishiwata C, Kako M, Ujii N, Endo C, Matsui A. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. *N Engl J Med* 1994; **330**: 744-750 [PMID: 8107740]
- 75 Resti M, Jara P, Hierro L, Azzari C, Giacchino R, Zuin G, Zancan L, Pedditzi S, Bortolotti F. Clinical features and progression of perinatally acquired hepatitis C virus infection. *J Med Virol* 2003; **70**: 373-377 [PMID: 12766999]
- 76 Yeung LT, To T, King SM, Roberts EA. Spontaneous clearance of childhood hepatitis C virus infection. *J Viral Hepat* 2007; **14**: 797-805 [PMID: 17927616]
- 77 Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20**: 17-35 [PMID: 10895429]
- 78 Vogt M, Lang T, Frösner G, Klingler C, Sendl AF, Zeller A, Wiebecke B, Langer B, Meisner H, Hess J. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med* 1999; **341**: 866-870 [PMID: 10498458]
- 79 Guido M, Bortolotti F, Leandro G, Jara P, Hierro L, Larrauri J, Barbera C, Giacchino R, Zancan L, Balli F, Crivellaro C, Cristina E, Pucci A, Rugge M. Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? *Am J Gastroenterol* 2003; **98**: 660-663 [PMID: 12650803]
- 80 European Paediatric Hepatitis C Virus Network. Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. *Clin Infect Dis* 2005; **41**: 45-51 [PMID: 15937762]
- 81 Larghi A, Zuin M, Crosignani A, Ribero ML, Pipia C, Battezzati PM, Binelli G, Donato F, Zanetti AR, Podda M, Tagger A. Outcome of an outbreak of acute hepatitis C among healthy volunteers participating in pharmacokinetics studies. *Hepatology* 2002; **36**: 993-1000 [PMID: 12297849]
- 82 Abdel-Hady M, Bunn SK, Sira J, Brown RM, Brundler MA, Davies P, Kelly DA. Chronic hepatitis C in children--review of natural history at a National Centre. *J Viral Hepat* 2011; **18**: e535-e540 [PMID: 21914074 DOI: 10.1111/j.1365-2893.2011.01456.x]
- 83 Jhaveri R. Diagnosis and management of hepatitis C virus-infected children. *Pediatr Infect Dis J* 2011; **30**: 983-985 [PMID: 21997662 DOI: 10.1097/INF.0b013e318236ac37]
- 84 Castellino S, Lensing S, Riely C, Rai SN, Davila R, Hayden RT, Fleckenstein J, Levstik M, Taylor S, Dean PJ, Kippenbrock S, Pope J, Carr J, Strickland DK, Hudson MM. The epidemiology of chronic hepatitis C infection in survivors of childhood cancer: an update of the St Jude Children's Research Hospital hepatitis C seropositive cohort. *Blood* 2004; **103**: 2460-2466 [PMID: 14684419]
- 85 Bortolotti F, Verucchi G, Cammà C, Cabibbo G, Zancan L, Indolfi G, Giacchino R, Marcellini M, Marazzi MG, Barbera C, Maggiore G, Vajro P, Bartolacci S, Balli F, Maccabruni A, Guido M. Long-term course of chronic hepatitis C in children: from viral clearance to end-stage liver disease. *Gastroenterology* 2008; **134**: 1900-1907 [PMID: 18439604 DOI: 10.1053/j.gastro.2008.02.082]
- 86 Mohan P, Colvin C, Glymph C, Chandra RR, Kleiner DE, Patel KM, Luban NL, Alter HJ. Clinical spectrum and histopathologic features of chronic hepatitis C infection in children. *J Pediatr* 2007; **150**: 168-174, 174.e1 [PMID: 17236895]
- 87 Davison SM, Mieli-Vergani G, Sira J, Kelly DA. Perinatal hepatitis C virus infection: diagnosis and management. *Arch Dis Child* 2006; **91**: 781-785 [PMID: 16923861]
- 88 Chutaputti A. Adverse effects and other safety aspects of the hepatitis C antivirals. *J Gastroenterol Hepatol* 2000; **15** Suppl: E156-E163 [PMID: 10921400]
- 89 Valladares G, Chacaltana A, Sjogren MH. The management of HCV-infected pregnant women. *Ann Hepatol* 2010; **9** Suppl: 92-97 [PMID: 20714003]
- 90 Ghamar Chehreh ME, Tabatabaei SV, Khazanehdari S, Alavian SM. Effect of cesarean section on the risk of perinatal transmission of hepatitis C virus from HCV-RNA+/HIV-mothers: a meta-analysis. *Arch Gynecol Obstet* 2011; **283**: 255-260 [PMID: 20652289 DOI: 10.1007/s00404-010-1588-9]
- 91 Arshad M, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period--are they opportunities for treatment? *J Viral Hepat* 2011; **18**: 229-236 [PMID: 21392169 DOI: 10.1111/j.1365-2893.2010.01413.x]
- 92 Mack CL, Gonzalez-Peralta RP, Gupta N, Leung D, Narke-wicz MR, Roberts EA, Rosenthal P, Schwarz KB. NASP-GHAN practice guidelines: Diagnosis and management of hepatitis C infection in infants, children, and adolescents. *J Pediatr Gastroenterol Nutr* 2012; **54**: 838-855 [PMID: 22487950 DOI: 10.1097/MPG.0b013e318258328d]
- 93 Wirth S, Ribes-Koninckx C, Calzadeo MA, Bortolotti F, Zancan L, Jara P, Shelton M, Kerkar N, Galoppo M, Pedreira A, Rodriguez-Baez N, Ciocca M, Lachaux A, Lacaille F, Lang T, Kullmer U, Huber WD, Gonzalez T, Pollack H, Alonso E, Broue P, Ramakrishna J, Neigut D, Valle-Segarra AD, Hunter B, Goodman Z, Xu CR, Zheng H, Novello S, Sniukiene V, Brass C, Albrecht JK. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. *J Hepatol* 2010; **52**: 501-507 [PMID: 20189674 DOI: 10.1016/j.jhep.2010.01.016]
- 94 Schwarz KB, Gonzalez-Peralta RP, Murray KF, Molleston JP, Haber BA, Jonas MM, Rosenthal P, Mohan P, Balistreri WF, Narkewicz MR, Smith L, Lobritto SJ, Rossi S, Valsamakis A, Goodman Z, Robuck PR, Barton BA. The combination of ribavirin and peginterferon is superior to peginterferon and



- placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011; **140**: 450-458.e1 [PMID: 21036173 DOI: 10.1053/j.gastro.2010.10.047]
- 95 **American College of Obstetricians and Gynecologists.** ACOG Practice Bulletin No. 86: Viral hepatitis in pregnancy. *Obstet Gynecol* 2007; **110**: 941-956 [PMID: 17906043]
- 96 Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 1998; **47**: 1-39 [PMID: 9790221]
- 97 **Giles M,** Hellard M, Sasadeusz J. Hepatitis C and pregnancy: an update. *Aust N Z J Obstet Gynaecol* 2003; **43**: 290-293 [PMID: 14714713]
- 98 **Blasig A,** Wagner EC, Pi D, Bigham M, Remple VP, Craib KJ, Doyle P, Dobson S, Yoshida EM, Patrick D, Krajden M, Money DM. Hepatitis C infection among pregnant women in British Columbia: reported prevalence and critical appraisal of current prenatal screening methods. *Can J Public Health* 2011; **102**: 98-102 [PMID: 21608379]
- 99 **Pinto CS,** Martins RM, Andrade SM, Stief AC, Oliveira RD, Castro AR. Hepatitis C virus infection among pregnant women in Central-Western Brazil, 2005-2007. *Rev Saude Publica* 2011; **45**: 974-976 [PMID: 21829975]
- 100 **Ward C,** Tudor-Williams G, Cotzias T, Hargreaves S, Regan L, Foster GR. Prevalence of hepatitis C among pregnant women attending an inner London obstetric department: uptake and acceptability of named antenatal testing. *Gut* 2000; **47**: 277-280 [PMID: 10896922]
- 101 **Delgado-Borrego A,** Smith L, Jonas MM, Hall CA, Negre B, Jordan SH, Ogrodowicz M, Raza R, Ludwig DA, Miller T, Lipshultz SE, Gonzalez-Peralta R, Chung RT. Expected and actual case ascertainment and treatment rates for children infected with hepatitis C in Florida and the United States: epidemiologic evidence from statewide and nationwide surveys. *J Pediatr* 2012; **161**: 915-921 [PMID: 22765955 DOI: 10.1016/j.jpeds.2012.05.002]
- 102 **Smith BD,** Morgan RL, Beckett GA, Falck-Ytter Y, Holtzman D, Teo CG, Jewett A, Baack B, Rein DB, Patel N, Alter M, Yartel A, Ward JW. Recommendations for the identification of chronic hepatitis C virus infection among persons born during 1945-1965. *MMWR Recomm Rep* 2012; **61**: 1-32 [PMID: 22895429]
- 103 **Plunkett BA,** Grobman WA. Routine hepatitis C virus screening in pregnancy: a cost-effectiveness analysis. *Am J Obstet Gynecol* 2005; **192**: 1153-1161 [PMID: 15846195]
- 104 **Wilson JM,** Jungner YG. Principles and practices of screening for disease. *Bol Oficina Sanit Panam* 1968; **65**: 281-393
- 105 **Jonas MM.** Children with hepatitis C. *Hepatology* 2002; **36**: S173-S178 [PMID: 12407591]
- 106 **Palomba E,** Manzini P, Fiammengo P, Maderni P, Saracco G, Tovo PA. Natural history of perinatal hepatitis C virus infection. *Clin Infect Dis* 1996; **23**: 47-50 [PMID: 8816128]
- 107 **Farci P,** Quinti I, Farci S, Alter HJ, Strazzera R, Palomba E, Coiana A, Cao D, Casadei AM, Ledda R, Iorio R, Vegnente A, Diaz G, Tovo PA. Evolution of hepatitis C viral quasiespecies and hepatic injury in perinatally infected children followed prospectively. *Proc Natl Acad Sci USA* 2006; **103**: 8475-8480 [PMID: 16707577]
- 108 **Albeldawi M,** Ruiz-Rodriguez E, Carey WD. Hepatitis C virus: Prevention, screening, and interpretation of assays. *Cleve Clin J Med* 2010; **77**: 616-626 [PMID: 20810872 DOI: 10.3949/ccjm.77a.09162]
- 109 **Ghany MG,** Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

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## Nucleos(t)ide analogues to treat hepatitis B virus-related hepatocellular carcinoma after radical resection

Yang Ke, Lin Wang, Le-Qun Li, Jian-Hong Zhong

Yang Ke, Le-Qun Li, Jian-Hong Zhong, Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Yang Ke, Lin Wang, Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Kunming Medical University, Kunming 650101, Yunnan Province, China

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Correspondence to: Jian-Hong Zhong, MD, PhD, Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, He Di Rd. #71, Nanning 530021, Guangxi Zhuang Autonomous Region, China. zhongjianhong66@163.com  
 Telephone: +86-771-5330855 Fax: +86-771-5312000

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### Abstract

Significant advances have been made in nucleos(t)ide analogue (NA) therapy to treat chronic hepatitis B, and this therapy reduces the risk of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) in some patients. However, whether NAs can also prevent recurrence after radical resection of HBV-related HCC remains controversial and is an important question, given that most patients will experience recurrence within a few years of curative surgery. Here we systematically reviewed the literature since 2004 on outcomes after administering NAs to patients with HBV-related HCC following radical resection. We focused on treatment indications, duration, effects on recurrence-free survival and overall survival, and the management of NA resistance. We find that patients with HCC should strongly consider NA therapy if they are positive for HBV-DNA, and that the available evidence suggests that postoperative NA therapy can increase both recurrence-free and overall survival. To minimize drug resistance, clinicians should opt for potent analogues with higher resistance

barriers, and they should monitor the patient carefully for emergence of NA-resistant HBV.

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**Key words:** Antiviral therapy; Hepatitis B virus; Hepatocellular carcinoma; Liver resection; Nucleos(t)ide analogue; Survival rate

**Core tip:** Significant advances have been made in nucleos(t)ide analogue (NA) therapy to treat chronic hepatitis B. However, for patients undergoing radical resection for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC), a number of important questions remain undefined, including when NA therapy should be initiated, how long the treatment should continue, and whether NAs can prevent recurrence after radical resection. Here we review the available evidence on these questions in the Medline database. We focus on NA treatment indications, duration, effects on recurrence-free survival and overall survival, and management of NA resistance in patients with HBV-related HCC.

Ke Y, Wang L, Li LQ, Zhong JH. Nucleos(t)ide analogues to treat hepatitis B virus-related hepatocellular carcinoma after radical resection. *World J Hepatol* 2014; 6(9): 652-659 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v6/i9/652.htm>  
 DOI: <http://dx.doi.org/10.4254/wjh.v6.i9.652>

### INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most frequent cause of cancer-related death in the world<sup>[1]</sup>. Hepatic resection, percutaneous ethanol injection, radiofrequency ablation are recognized as radical treatment options for HCC and are highly effective at removing tumors; however, patients' prognosis after radical resection remains poor, due to the high recurrence rate<sup>[1,2]</sup>. HCC recurrence occurs in up to

41%-50% of patients within 2 years after resection (early recurrence) and in up to 20% of patients more than 2 years later (late recurrence)<sup>[3,4]</sup>. Most early recurrence appears to reflect diffusion of primary tumors, while most late recurrence stems from *de novo* tumors spontaneously arising in the remnant diseased liver<sup>[3-5]</sup>.

In China and Sub-Saharan Africa, the major risk factor for HCC is hepatitis B virus (HBV) infection. Therefore investigators reasoned that the same nucleos(t)ide analogues (NAs) that have been proven so effective against chronic HBV infection may also benefit patients with HBV-related HCC. Indeed, randomized controlled trials (RCTs)<sup>[6]</sup> and large retrospective studies<sup>[7-9]</sup> have shown that NAs can dramatically reduce the risk of HCC in patients with chronic HBV infection or cirrhosis. While this suggests that NAs are effective against primary HCC, the question of whether they can also prevent HCC recurrence after radical resection remains controversial<sup>[10]</sup>.

Here we systematically reviewed the literature on this question by searching the Medline database for articles published since 2004 on outcomes of NA therapy in patients with HBV-related HCC. We used the following search terms: “nucleoside analogue”, “nucleoside analog”, “nucleotide analogue”, “nucleotide analog”, “antiviral therapy”, “hepatitis B virus”, “hepatocellular carcinoma”, “liver resection”, and “survival rate”. We focused on treatment indications, duration, effects on recurrence-free survival and overall survival, and the development of NA resistance.

## TYPES OF NAS

Five types of oral NAs have been used in clinical practice: lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (LdT), entecavir (ETV) and tenofovir disoproxil fumarate (TDF). LAM and LdT are L-nucleoside analogues, ADV and TDF are acyclic adenine nucleotide analogues, and ETV is a cyclopentyl guanosine analogue<sup>[11]</sup>. All 5 of these NA types can be phosphorylated in cells, and subsequently compete with natural nucleotides to be incorporated into viral DNA by HBV polymerase/reverse transcriptase. Since the analogues cannot be extended by HBV polymerase, they cause premature termination of genome replication. Studies suggest that ETV, TDF, and LdT are similarly effective at suppressing HBV-DNA synthesis and are more potent than LAM and ADV<sup>[11]</sup>, although none can completely eradicate HBV due to the persistence of covalently closed circular DNA in the nuclei of infected hepatocytes<sup>[12]</sup>.

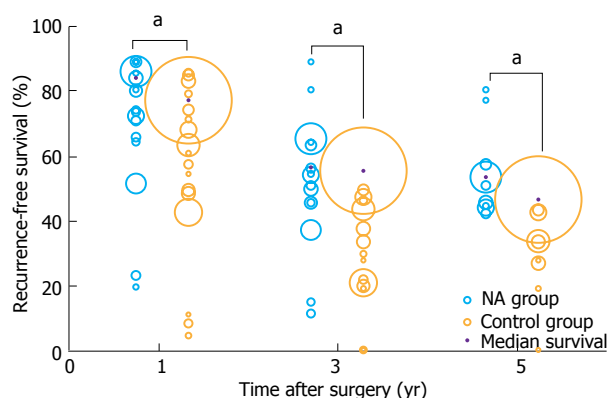
## INDICATIONS AND DURATION OF NA THERAPY AFTER HCC SURGERY

Nowadays there are Asian-Pacific consensus<sup>[11]</sup>, Chinese Medical Association guideline<sup>[13]</sup>, American Association for the Study of Liver Disease (AASLD) guideline<sup>[14]</sup>, European Association for the Study of Liver (EASL)

guideline<sup>[15]</sup>, Treatment Algorithm in the United States<sup>[16]</sup> and Asian-American guideline<sup>[17]</sup> related to the treatment of chronic hepatitis B infection. In these guidelines<sup>[11,13-17]</sup>, the criteria for initiating treatment such as ALT level and HBV-DNA amount are different. Current Asian guidelines<sup>[11,13]</sup> recommend that NA therapy be considered if the ALT level is > 2-fold greater than the upper limit of the normal range, and the HBV-DNA level is either > 20000 IU/mL if the patient is HBeAg-positive or > 2000 IU/mL if the patient is HBeAg-negative. In America, with the same criteria about ALT level, NA therapy is recommended to patients if their HBV-DNA level is > 20000 IU/mL<sup>[14]</sup>. While a panel of Asian-American physicians with expertise in hepatitis B treatment has suggested<sup>[17]</sup> that Asia Americans should be considered for treatment when they have HBV-DNA levels above 2000 IU/mL, and serum ALT levels above the upper limit of the normal range, and so did EASL guidelines<sup>[15]</sup> in the criteria of ALT level and HBV-DNA amount, which are stricter than AASLD guideline<sup>[14]</sup>.

Recommended treatment duration also varies depending on these guidelines<sup>[11,13-15]</sup>. In HBeAg-positive patients who show HBeAg seroconversion and undetectable levels of HBV-DNA, Asian-Pacific guideline<sup>[11]</sup> recommends that NA treatment can be discontinued after 12 mo of consolidation therapy, while AASLD guideline<sup>[14]</sup> recommends the duration of consolidation therapy be at least 6 mo. In HBeAg-negative patients, both Asian-Pacific and AASLD guidelines recommend NA treatment should ideally be stopped when HBsAg is no longer detectable<sup>[11,14]</sup>, while Asian-Pacific guideline<sup>[11]</sup> advises if the patient remains HBsAg-positive, NA treatment can be discontinued after at least 2 years of therapy when test results show undetectable HBV-DNA levels on 3 separate occasions 6 mo apart. EASL guideline<sup>[15]</sup> suggests that in both HBeAg-positive and HBeAg-negative patients sustained off-treatment HBsAg loss is the ideal end point. Sustained off-treatment virological and biochemical response in HBeAg-negative patients (including HBeAg-positive patients at baseline with durable anti-HBe seroconversion) is the second, and a maintained undetectable HBV-DNA under long-term antiviral therapy in HBeAg-positive patients without anti-HBe seroconversion and in HBeAg-negative patients is the next most desirable end point.

Since these guidelines<sup>[11,13-17]</sup> were different from each other and were developed for patients whose major disease was chronic HBV infection, it is unclear whether they are optimal for patients with HBV-related HCC. Given the need to reduce HBV replication as much as possible in these patients, particularly before drug resistance emerges, the Chinese Medical Association<sup>[18]</sup> recommends that the threshold of viremia to initiate NA therapy for patients with HBV-related HCC should be lower than the threshold for patients without HCC, and that patients with HBV-related HCC should take NA therapy as long as they show detectable levels of HBV-DNA, regardless of ALT levels. Going even further, some investigators<sup>[19]</sup> have suggested routine prophylactic



**Figure 1** Bubble plot of recurrence-free survival in patients receiving nucleos(t)ide analogue therapy or not after radical resection to treat hepatitis B virus-related hepatocellular carcinoma. Bubble size reflects relative cohort size. <sup>a</sup> $P < 0.05$ : NA group vs Control group. NA: Nucleos(t)ide analogue.

NA therapy for HCC patients with HBV-DNA levels  $< 2000$  IU/mL before liver resection. The aim is to prevent HBV reactivation after liver resection, which occurs in as many as 19% of patients within the first 1 year and which can severely reduce liver function and survival<sup>[19]</sup>.

Since NA therapy cannot completely eradicate HBV, some investigators have advocated lifelong treatment, regardless of undetectable levels of HBV-DNA and HBeAg seroconversion in HBeAg-positive patients or HBsAg loss in HBeAg-negative patients. Those authors argue that long-term therapy may help prevent hepatitis flare-ups and inhibit hepatocarcinogenesis to the greatest extent<sup>[20]</sup>, although there is not sufficient evidence nowadays.

## POSTOPERATIVE NA THERAPY AND RECURRENCE-FREE SURVIVAL

Our extensive online search in the Medline database identified 19 studies published since 2004 that investigated outcomes of postoperative NA therapy in patients with HBV-related HCC. These references comprise 17 retrospective studies<sup>[21-37]</sup> and 2 RCTs<sup>[38,39]</sup>. Most of studies come from Asia, including Chinese mainland, Japan, Hong Kong and Tai Wan, which reflects HBV epidemiology and the high incidence of HBV-related HCC in this region. One study from the United States has a small number of patients appeared first in 2011<sup>[36]</sup> and further follow up published in 2014 with more cases and a longer follow up over 12 years<sup>[37]</sup>. Of the 19 included studies, besides patients who underwent hepatic resection (6705, 96.7%), NA therapy were also applied for patients with ablative procedures as follows: radiofrequency ablation (176, 2.5%), percutaneous ethanol injection (7, 0.1%), and transarterial chemoembolization (49, 0.7%). Patients' characteristics in these studies are shown in Table 1. The outcomes data are shown in the Table 2.

All 19 studies reported data on recurrence-free survival after radical surgery. Several retrospective studies<sup>[21-23,26-29,33,35]</sup> showed that NA treatment did not lead to significantly higher recurrence-free survival than non-NA

treatment, while other retrospective studies<sup>[24,25,30-32,34,36,37]</sup> and the RCTs<sup>[38,39]</sup> showed that NA therapy was associated with significantly higher recurrence-free survival than non-NA treatment.

To synthesize these findings quantitatively, we generated bubble plots of 1-, 3-, and 5-year recurrence-free survival, with bubble size proportional to the size of the study cohort (Figure 1). We also compared median recurrence-free survival between NA and non-NA groups using the Mann-Whitney *U* test. The NA group (1468 patients) showed a median recurrence-free survival of 85.0% (range 19.7%-90.0%) at 1 year, 57.0% (range 11.4%-90.0%) at 3 years, and 54.0% (range 42.6%-81.3%) at 5 years. These median survival rates were significantly higher than the corresponding values in the non-NA group (5541 patients): 78.0% (range 4.5%-86.6%) at 1 year, 56.0% (range 0%-56.0%) at 3 years, and 47.0% (range 0%-47.0%) at 5 years (all  $P < 0.001$ ).

Next we examined whether, based on the available evidence, NA therapy prevents early recurrence, late recurrence, or both. Studies have shown that tumor factors are associated with early HCC recurrence, while high viral loads and hepatic inflammatory activity are associated with late HCC recurrence<sup>[3,4]</sup>. NAs can suppress HBV-DNA replication and promote ALT normalization but cannot affect tumor factors directly, so in theory NAs may prevent late HCC recurrence but have minimal effect on early HCC recurrence. Several retrospective studies and a RCT<sup>[27,33,35,39]</sup> support this idea. However, the other RCT<sup>[38]</sup> in our review found that NA therapy significantly decreased early HCC recurrence, while it did not report outcomes on late HCC recurrence. NA therapy may inhibit early HCC recurrence, which usually arises due to diffusion of the primary tumor, by reducing high HBV load and HBV mutations, all of which are associated with HCC metastasis and growth<sup>[40-42]</sup>, as well as by inhibiting HBxAg, which promotes HCC invasiveness and metastatic potential<sup>[43,44]</sup>. Further studies are urgently needed to clarify whether and how NA therapy affects risk of HCC recurrence, since the results of RCT<sup>[38]</sup> in our review may overestimate the NA efficacy because the control group at baseline had significantly higher rates of cirrhosis, lower rates of tumor encapsulation, and higher rates of HBeAg positivity than the NA group, as well as poorer tumor differentiation and higher AFP levels.

## POSTOPERATIVE NA THERAPY AND OVERALL SURVIVAL

A total of 15 studies reported data on overall survival after radical surgery. Twelve of them, including the RCTs<sup>[22,27,29-34,36-39]</sup>, concluded that NA treatment leads to significantly higher overall survival than non-NA treatment, but 3 studies<sup>[21,23,26]</sup> concluded that NA therapy does not lead to significantly higher overall survival.

The 1-, 3-, and 5-year overall survival rates were summarized using bubble plots (Figure 2), and median rates were compared between NA and non-NA groups using



Table 1 Characteristics of patients with hepatitis B virus-related hepatocellular carcinoma treated with nucleos(t)ide analogues or not after radical resection

Ref.	Country or region	No. of patient <sup>1</sup>	Mean age (yr) <sup>1</sup>	TNM stage (I/II/III/IV) (n)	Multiple tumor (%) <sup>1</sup>	Mean tumor size (cm) <sup>1</sup>	Portal vein invasion (%) <sup>1</sup>	Mean HBV-DNA level (log <sub>10</sub> copies/mL) <sup>1</sup>	Mean ALT (U/L) <sup>1</sup>	Cirrhosis (%) <sup>1</sup>	Initial treatment for HCC, (Ope/RFA/PEI/TACE)	NA therapy	Mean antiviral treatment duration (mo)	Mean follow-up duration (mo) <sup>1</sup>
Piao <i>et al</i> <sup>[21]</sup>	Japan	30 vs 40	59 vs 58	31/25/11/3	N/A	2.3 vs 2.5 <sup>2</sup>	N/A	6.1 vs 6.5 <sup>2</sup>	88 vs 62	N/A	22/16/0/32	LAM	N/A	24
Shuqun <i>et al</i> <sup>[22]</sup>	Chinese mainland	16 vs 17	48.3 vs 48.5	N/A	N/A	≥ 5 cm: 56.2% vs 70.6%	37.5 vs 23.5	N/A	N/A	100 vs 94.1	33/0/0/0	LAM	12	12-36
Kuzuya <i>et al</i> <sup>[23]</sup>	Japan	16 vs 33	59.8 vs 61.1	25/19/5/0	N/A	N/A	N/A	6.2 vs 4.1 <sup>2</sup>	56.6 vs 54.2	N/A	31/18/0/0	LAM	22.7	38.0 vs 32.6
Kubo <i>et al</i> <sup>[24]</sup>	Japan	14 vs 10	55 vs 55	5/9/10/0	N/A	2.4 vs 2.8	28.6 vs 40.0	6.0 vs 6.0	53 vs 56 <sup>2</sup>	42.9 vs 40.0	24/0/0/0	LAM	32	36.7 vs 7.3 <sup>2</sup>
Hung <i>et al</i> <sup>[25]</sup>	Hong Kong	10 vs 62	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	72/0/0/0	LAM	N/A	18.9 <sup>2</sup>
Yoshida <i>et al</i> <sup>[26]</sup>	Japan	33 vs 71	57 vs 59	I + II: 57.6% vs 73.2%	N/A	2.6 vs 2.8	N/A	≥ 3.7: 100% vs 63%	54 vs 36 <sup>2</sup>	N/A	0/104/0/0	LAM	N/A	33 vs 47
Koda <i>et al</i> <sup>[27]</sup>	Japan	30 vs 20	59 vs 60	19/20/11/0	N/A	N/A	N/A	5.7 vs 5.2	78 vs 54	N/A	12/24/5/9	28LAM + 2ETV	28.6	28.6 vs 36.3
Chuma <i>et al</i> <sup>[28]</sup>	Japan	20 vs 30	55.7 vs 55.6	19/27/4/0	25.0 vs 23.3	1.7 vs 2.1	N/A	6.0 vs 5.9 <sup>2</sup>	43.1 vs 37.7	55.0 vs 53.3	10/10/0/0	15LMA + 5ETV	N/A	35.5 vs 49.2 <sup>2</sup>
Li <i>et al</i> <sup>[29]</sup>	Chinese mainland	43 vs 36	46 vs 45	13/27/39/0	N/A	7.1 vs 8.5	30.2 vs 27.8	6.5 vs 7.3	60.8 vs 56.5	55.8 vs 69.4	79/0/0/0	LAM	N/A	12 vs 12
Chan <i>et al</i> <sup>[30]</sup>	Hong Kong	42 vs 94	57 vs 55 <sup>2</sup>	39/32/64/0	N/A	9.3 vs 9.0 <sup>2</sup>	11.9 vs 18.1	N/A	58.0 vs 42.5 <sup>2</sup>	73.8 vs 56.4	136/0/0/0	38LAM + 4ETV	N/A	N/A
Wu <i>et al</i> <sup>[31]</sup>	Tai Wan	518 vs 4051	54.4 vs 54.6	N/A	N/A	N/A	N/A	N/A	N/A	48.6 vs 38.7	4569/0/0/0	159LAM + 292ETV + 361dT + 31Combined	17.4	31.7 vs 26.2
Urata <i>et al</i> <sup>[32]</sup>	Japan	46 vs 13	57 vs 58	N/A	28.3 vs 61.5	2.8 vs 3.4	34.8 vs 46.2	4.7 vs 6.1	46.8 vs 58.0	45.7 vs 30.8	59/0/0/0	22LAM + 24ETV	N/A	36.2 <sup>2</sup>
Ke <i>et al</i> <sup>[33]</sup>	Chinese mainland	141 vs 141	48.9 vs 49.7	N/A	27.7 vs 24.1	4.5 vs 5.0 <sup>2</sup>	7.8 vs 7.1	4.9 vs 4.7	39 vs 42	81.6 vs 81.6	282/0/0/0	LAM	12	24 vs 23
Yin <i>et al</i> <sup>[38]</sup>	Chinese mainland	81 vs 82	47.9 vs 49.3	N/A	12.3 vs 22.0	≥ 3 cm: 86.4% vs 93.9%	3.7 vs 7.3	4.9 vs 4.6	47.3 vs 37.5	24.7 vs 28.0	163/0/0/0	LAM	N/A	39.9 <sup>2</sup>
Su <i>et al</i> <sup>[34]</sup>	Tai Wan	62 vs 271	52 vs 58 <sup>2</sup>	N/A	22.6 vs 46.9	2.7 vs 4.2 <sup>2</sup>	11.3 vs 20.0	5.9 vs 5.5 <sup>2</sup>	45 vs 42 <sup>2</sup>	33.7 vs 45.8	333/0/0/0	40LAM + 19ETV + 3PEG-IFN	N/A	45.9 <sup>2</sup>
Yan <i>et al</i> <sup>[35]</sup>	Chinese mainland	35 vs 25	45 vs 47	22/29/9/0	N/A	4.7 vs 5.0	65.7 vs 68.0	> 5: 54.3% vs 72.0%	41.5 vs 35.8	N/A	60/0/0/0	LAM	N/A	N/A
Hann <i>et al</i> <sup>[37]</sup>	The United States	16 vs 9	57 vs 53 <sup>2</sup>	N/A	0 vs 0	2.7 vs 3.0 <sup>2</sup>	0 vs 0	5.4 vs 6.9 <sup>2</sup>	N/A	N/A	3/4/2/8/ others <sup>3</sup>	8(LAM + TDF) + 3(LAM + ADV) + 2(TLV + TDF) + 2TDF + 1LAM	N/A	60.2
Huang <i>et al</i> <sup>[39]</sup>	Chinese mainland	100 vs 100	50.6 vs 50.5	N/A	17 vs 16	4.9 vs 5.1	0 vs 0	> 3.3: 100% vs 100%	52.6 vs 51.4	N/A	200/0/0/0	ADV	N/A	60 <sup>2</sup>

<sup>1</sup>Patients who received postoperative NA treatment vs patients who received no postoperative NA treatment; <sup>2</sup>Median values; <sup>3</sup>Two patients received resection and RFA for their initial treatment; Three patients received RFA and TACE; One patient received RFA, PEI and TACE; Two patients received cryoablation. Boldfaced data come from randomized controlled trials in our review<sup>[38,39]</sup>. ADV: Adefovir dipivoxil; ETV: Entecavir; LAM: Lamivudine; LdT: Telbivudine; N/A: Not available; Ope: Operation; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation; TACE: Transarterial chemoembolization; NA: Nucleos(t)ide analogue.

**Table 2** Survival outcomes of patients with hepatitis B virus-related hepatocellular carcinoma treated with nucleos(t)ide analogues or not after radical resection

Year of publication	Ref.	Group	n	Overall survival rate (%)				Recurrence-free survival rate (%)			
				1 yr	3 yr	5 yr	P	1 yr	3 yr	5 yr	P
2005	Piao <i>et al</i> <sup>[21]</sup>	NAs	30	100	91.3	N/A	0.12	75	46	N/A	> 0.05
		Control	40	92.4	66	N/A		58	22	N/A	
2006	Shuqun <i>et al</i> <sup>[22]</sup>	NAs	16	24	N/A	N/A	0.0053	19.7	N/A	N/A	> 0.05
		Control	17	0	N/A	N/A		4.5	N/A	N/A	
2007	Kuzuya <i>et al</i> <sup>[23]</sup>	NAs	16	100	100	N/A	0.063	86.5	64.9	N/A	0.622
		Control	33	86.6	46.8	N/A		86.6	46.8	N/A	
2007	Kubo <i>et al</i> <sup>[24]</sup>	NAs	14	N/A	N/A	N/A	N/A	90	90	78	0.0086
		Control	10	N/A	N/A	N/A		55	28	28	
2008	Hung <i>et al</i> <sup>[25]</sup>	NAs	10	N/A	N/A	N/A	N/A	90	N/A	N/A	0.03
		Control	62	N/A	N/A	N/A		75	N/A	N/A	
2008	Yoshida <i>et al</i> <sup>[26]</sup>	NAs	33	100	80	59	> 0.05	N/A	N/A	N/A	> 0.05
		Control	71	100	85	70		N/A	N/A	N/A	
2009	Koda <i>et al</i> <sup>[27]</sup>	NAs	30	96	76	76	0.02	65	15	N/A	> 0.05
		Control	20	86	48	32		72	30	N/A	
2009	Chuma <i>et al</i> <sup>[28]</sup>	NAs	20	N/A	N/A	N/A	N/A	90	55	45	> 0.05
		Control	64	N/A	N/A	N/A		85.9	50	43.7	
2010	Li <i>et al</i> <sup>[29]</sup>	NAs	43	41.9	N/A	N/A	0.0094	23.3	N/A	N/A	0.072
		Control	36	33.3	N/A	N/A		8.3	N/A	N/A	
2011	Chan <i>et al</i> <sup>[30]</sup>	NAs	42	88.1	79.1	71.2	0.005	66.5	51.4	51.4	0.05
		Control	94	76.5	47.5	43.5		48.9	33.8	33.8	
2012	Wu <i>et al</i> <sup>[31]</sup>	NAs	518	94	81	73	0.002	87	66	54	< 0.001
		Control	4051	91	74	62		78	56	47	
2012	Urata <i>et al</i> <sup>[32]</sup>	NAs	46	100	97.1	89.7	0.0025	71.6	56.8	42.6	0.0478
		Control	13	84.6	68.4	59.8		61.5	19.2	19.2	
2013	Ke <i>et al</i> <sup>[33]</sup>	NAs	141	92.1	84.4	79.1	0.009	73.1	54.7	44.5	0.503
		Control	141	89.6	66.3	52.1		68.8	47.8	43	
2013	Yin <i>et al</i> <sup>[38]</sup>	NAs	81	98	88	N/A	< 0.001	81	46	N/A	< 0.001
		Control	82	86	51	N/A		50	20	N/A	
		NAs	215	84	60	N/A	0.04	52	37.5	N/A	< 0.001
		Control	402	75	50	N/A		43	21	N/A	
2013	Su <i>et al</i> <sup>[34]</sup>	NAs	62	99	96	89	< 0.001	90	64	58	< 0.001
		Control	271	84	64	49		64	44	34	
2013	Yan <i>et al</i> <sup>[35]</sup>	NAs	35	N/A	N/A	N/A	N/A	74.3	11.4	N/A	0.283
		Control	25	N/A	N/A	N/A		80	0	N/A	
2014	Hann <i>et al</i> <sup>[37]</sup>	NAs	16	100	93.8	86.5	< 0.001	81.3	81.3	81.3	< 0.001
		Control	9	55.6	0	0		11.1	0	0	
2014	Huang <i>et al</i> <sup>[39]</sup>	NAs	100	96	77.6	63.1	0.001	85	50.3	46.1	0.026
		Control	100	94	67.4	41.5		84	37.9	27.1	

Boldfaced data come from randomized controlled trials in our review<sup>[38,39]</sup>. N/A: Not applicable; NA: Nucleos(t)ide analogue.

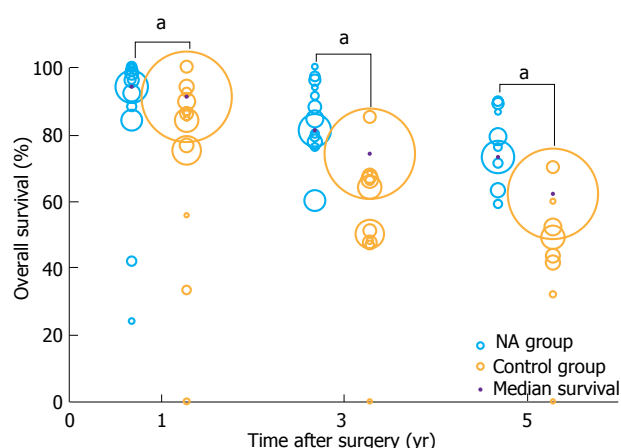
**Table 3** Mutations of the hepatitis B virus polymerase gene arising after initial therapy with one nucleos(t)ide analogue and resulting in cross-resistance to other nucleos(t)ide analogues

Initial NA therapy	Mutational sites after initial NA therapy	Cross-resistance data				
		LAM	LdT	ETV	ADV	TDF
	Wild-type	S	S	S	S	S
LAM or LdT	M204I/V	R	R	I	S	S
ADV	N236T	S	S	S	R	I
LAM or LdT or ADV	A181T/V	R	R	S	R	I
ADV or TDF	A181T/V + N236T <sup>1</sup>	R	R	S	R	R
ETV	L181M + M204V/I ± I169 ± T184 ± S202 ± M250V <sup>2</sup>	R	R	R	S	S

<sup>1</sup>Resistance to ADV or TDF is associated with the substitution A181T/V and/or N235T in HBV polymerase gene; <sup>2</sup>Resistance to ETV is associated with substitutions at I169, T184, S202 or M250V, and with the simultaneous substitutions at L181M plus M204V/I in HBV polymerase gene. Data come from ref. <sup>[11]</sup>. ADV: Adefovir dipivoxil; ETV: Entecavir; I: Intermediate; LAM: Lamivudine; LdT: Telbivudine; NA: Nucleos(t)ide analogue; R: Resistant; S: Sensitive; TDF: Tenofovir disoproxil fumarate.

the Mann-Whitney *U* test. Median survival in the NA group (1468 patients) was 94.0% (range 24.0%-100.0%) at 1 year, 81.0% (range 60.0%-100.0%) at 3 years, and 73.0% (range 59.0%-89.7%) at 5 years. These values were

significantly higher than the corresponding ones for the non-NA group (5200 patients): 91.0% (range 0-100.0%) at 1 year, 74.0% (range 0-85.0%) at 3 years, and 62.0% (range 0%-70.0%) at 5 years (all *P* < 0.001).



**Figure 2** Bubble plot of overall survival in patients receiving nucleos(t)ide analogue therapy or not after radical resection to treat hepatitis B virus-related hepatocellular carcinoma. Bubble size reflects relative cohort size. <sup>a</sup> $P < 0.05$ : NA group vs Control group. NA: Nucleos(t)ide analogue.

Investigators have attributed this survival benefit to 3 factors. First, NA therapy can efficiently suppress HBV replication and reactivation, ease liver inflammation and fibrosis, impede progression of liver disease, and prevent liver failure<sup>[21-23,27,29,33,38,45]</sup>. Second, liver function improvement after NA therapy increases the possibility of curative re-treatment and allows surgeons to remove a larger liver region after recurrence, which means lower risk of residual tumors<sup>[23,29,33,45]</sup>. Third, NA therapy can reduce recurrence, helping to increase overall survival<sup>[24,25,30-32,34,36-38]</sup>.

To define more precisely which patients with HBV-related HCC may benefit from NA therapy, we retrospectively studied its efficacy in patients with HCC in different stages of the Barcelona Clinic Liver Cancer (BCLC) system<sup>[33]</sup>. We found that NA therapy provided significant survival benefit to patients with BCLC stage A or B disease, but not to patients with BCLC-C disease. These results are similar to those reported in 2 larger retrospective studies<sup>[30,34]</sup>. This may reflect the poor prognosis of BCLC-C patients, whose short survival provides insufficient time for NA therapy to be effective.

## MANAGEMENT OF NA RESISTANCE IN HBV-RELATED HCC PATIENTS

One of the major problems associated with long-term NA therapy is the emergence of NA-resistant HBV strains<sup>[21,23,27]</sup>. Such resistance increases not only the risk of breakthrough hepatitis and liver failure, but also the difficulty and cost of subsequent treatment. LAM has the worst antiviral resistance profile among NAs, and LAM resistance is caused by mutations of the YMDD region in the active site of the HBV polymerase/reverse transcriptase gene<sup>[11]</sup>. One study<sup>[27]</sup> reported YMDD mutations in 11 of 28 patients after  $28.6 \pm 16.7$  mo of LAM administration. Of those 11 patients, 6 exhibited breakthrough hepatitis; fortunately none of them experienced fatal liver failure because they were immediately given ADV or ETV.

To prevent NA resistance and manage its clinical ef-

fects in patients with HBV-related HCC, clinicians should obtain a thorough medical history for NA candidates. Patients who previously received NA therapy and developed resistance should receive potent NA not associated with cross-resistance (Table 3) in order to reduce the risk of eliciting multiple drug-resistant viral strains<sup>[12]</sup>. For patients who have never received any NA therapy, potent drugs with high resistance barriers, such as ETV and TDV, may be the best choice<sup>[12]</sup>. Clinicians should also not rush to incorrect conclusions about NA resistance, since about 40% of cases of HBV-related breakthrough hepatitis occur simply because of poor patient adherence to NA therapy rather than NA resistance<sup>[46]</sup>. On the other hand, drug resistance should be considered if regular follow-up tests of HBV-DNA levels and liver function every 2-3 mo give abnormal results and other possible causes can be excluded. In such cases, an appropriate rescue therapy using potent NAs without cross-resistance should be given as soon as genotypic drug resistance is confirmed<sup>[11]</sup>.

## CONCLUSION

Given the serious clinical consequences of uncontrolled HBV replication, patients with HBV-related HCC should consider taking NA if they are positive for HBV-DNA. Because NA therapy cannot completely eradicate HBV, patients should prepare for the possibility that they may require lifelong treatment. With the currently advanced techniques of the loco-regional ablations such as radio-frequency ablation, microwave ablation and others, NA therapy also applies for HCC patients who underwent such procedures in addition to surgical resection, and a significant body of evidence suggests that postoperative NA therapy in patients with HBV-related HCC improves both recurrence-free survival and overall survival.

Every coin has two sides. Emergence of NA-resistant HBV strains is a significant concern, highlighting the importance of regular monitoring of HBV-DNA levels and liver function during NA therapy. The most potent NAs with high resistance barriers, such as EVT and TDF, may be the best choice for NA-naïve patients. In case of drug resistance, rescue therapy should be carried out using potent NAs not associated with cross-resistance.

## REFERENCES

- 1 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140-6736(11)61347-0]
- 2 **Zhong JH**, Li H, Li LQ, You XM, Zhang Y, Zhao YN, Liu JY, Xiang BD, Wu GB. Adjuvant therapy options following curative treatment of hepatocellular carcinoma: a systematic review of randomized trials. *Eur J Surg Oncol* 2012; **38**: 286-295 [PMID: 22281155 DOI: 10.1016/j.ejso.2012.01.006]
- 3 **Du ZG**, Wei YG, Chen KF, Li B. Risk factors associated with early and late recurrence after curative resection of hepatocellular carcinoma: a single institution's experience with 398 consecutive patients. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 153-161 [PMID: 24686542]
- 4 **Wu JC**, Huang YH, Chau GY, Su CW, Lai CR, Lee PC, Huo

- TI, Sheen IJ, Lee SD, Lui WY. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. *J Hepatol* 2009; **51**: 890-897 [PMID: 19747749 DOI: 10.1016/j.jhep.2009.07.009]
- 5 **Zhong JH**, Ma L, Li LQ. Postoperative therapy options for hepatocellular carcinoma. *Scand J Gastroenterol* 2014; **49**: 649-661 [PMID: 24716523 DOI: 10.3109/00365521.2014.905626]
- 6 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531 [PMID: 15470215 DOI: 10.1056/NEJMoa033364]
- 7 **Matsumoto A**, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, Okita K, Hayashi N, Okanoue T, Iino S, Tanikawa K. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; **32**: 173-184 [PMID: 16024289 DOI: 10.1016/j.hepres.2005.02.006]
- 8 **Hosaka T**, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013; **58**: 98-107 [PMID: 23213040 DOI: 10.1002/hep.26180]
- 9 **Wong GL**, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Lai JW, Lo AO, Chan HY, Wong VW. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. *Hepatology* 2013; **58**: 1537-1547 [PMID: 23389810 DOI: 10.1002/hep.26301]
- 10 **Zhong JH**, Li le Q, Wu LC. Lamivudine with or without adefovir dipivoxil for postoperative hepatocellular carcinoma. *Cochrane Database Syst Rev* 2011; **(12)**: CD008713 [PMID: 22161435 DOI: 10.1002/14651858.CD008713.pub2]
- 11 **Liaw YF**, Kao JH, Piratvisuth T, Chan HLY, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarapurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012; **6**: 531-561 [DOI: 10.1007/s12072-012-9365-4]
- 12 **Santantonio TA**, Fasano M. Chronic hepatitis B: Advances in treatment. *World J Hepatol* 2014; **6**: 284-292 [PMID: 24868322 DOI: 10.4254/wjh.v6.i5.284]
- 13 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases**, Chinese Medical Association. The guideline of prevention and treatment for chronic hepatitis B (2010 version). *Zhonghua Ganzhangbing Zazhi* 2011; **19**: 13-24 [PMID: 21272453 DOI: 10.3760/cma.j.issn.1007-3418.2011.01.007]
- 14 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
- 15 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
- 16 **Keeffe EB**, Dieterich DT, Han SH, Jacobson IM, Martin P, Schiff ER, Tobias H. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: 2008 update. *Clin Gastroenterol Hepatol* 2008; **6**: 1315-1341; quiz 1286 [PMID: 18845489 DOI: 10.1016/j.cgh.2008.08.021]
- 17 **Tong MJ**, Pan CQ, Hann HW, Kowdley KV, Han SH, Min AD, Leduc TS. The management of chronic hepatitis B in Asian Americans. *Dig Dis Sci* 2011; **56**: 3143-3162 [PMID: 21935699 DOI: 10.1007/s10620-011-1841-5]
- 18 Recommendation on antiviral therapy to hepatitis B/C virus related hepatocellular carcinoma. *Zhonghua Ganzhangbing Zazhi* 2013; **21**: 96-100 [PMID: 24000462]
- 19 **Huang G**, Lai EC, Lau WY, Zhou WP, Shen F, Pan ZY, Fu SY, Wu MC. Posthepatectomy HBV reactivation in hepatitis B-related hepatocellular carcinoma influences postoperative survival in patients with preoperative low HBV-DNA levels. *Ann Surg* 2013; **257**: 490-505 [PMID: 22868358 DOI: 10.1097/SLA.0b013e318262b218]
- 20 **Wei Q**, Xu X, Ling Q, Zheng S. Indefinite antiviral therapy may be required after surgical resection for hepatocellular carcinoma complicating chronic hepatitis B. *J Res Med Sci* 2013; **18**: 726-730 [PMID: 24379852]
- 21 **Piao CY**, Fujioka S, Iwasaki Y, Fujio K, Kaneyoshi T, Araki Y, Hashimoto K, Senoh T, Terada R, Nishida T, Kobashi H, Sakaguchi K, Shiratori Y. Lamivudine treatment in patients with HBV-related hepatocellular carcinoma—using an untreated, matched control cohort. *Acta Med Okayama* 2005; **59**: 217-224 [PMID: 16286955]
- 22 **Shuqun C**, Mengchao W, Han C, Feng S, Jiahe Y, Wenming C, Zhengfeng Y, Yuxiang Z, Peijun W. Antiviral therapy using lamivudine and thymosin alpha1 for hepatocellular carcinoma coexisting with chronic hepatitis B infection. *Hepato-gastroenterology* 2006; **53**: 249-252 [PMID: 16608033]
- 23 **Kuzuya T**, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, Itoh A, Ishigami M, Hayashi K, Honda T, Goto H. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1929-1935 [PMID: 17914972 DOI: 10.1111/j.1440-1746.2006.04707.x]
- 24 **Kubo S**, Tanaka H, Takemura S, Yamamoto S, Hai S, Ichikawa T, Kodai S, Shinkawa H, Sakaguchi H, Tamori A, Habu D, Nishiguchi S. Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus. *Hepatology* 2007; **37**: 94-100 [PMID: 17300703 DOI: 10.1111/j.1872-034X.2007.00013.x]
- 25 **Hung IF**, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at the time of resection. *Am J Gastroenterol* 2008; **103**: 1663-1673 [PMID: 18616655 DOI: 10.1111/j.1572-0241.2008.01872.x]
- 26 **Yoshida H**, Yoshida H, Goto E, Sato T, Ohki T, Masuzaki R, Tateishi R, Goto T, Shiina S, Kawabe T, Omata M. Safety and efficacy of lamivudine after radiofrequency ablation in patients with hepatitis B virus-related hepatocellular carcinoma. *Hepatol Int* 2008; **2**: 89-94 [PMID: 19669283 DOI: 10.1007/s12072-007-9020-7]
- 27 **Koda M**, Nagahara T, Matono T, Sugihara T, Mandai M, Ueki M, Ohyama K, Hosho K, Okano J, Kishimoto Y, Kono M, Maruyama S, Murawaki Y. Nucleotide analogs for patients with HBV-related hepatocellular carcinoma increase the survival rate through improved liver function. *Intern Med* 2009; **48**: 11-17 [PMID: 19122351]
- 28 **Chuma M**, Hige S, Kamiyama T, Meguro T, Nagasaka A, Nakanishi K, Yamamoto Y, Nakanishi M, Kohara T, Shio T, Yamamoto K, Horimoto H, Kobayashi T, Yokoo H, Matsushita M, Todo S, Asaka M. The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma. *J Gastroenterol* 2009; **44**: 991-999 [PMID: 19554391 DOI: 10.1007/s00535-009-0093-z]
- 29 **Li N**, Lai EC, Shi J, Guo WX, Xue J, Huang B, Lau WY, Wu MC, Cheng SQ. A comparative study of antiviral therapy after resection of hepatocellular carcinoma in the immune-active phase of hepatitis B virus infection. *Ann Surg Oncol* 2010; **17**: 179-185 [PMID: 19727956 DOI: 10.1245/s10434-009-0694-z]
- 30 **Chan AC**, Chok KS, Yuen WK, Chan SC, Poon RT, Lo CM, Fan ST. Impact of antiviral therapy on the survival of patients after major hepatectomy for hepatitis B virus-related hepatocellular carcinoma. *Arch Surg* 2011; **146**: 675-681 [PMID: 21690443 DOI: 10.1001/archsurg.2011.125]
- 31 **Wu CY**, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, Lin JT. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. *JAMA* 2012; **308**: 1906-1914 [PMID: 22436845 DOI: 10.1007/s12072-012-9365-4]



- 23162861]
- 32 **Urata Y**, Kubo S, Takemura S, Uenishi T, Kodai S, Shinkawa H, Sakae M, Kaneda K, Ohata K, Nozawa A, Suehiro S. Effects of antiviral therapy on long-term outcome after liver resection for hepatitis B virus-related hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2012; **19**: 685-696 [PMID: 22203455 DOI: 10.1007/s00534-011-0489-z]
  - 33 **Ke Y**, Ma L, You XM, Huang SX, Liang YR, Xiang BD, Li LQ, Zhong JH. Antiviral therapy for hepatitis B virus-related hepatocellular carcinoma after radical hepatectomy. *Cancer Biol Med* 2013; **10**: 158-164 [PMID: 24379991 DOI: 10.7497/j.issn.2095-3941.2013.03.006]
  - 34 **Su CW**, Chiou YW, Tsai YH, Teng RD, Chau GY, Lei HJ, Hung HH, Huo TI, Wu JC. The Influence of Hepatitis B Viral Load and Pre-S Deletion Mutations on Post-Operative Recurrence of Hepatocellular Carcinoma and the Tertiary Preventive Effects by Anti-Viral Therapy. *PLoS One* 2013; **8**: e66457 [PMID: 23805222 DOI: 10.1371/journal.pone.0066457]
  - 35 **Yan Q**, Ni J, Zhang GL, Yao X, Yuan WB, Zhou L, Zheng SS. Efficacy of postoperative antiviral combined transcatheter arterial chemoembolization therapy in prevention of hepatitis B-related hepatocellular carcinoma recurrence. *Chin Med J (Engl)* 2013; **126**: 855-859 [PMID: 23489790]
  - 36 **Hann HW**, Bergin D, Coben R, DiMarino AJ. Prevention of new hepatocellular carcinoma with concomitant antiviral therapy in chronic hepatitis B patients whose initial tumor was successfully ablated. *Int J Cancer* 2011; **128**: 739-742 [PMID: 20473872 DOI: 10.1002/ijc.25382]
  - 37 **Hann HW**, Coben R, Brown D, Needleman L, Rosato E, Min A, Hann RS, Park KB, Dunn S, DiMarino AJ. A long-term study of the effects of antiviral therapy on survival of patients with HBV-associated hepatocellular carcinoma (HCC) following local tumor ablation. *Cancer Med* 2014; **3**: 390-396 [PMID: 24519810 DOI: 10.1002/cam4.197]
  - 38 **Yin J**, Li N, Han Y, Xue J, Deng Y, Shi J, Guo W, Zhang H, Wang H, Cheng S, Cao G. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. *J Clin Oncol* 2013; **31**: 3647-3655 [PMID: 24002499 DOI: 10.1200/JCO.2012.48.5896]
  - 39 **Huang G**, Lau WY, Wang ZG, Pan ZY, Yuan SX, Shen F, Zhou WP, Wu MC. Antiviral Therapy Improves Post-operative Survival in Patients With Hepatocellular Carcinoma: A Randomized Controlled Trial. *Ann Surg* 2014 Jul 28; Epub ahead of print [PMID: 25072444 DOI: 10.1097/SLA.0000000000000858]
  - 40 **Huang Y**, Wang Z, An S, Zhou B, Zhou Y, Chan HL, Hou J. Role of hepatitis B virus genotypes and quantitative HBV DNA in metastasis and recurrence of hepatocellular carcinoma. *J Med Virol* 2008; **80**: 591-597 [PMID: 18297705 DOI: 10.1002/jmv.21117]
  - 41 **Huang Y**, Tong S, Tai AW, Hussain M, Lok AS. Hepatitis B virus core promoter mutations contribute to hepatocarcinogenesis by deregulating SKP2 and its target, p21. *Gastroenterology* 2011; **141**: 1412-1421, 1412-1421 [PMID: 21704589 DOI: 10.1053/j.gastro.2011.06.048]
  - 42 **Mun HS**, Lee SA, Kim H, Hwang ES, Kook YH, Kim BJ. Novel F141L pre-S2 mutation in hepatitis B virus increases the risk of hepatocellular carcinoma in patients with chronic genotype C infections. *J Virol* 2011; **85**: 123-132 [PMID: 20962085 DOI: 10.1128/JVI.01524-10]
  - 43 **Ou DP**, Tao YM, Tang FQ, Yang LY. The hepatitis B virus X protein promotes hepatocellular carcinoma metastasis by upregulation of matrix metalloproteinases. *Int J Cancer* 2007; **120**: 1208-1214 [PMID: 17187364 DOI: 10.1002/ijc.22452]
  - 44 **Liu H**, Xu L, He H, Zhu Y, Liu J, Wang S, Chen L, Wu Q, Xu J, Gu J. Hepatitis B virus X protein promotes hepatoma cell invasion and metastasis by stabilizing Snail protein. *Cancer Sci* 2012; **103**: 2072-2081 [PMID: 22957763 DOI: 10.1111/cas.12017]
  - 45 **Kim JH**, Park JW, Koh DW, Lee WJ, Kim CM. Efficacy of lamivudine on hepatitis B viral status and liver function in patients with hepatitis B virus-related hepatocellular carcinoma. *Liver Int* 2009; **29**: 203-207 [PMID: 18662281 DOI: 10.1111/j.1478-3231.2008.01828.x]
  - 46 **Hongthanakorn C**, Chotiayaputta W, Oberhelman K, Fontana RJ, Marrero JA, Licari T, Lok AS. Virological breakthrough and resistance in patients with chronic hepatitis B receiving nucleos(t)ide analogues in clinical practice. *Hepatology* 2011; **53**: 1854-1863 [PMID: 21618260 DOI: 10.1002/hep.24318]

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## Telaprevir- and boceprevir-based tritherapies in real practice for F3-F4 pretreated hepatitis C virus patients

Delphine Bonnet, Matthieu Guivarch, Emilie Bérard, Jean-Marc Combis, Andre Jean Remy, Andre Glibert, Jean-Louis Payen, Sophie Metivier, Karl Barange, Herve Desmorat, Anaïs Palacin, Florence Nicot, Florence Abravanel, Laurent Alric

Delphine Bonnet, Matthieu Guivarch, Anaïs Palacin, Laurent Alric, Internal Medicine-Digestive Department, Purpan University Hospital, 31059 Toulouse cedex 9, France

Emilie Bérard, Department of Epidemiology, Health Economics and Public Health, Toulouse University Hospital, 31073 Toulouse, France

Emilie Bérard, UMR-1027 INSERM-Toulouse III University, 31062 Toulouse, France

Jean-Marc Combis, Clinique Ambroise Pare, 31300 Toulouse, France

Andre Jean Remy, Perpignan General Hospital, 66000 Perpignan, France

Andre Glibert, Tarbes General Hospital, 65000 Tarbes, France

Jean-Louis Payen, Montauban General Hospital, 82000 Montauban, France

Sophie Metivier, Karl Barange, Hepatogastroenterology-Digestive Department, Purpan University Hospital, 31059 Toulouse cedex 9, France

Herve Desmorat, Clinique du Parc, 31078 Toulouse Cedex 4, France

Florence Nicot, Florence Abravanel, Virology Unit INSERM U1043, 31059 Toulouse cedex 9, France

Laurent Alric, UMR152 IRD Toulouse III University, 31400 Toulouse, France

**Author contributions:** Bonnet D and Guivarch M equally contributed to the work; Bonnet D contributed to planning and conducting the study, collecting and interpreting the data, drafting the manuscript; Guivarch M contributed to planning and conducting the study, collecting and interpreting the data, drafting the manuscript; Bérard E contributed to biostatistical analysis and interpreting the data, drafting the manuscript; Combis JM, Remy AJ, Glibert A, Payen JL, Desmorat H, Metivier S and Barange K contributed to treatment of patients; Palacin A contributed to collecting data; Nicot F contributed to viral monitoring; Abravanel F contributed to viral monitoring; Alric L contributed to treatment of patients, planning and conducting the study, interpreting the data, drafting the manuscript.

**Correspondence to:** Laurent Alric, Professor, Internal Medicine, Digestive Department, Pavillon Dieulafoy, CHU Purpan, TSA 40031, Toulouse 31059 cedex, France. [alric.l@chu-purpan-toulouse.fr](mailto:alric.l@chu-purpan-toulouse.fr)

Telephone: +33-5-61779551 Fax: +33-5-61772230

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### Abstract

**AIM:** To assess, in a routine practice setting, the sustained virologic response (SVR) to telaprevir (TPV) or boceprevir (BOC) in hepatitis C virus (HCV) null-responders or relapsers with severe liver fibrosis.

**METHODS:** One hundred twenty-five patients were treated prospectively for 48 wk with TPV or BOC + pegylated-interferon (peg-INF)  $\alpha 2a$  + ribavirin (PR) according to standard treatment schedules without randomization. These patients were treated in routine practice settings in 10 public or private health care centers, and the data were prospectively collected. Only patients with severe liver fibrosis (Metavir scores of F3 or F4 upon liver biopsy or liver stiffness assessed by elastography), genotype 1 HCV and who were null-responders or relapsers to prior PR combination therapy were included in this study.

**RESULTS:** The Metavir fibrosis scores were F3 in 35 (28%) and F4 in 90 (72%) of the patients. In total, 62.9% of the patients were null-responders and 37.1% relapsers to the previous PR therapy. The overall SVR rate at 24 wk post-treatment withdrawal was 59.8%. The SVR was 65.9% in the TPV group and 44.1% in the BOC group. Independent predictive factors of an SVR included a response to previous treatment, relapsers *vs* null-responders [OR = 3.9; (1.4, 10.6),  $P = 0.0084$ ], a rapid virological response (RVR) [OR 6.9 (2.6, 18.2),  $P = 0.001$ ] and liver stiffness lower than 21.3 kPa [OR = 8.2 (2.3, 29.5),  $P = 0.001$ ]. During treatment, 63 patients (50.8%) had at least one severe adverse event

(SAE) of grade 3 or 4. A multivariate analysis identified two factors associated with SAEs: female gender [OR = 2.4 (1.1, 5.6),  $P = 0.037$ ] and a platelet count below  $150 \times 10^3/\text{mm}^3$  [OR = 5.3 (2.3, 12.4),  $P \leq 0.001$ ].

**CONCLUSION:** More than half of these difficult-to-treat patients achieved an SVR and had SAEs in an actual practice setting. The SVR rate was influenced by the response to previous PR treatment, the RVR and liver stiffness.

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**Key words:** Hepatitis C virus; Hepatitis C; Antiviral therapy; Protease inhibitors; Fibroscan; Liver stiffness; Cirrhosis; Boceprevir; Telaprevir; Ribavirin

**Core tip:** To the best of our knowledge, this study marks the first time that a significant link has been shown between a sustained virological response to triple therapy and the liver stiffness measured by elastography at baseline. We also demonstrate that triple therapy is poorly tolerated. Two factors predict the development of serious adverse events: female gender and an initial platelet count of less than  $150000/\text{mm}^3$ ; these factors facilitate the identification of at-risk patients.

Bonnet D, Guivarch M, Bérard E, Combis JM, Remy AJ, Glibert A, Payen JL, Metivier S, Barange K, Desmorat H, Palacin A, Nicot F, Abravanel F, Alric L. Telaprevir- and boceprevir-based tritherapies in real practice for F3-F4 pretreated hepatitis C virus patients. *World J Hepatol* 2014; 6(9): 660-669 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v6/i9/660.htm> DOI: <http://dx.doi.org/10.4254/wjh.v6.i9.660>

## INTRODUCTION

Approximately 80% of patients infected with the hepatitis C virus (HCV) develop chronic infections that could lead to cirrhosis and hepatocellular carcinoma<sup>[1]</sup>. Combination therapy with pegylated-interferon (peg-IFN)  $\alpha 2a$  + ribavirin (PR) was the first demonstrably effective treatment<sup>[2,3]</sup>. The current combination of PR with protease inhibitors (PIs) such as telaprevir (TPV) or boceprevir (BOC) is clearly more beneficial for HCV genotype 1 patients<sup>[4-7]</sup>. The majority of HCV genotype 1 patients demonstrate a sustained virological response (SVR) to TPV (69% to 75%) and BOC triple therapy (68% to 75%)<sup>[4-7]</sup>. However, only 65% of genotype 1 HCV patients who were previously unresponsive to PR therapy produced an SVR to TPV triple therapy; only 66% of these unresponsive patients produced an SVR to BOC triple therapy<sup>[8-10]</sup>. Some predictive factors, such as high baseline viral load, HCV genotype 1a, IL-28B T/T polymorphism and severe liver fibrosis, have been associated with a poor response to antiviral treatment with PI<sup>[6,10]</sup>. Moreover, the data on the benefit of retreating

HCV genotype 1 cirrhotic patients who did not respond to a standard PR regimen with triple therapy are inconclusive<sup>[8-10]</sup>. A study on small subgroups of null-responder patients with severe liver fibrosis given TPV triple therapy determined that 39% of Metavir F3 patients and only 14% of patients with Metavir F4 produced an SVR<sup>[8]</sup>. Therefore, guidelines and new treatment strategies are required that consider the cost and adverse effects of TPV and BOC combined with PR for these difficult-to-treat patients. In these pivotal studies performed exclusively in academic centers, many patients experienced adverse effects despite restricting inclusion criteria and strict observance of treatment rules<sup>[5-10]</sup>. In addition, because very few non-responder patients with severe liver fibrosis were included in these studies, the occurrence of severe adverse effects (SAEs) in this specific population remains unclear. Therefore, we need to evaluate (in actual practice settings) the efficacy and safety profile of PI triple therapy in pretreated HCV genotype 1 patients with severe liver fibrosis.

This study assesses the SVR and safety profiles of triple therapy with TPV or BOC combined with PR in HCV genotype 1 patients with severe liver fibrosis (Metavir F3 or F4) who had previously failed to adequately respond to the standard PR treatment. This observational non-randomized prospective cohort study was performed in actual practice settings.

## MATERIALS AND METHODS

### Study design and patients

This study used a non-randomized multicenter prospective observational cohort from a Midi-Pyrénées network (HEPATOMIP) of hepatogastroenterology practitioners working in the Toulouse University hospital and in 9 general hospitals or private clinics. The cohort included 125 consecutive HCV genotype 1 null-responder or relapsers patients with severe liver fibrosis who were seen between February 2011 and January 2012. Only those patients with severe liver fibrosis having a Metavir fibrosis score of F3 or F4 were included.

All of the patients were infected with HCV genotype 1 and did not achieve an SVR with previous standard treatments with peg-IFN  $\alpha 2a$  or  $2b$  + ribavirin, described as follows<sup>[11]</sup>: (1) relapsers were defined as patients who achieved undetectable HCV RNA levels at the end of 48 wk of PR treatment and then subsequently relapsed; and (2) null-responders failed to achieve a decrease of at least 2 log HCV RNA IU/mL during PR treatment given for at least 24 wk.

Partial responders to previous therapy were not included in the study. After an interval of at least 6 mo, patients were given either 12 wk of TPV (750 mg every 8 h, Janssen-Cilag, Issy les Moulineaux, France) combined with PR (Roche, Meylan, France) followed by 36 wk of PR or 4 wk (lead-in phase) of PR followed by 44 wk of PR and BOC (800 mg every 8 h (MSD, Courbevoie, France) according to French label guidelines<sup>[12]</sup> (French National Agency of drugs and health products security,

ANSM cohort temporary use authorization n° 324 and n° 330). In this observational cohort, TPV or BOC triple therapy was selected by each physician; however, all of the patients received the same schedule of PR, as follows: peg-IFN  $\alpha$ 2a (180 g/wk) + ribavirin (1000 to 1200 mg/d, depending on body weight). The Toulouse University review board approved this cohort, and all of the patients provided written informed consent.

A quantification of the HCV RNA level was performed at baseline, then every 4 wk during triple therapy and at 12 and 24 wk following treatment withdrawal using real-time polymerase chain reaction (COBAS AmpliCor/TaqMan, Roche Diagnostics, Basel, Switzerland) with a lower detection limit of 15 IU/mL. Fibrosis was evaluated by a liver biopsy or by measuring the liver stiffness (LS) according to the manufacturer's instructions (Fibroscan, Echosens). The results were expressed in kilopascals (kPa). Metavir F3 was defined by a liver stiffness of 9.5–12.4 kPa and Metavir F4 cirrhotic patients were defined by values of up to 12.5 kPa.

### Efficacy

The response to triple therapy could be summarized as: (1) A rapid virological response (RVR), *i.e.*, negative for HCV RNA after 4 weeks of triple therapy (defined as week 4 for the TPV group and week 8 for the BOC group); (2) A virological response (VR), *i.e.*, negative for HCV RNA at the end of triple therapy; and (3) or a sustained virological response (SVR), *i.e.*, negative for HCV RNA 24 wk after the end of treatment.

### Safety and adverse events

All of the patients were seen by their physicians at baseline, every 2 wk during the first 2 mo, every 4 wk during the following phase of therapy and then every 4, 12, and 24 wk after treatment withdrawal. Adverse events were graded by investigators according to a modified World Health Organization grading system. Non-life-threatening adverse events and hematological disorders were managed according to the French association of the study of the liver (AFEF) by reducing the ribavirin dose and/or giving erythropoietin (EPO) at the discretion of the physician<sup>[11]</sup>. EPO was recommended when the patient's hemoglobin (Hb) level dropped to less than 10 g/dL, despite a previous reduction in the ribavirin dose by 200 mg/d.

### Statistical analysis

Statistical analyses were performed using STATA software, release 11.2 (STATA Corporation, College Station, TX, United States). Numbers and frequencies were used for the described qualitative data, and means  $\pm$  SD or medians (inter-quartile range: IQR) were used when the normality assumption was not met for quantitative data. The qualitative variables were compared between groups (TPV and BOC groups; SVR and no-SVR groups; SAEs and no-SAEs groups) using the  $\chi^2$  test (or Fisher's exact test for small expected numbers). Student's *t* test was used to compare the distribution of quantitative data.

Alternatively, the Mann-Whitney test was used when the distribution was not normal or when homoscedasticity was rejected. We assessed the accuracy of liver stiffness to predict an SVR according to receiver operating characteristic (ROC) curves (plotting sensitivity *vs* 1-specificity at various cut-off settings), and we defined the optimal liver stiffness cut-off value of 21.3 kPa according to the best rate of correctly classified subjects  $\{[(\text{true positives} + \text{true negatives})/\text{total}]; 69.2\%\}$ . Odds ratios (ORs) for SVR or SAE and 95% CIs were assessed using a logistic regression model. The variables initially included in the model were those associated with SVR or SAE in the univariate analysis (*P* value < 0.20). A backward procedure was applied to assess variables that were significantly and independently associated with SVR (or SAE) (*P* value < 0.05). Because the linearity hypothesis was not fully respected, the following continuous variables were transformed into ordered data: liver stiffness (< 21.3 kPa *vs*  $\geq$  21.3 kPa) for the SVR model, platelet count (<  $150 \times 10^3/\text{mm}^3$  *vs*  $\geq 150 \times 10^3/\text{mm}^3$ ) for the SAE model. Interactions between independent covariates were tested in the final regression models, and none of these interactions was significant. All of the reported *P* values are two-sided, and the significance threshold was set at < 0.05.

## RESULTS

### Characteristics of patients at baseline

This prospective cohort included 125 HCV genotype 1 patients (Table 1). None of the patients had responded to previous treatment with standard PR combination therapy. There were 46/124 (37.1%) previous relapsers and 78/124 (62.49%) null-responders, and there were more men (64.8%) than women (35.2%). HCV subtype 1b (56.8%) infections were more frequent than HCV subtype 1a (31.2%) infections, although the HCV genotype was not defined as 1b or 1a in 12% of cases. As expected in this population of relapsers and null-responders to prior antiviral therapy, only 15.4% of the patients had an IL-28B genotype C/C. All of the patients had severe liver fibrosis: 28% were Metavir F3 and 72% were cirrhotic, with a Metavir F4 score. All except 2 of the cirrhotic patients were classified as Child-Pugh class A. Triple therapy was not randomized; TPV or BOC was selected by the patient's physician, with 72% of the patients treated with TPV and 28% treated with BOC. We observed no difference in the subsequent parameters for the two groups.

### Virologic response to triple therapy

The overall SVR rate (Table 2) was 59.8% (73/122 patients). From the overall population, 92 patients (75.4%) had undetectable HCV RNA levels at the end of triple therapy, and 19 patients (20.6%) relapsed during the post-treatment follow-up. Three of the 57 patients (5.2%) with negative HCV RNA levels at 12 wk after the end of triple therapy suffered a late relapse after the twelfth week. These three patients were null-responders to previous PR treatment, and one of these patients had a RVR during



**Table 1** Demographic and baseline characteristics *n* (%)

Triple therapy: Peg-IFN $\alpha$ 2a + Ribavirin + Protease inhibitor,	125
Telaprevir	90 (72)
Boceprevir	35 (28)
Ribavirin dosage mg/kg, mean (SD)	14.4 (2.1)
Age, yr, mean (SD)	56.2 (9.7)
Gender	
Male	81 (64.8)
Female	44 (35.2)
HCV genotype	
1a	39 (31.2)
1b	71 (56.8)
Undetermined subtype 1	15 (12)
<i>IL28B</i> genotype (rs12979860)	
C/T or T/T	88 (84.6)
Viral load, mean (log <sub>10</sub> IU/ mL)	6.3 (0.7)
Prior response to anti-viral therapy	
Relapsers	46 (37.1)
Null-responders	78 (62.9)
Liver fibrosis grade	
Metavir F3	35 (28)
Metavir F4	90 (72)
Child-Pugh score	
A	123 (98.4)
B	1 (0.8)
C	1 (0.8)
Liver stiffness values (kPa)	
Mean (SD)	17.5 (10.3)
Median (IQR)	14.3 (10.4-20.6)
Oesophageal varices	
None	92 (75.4)
Grade 1	16 (13.1)
Grade 2 or 3	14 (11.5)
Mean hemoglobin level (g/dL, mean (SD))	15.1 (1.6)
Mean platelet count $\times 10^3/\text{mm}^3$ , mean (SD)	165.69 (64.9)
Mean neutrophil count $\times 10^3/\text{mm}^3$ , mean (SD)	3.4 (1.2)

Relapsers were defined as patients who had undetectable levels of HCV RNA at the end of prior treatment and subsequently relapsed. Null-responders failed to achieve a decline of at least 2 log HCV RNA IU/mL during peg-IFN  $\alpha$ 2a + ribavirin treatment after a minimum duration of 24 wk. Peg-IFN: Pegylated-interferon; HCV: Hepatitis C virus; IQR: Inter-quartile range.

triple therapy. The remaining 73 patients maintained their SVR until the end of the follow-up period, 24 wk after triple therapy withdrawal. The SVR rate was higher (Table 2) in the TPV group (65.9%) than in the BOC group [44.1%;  $P = 0.0276$ , OR = 2.49 (1.1, 5.5), univariate analysis]. The SVR rate was not significantly influenced by the HCV subtype (1a or 1b), *IL-28B* genotype or viral load at baseline. Non-cirrhotic patients tended to have a better SVR than the patients with cirrhosis (Table 2); however, this difference did not reach statistical significance (68.6% for Metavir F3 and 56.3% for Metavir F4,  $P = 0.212$ ). Only 23 of the 52 patients (44%) in the subgroup of very difficult-to-treat patients (those with cirrhosis and a null-response to prior therapy) achieved an SVR. Among these SVR patients, 20/23 (87%) were given TPV triple therapy and 3/23 (13%) were given BOC triple therapy. Neither decreasing the ribavirin dosage nor anemia was associated with a loss of SVR (Table 2).

Patients who exhibited a RVR (Figure 1A), defined as a negative viral load 4 wk after the initiation of PI thera-

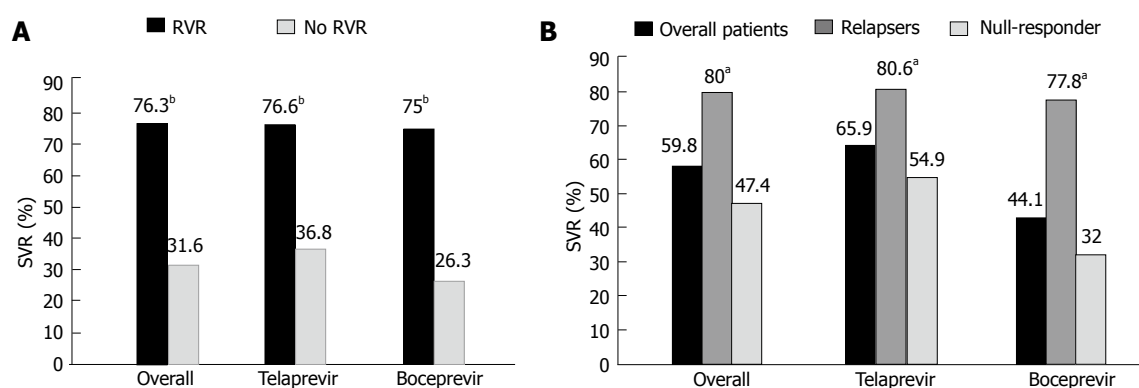
**Table 2** Factors associated with sustained virological response *n* (%)

	No sustained virological response <i>n</i> = 49	Sustained virological response <i>n</i> = 73	<i>P</i> value univariate analysis	<i>P</i> value multivariate analysis OR (95%CI)
Protease inhibitor				
Telaprevir	30 (34.1)	58 (65.9)	0.0276	NS <sup>1</sup>
Boceprevir	19 (55.9)	15 (44.1)		
Gender				
Male	27 (34.2)	52 (65.8)	0.0675	NS <sup>1</sup>
Female	22 (51.2)	21 (48.8)		
HCV genotype 1 subtype				
1a	17 (45.9)	20 (54.1)	0.8051	-
1b	26 (37.1)	44 (62.9)		
<i>IL28B</i> genotype, rs12979860, <i>n</i> (%)				
C/C	4 (25)	12 (75)	0.4420	-
C/T or T/T	40 (45.5)	48 (54.5)		
Response to prior therapy				0.008
Null-responders	40 (52.6)	36 (47.4)	0.0004	1.0
Relapsers	9 (20)	36 (80)		3.9 (1.4-10.6)
Grade of liver fibrosis				
Metavir F3	11 (31.4)	24 (68.6)	0.2118	-
Metavir F4	38 (43.7)	49 (56.3)		
Liver stiffness value (kPa)				
Median, kPa (IQR)	17.3 (11.5-28.8)	13.9 (9.4-19.7)	0.0296	0.001
< 21.3 kPa	21 (30)	49 (70)	0.002	8.2 (2.3-29.5)
$\geq 21.3$ kPa	14 (66.7)	7 (33.3)		1
Rapid virological response	18 (23.7)	58 (76.3)	$\leq 0.0001$	$\leq 0.001$
No rapid virological response	26 (68.4)	12 (31.6)		6.9 (2.6-18.2)
Decrease of ribavirin dosage	18 (37.5)	30 (62.5)	0.6287	-
No decrease of ribavirin dosage	31 (41.9)	43 (58.1)		

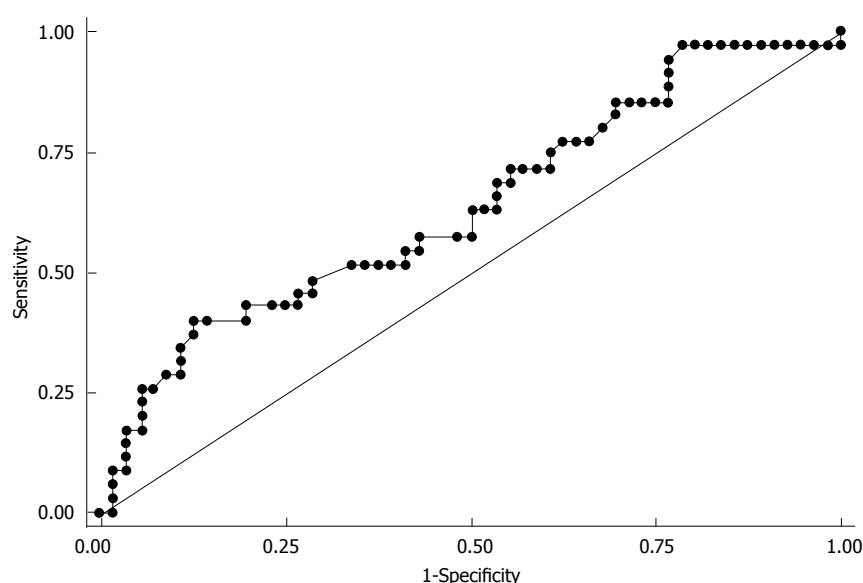
Sustained virological response (SVR) was analyzed 24 wk after triple therapy withdrawal for 122 patients. Rapid virological response (RVR) under triple therapy was defined as undetectable HCV RNA levels following 4 wk of antiviral triple therapy (*i.e.*, week 4 in the TPV group and week 8 in the BOC treatment group)<sup>1</sup>. These factors were initially included in the multivariate model ( $P$  value < 0.2 in the univariate analysis) and were not independently associated with SVR in the final multivariate model.

py, had a better SVR than the patients who did not have this rapid drop in the HCV RNA load (76.3% *vs* 36.4%, respectively,  $P < 0.0001$ ). In the overall population (Figure 1B), the relapsers to prior PR therapy had a better SVR than the null-responders (80% *vs* 47.4%,  $P = 0.0004$ ). This higher rate of SVR observed in the prior PR relapsers compared with the null-responders remained significant in the TPV or BOC subgroups (80.6% *vs* 54.9%, 77.8% *vs* 32%, respectively,  $P < 0.05$ ).

Overall, in the triple therapy population (Table 2), the liver stiffness (LS) values were significantly lower ( $P = 0.0296$ ) in the patients with an SVR [median: 13.9 kPa, IQR (9.4-19.7)] than in the patients who failed to have an SVR [median: 17.3 kPa, IQR (11.5-28.8)]. The corresponding area under the ROC curve (Figure 2) predicting SVR was 0.64 (0.52-0.76). The optimal LS cut-off value associated with an SVR was 21.3 kPa, which had a predic-



**Figure 1 Sustained virological response.** A: Influence of rapid virological response on sustained virological response. Patients with rapid virological response (RVR) vs patients without RVR, <sup>b</sup> $P < 0.001$ ; B: Influence of response to previous treatment on sustained virological response. Prior relapsers vs prior null-responders, <sup>a</sup> $P < 0.05$ . Sustained virological response was analyzed 24 wk after treatment withdrawal for 122 patients (overall numbers of patients: 88 in the telaprevir group, 34 in the boceprevir group).



**Figure 2 Diagnostic value of liver stiffness measurement to predict sustained virological response.** The area under the receiver operating characteristic curve was 0.64 (0.52-0.76).

tive positive value of 66.7% (43, 85) and a negative predictive value of 70% (57, 80). An SVR occurred in 70% of the patients with a LS below 21.3 kPa and in 33.3% of the patients with a LS of up to 21.3 kPa ( $P = 0.002$ ).

The logistic regression analysis (Table 2) showed that only three factors were independently associated with SVR. These factors were a relapse after PR treatment, the LS value, and a RVR to triple therapy. The SVR rate was greater in the prior relapsers than in the prior null-responders to PR therapy [OR = 3.9 (1.4, 10.6),  $P = 0.004$ ]. A LS of less than 21.3 kPa was associated with an improved response to triple therapy [OR = 8.2 (2.3, 29.5),  $P = 0.001$ ]. An SVR was associated with a RVR under triple therapy [OR = 6.9 (2.48, 18.2),  $P = 0.001$ ], defined as HCV RNA-negative after 4 wk of antiviral treatment (week 4 in the TPV group and week 8 in the BOC group). The multivariate analysis revealed no difference in the SVRs of the TPV and BOC groups.

### Safety

Adverse events  $\geq$  grade 1 occurred in 102/124 patients (82.2%) and were significantly more frequent in the pa-

tients receiving TPV ( $n = 79/89$ , 88.8%) compared with the patients receiving BOC ( $n = 23/35$ , 65.7%) [OR = 4.12 (1.4; 12),  $P = 0.0059$ ]. Approximately half of the patients (63: 50.8%) suffered a SAE  $\geq$  grade 3 during treatment (Table 3). These grade 3 or 4 SAEs were as follows: thrombocytopenia ( $n = 42$ , 66%), neutropenia ( $n = 21$ , 33%), anemia ( $n = 18$ , 28.5%), severe infection ( $n = 4$ , 6.3%), fatigue ( $n = 3$ , 4.7%), skin rash ( $n = 2$ , 3.2%), and hepatic failure ( $n = 2$ , 3.2%). The total percentage exceeds 100% because some subjects had several grade 3 or 4 SAEs. None of the patients died during treatment. Neither the fibrosis stage (F3 or F4) nor the protease inhibitor used (TPV or BOC) influenced the occurrence of SAEs. EPO use and blood transfusions were analyzed among the 125 patients. A total of 17 patients (13.6%) were given blood transfusions, and 65 patients (52%) received EPO. The frequencies of EPO use and blood transfusions in the TPV and BOC groups were not significantly different. Treatment was discontinued because of SAEs in 11 patients (8.9%).

The univariate analysis (Table 4) showed four factors associated with an SAE during triple therapy. Women had

**Table 3** Safety profile of triple therapy: Severe adverse events grade 3 or 4 *n* (%)

	Overall patients ( <i>n</i> = 124)	Telaprevir ( <i>n</i> = 89)	Boceprevir ( <i>n</i> = 35)	<i>P</i> value univariate analysis
Premature discontinuation due to SAE	11 (8)	10 (11.2)	1 (2.9)	0.178
Death	0	0	0	-
Severe adverse events grade 3/4	63 (50.8)	46 (51.7)	17 (48.6)	0.75
Infection	4 (3.2)	3 (3.4)	1 (2.9)	1
Liver decompensation	2 (1.6)	2 (2.2)	0	1
Fatigue	3 (2.4)	3 (3.4)	0	0.558
Skin rash	2 (1.6)	2 (2.2)	0	1
Kidney failure	1 (0.8)	1 (1.1)	0	1
Digestive adverse events	1 (0.8)	1 (1.1)	0	1
Thromboembolic events	1 (0.8)	1 (1.1)	0	1
Anemia	18 (14.5)	16 (18)	2 (5.7)	0.081
Neutropenia	21 (16.8)	14 (15.7)	7 (20)	0.568
Thrombocytopenia	42 (33.6)	32 (36)	10 (28.6)	0.434
Erythropoietin use <sup>1</sup>	65 (52)	45 (90)	20 (57.1)	0.473
Blood transfusion <sup>1</sup>	17 (13.6)	15 (16.7)	2 (5.7)	0.149

Only severe adverse events (SAEs) of grade 3 or 4 were reported in the table. SAEs were known for 124 patients. <sup>1</sup>Erythropoietin use and blood transfusions were analyzed among the 125 patients. A single subject might have several severe adverse effects.

SAEs more frequently (62.8%) than men (44.4%,  $P = 0.05$ ). The platelet counts (mean  $\pm$  SD) were lower in the patients who had a SAE ( $143.5 \pm 65.4 \times 10^3/\text{mm}^3$ ) than in the patients with no SAE  $191.1 \pm 54.9 \times 10^3/\text{mm}^3$ ,  $P \leq 0.0001$ ). SAEs were more frequent in patients with low levels of serum albumin (median:  $39.4 \pm 4.9$  and  $42 \pm 4.9$  g/L,  $P = 0.02$ ) or with a high bilirubin concentration [median: 13.1, IQR (9.1-19.1) and 10.8 (8-13.5) M/L,  $P = 0.036$ ]. The two factors that remained independently associated with SAE occurrence (Table 4) were being female [OR = 2.4 (1.1, 5.6),  $P = 0.037$ ] and a platelet count lower than  $150 \times 10^3/\text{mm}^3$  [OR 5.3 (2.3, 12.4),  $P \leq 0.001$ ]. A greater number of the patients with platelet counts lower than  $150 \times 10^3/\text{mm}^3$  (75.6%) experienced an SAE than did those with platelet counts higher than this cutoff (37.5%;  $P = 0.0001$ ).

## DISCUSSION

PI therapy has rarely been used to treat patients with severe liver fibrosis who failed to respond to prior treatment with PR. The few Metavir F3 or F4 patients treated were selected from within larger studies and do not always reflect the population seen in routine clinical practice<sup>[5-10]</sup>. The main objective of this study was to assess the effectiveness of triple therapy for difficult-to-treat patients with severe liver fibrosis who were null-responders or relapsers to prior PR treatment. It is necessary to understand how patients tolerate these treatments to identify the patients most at risk of suffering severe adverse side effects.

The overall SVR rate of 59.8% at 24 wk post-treat-

**Table 4** Factors associated with the occurrence of severe adverse events of grade 3 or 4 *n* (%)

	Severe adverse events <i>n</i> = 63	No severe adverse events <i>n</i> = 61	<i>P</i> value univariate analysis	<i>P</i> value multivariate Analysis OR (95%CI)
Protease inhibitor				
Telaprevir	47 (51.7)	43 (48.3)	0.7548	-
Boceprevir	17 (48.6)	18 (51.4)		
Genre				0.037
Male	36 (44.4)	45 (55.6)		1.0
Female	27 (62.8)	16 (37.2)	0.0518	2.4 (1.1-5.6)
Liver fibrosis				
Metavir F3	15 (42.9)	20 (57.1)	0.2667	-
Metavir F4	48 (53.9)	41 (46.1)		
Platelets				
Mean $\times 10^3/\text{mm}^3$ (SD)	143.5 (65.43)	191.1 (54.9)	$\leq 0.0001$	$\leq 0.001$
< $150 \times 10^3/\text{mm}^3$	34 (75.6)	11 (24.4)	0.0001	1.0
$\geq 150 \times 10^3/\text{mm}^3$	27 (37.5)	45 (62.5)		5.3 (2.3-12.4)
Albumin, mean, g/L, (SD)	39.4 (4.9)	42 (54.9)	0.0196	-
Bilirubin, median $\mu\text{M/L}$ , (IQR)	13.1 (9.1-19.1)	10.8 (8-13.5)	0.0359	-

The variables initially included in the logistic regression model were those associated with SAEs in the univariate analysis ( $P$  value < 0.20). SAEs: Severe adverse events; IQR: Inter-quartile range.

ment was satisfactory in this difficult-to-treat population. This high rate shows that PI could be used successfully in routine clinical practice, including for patients with severe liver fibrosis, with an efficacy equivalent to results obtained in controlled trials. The SVR rate for patients in the RESPOND-2 trial<sup>[10]</sup> with severe liver fibrosis (Metavir F3 or F4) who failed to respond to previous treatment and were retreated with BOC was 55.5%. This study, however, was carried out on a limited number of patients. The SVR rate for our patients treated with BOC was 44.1%. The lower response rate for our BOC-treated patients might be due to the type of response of these patients to the prior treatment. The majority of patients in the RESPOND-2 study<sup>[10]</sup> were relapsers (64%), whereas the majority (73.5%) of our patients were null-responders. The SVR rate for the patients in the REALIZE trial<sup>[8]</sup> who had severe liver fibrosis (Metavir F3 and F4) and who were given TPV triple therapy was 56%. The SVR for our patients given TPV triple therapy was 65.9%, which was better than that of the REALIZE patients. However, the SVR rate for those REALIZE patients<sup>[8]</sup> classified as Metavir F3 was similar (66.4%) to the rate for our patients.

We identified elements predicting an SVR. One of the main predictive factors of the virological response to triple therapy was the type of response to the previous PR treatment. Relapsers on the previous treatment had very high SVR rates (80%), whereas the SVR of null-responders was only 47.4%. In the overall population, the type of response to a previous treatment was independently associated with the SVR to triple therapy. This influence

of the type of virological response to PR treatment on the SVR to triple therapy was also described in phase III studies for patients treated with both TPV and BOC<sup>[8-10]</sup>. We observed an unexpectedly high SVR (54.9%) with TPV triple therapy in our previous null-responders (28 of 51 patients). This rate is approximately twofold higher than the SVR rate observed in the REALIZE study in the same population of null-responders with severe liver fibrosis<sup>[8]</sup>. A more detailed comparison of the characteristics of cirrhotic patients in phase III studies and our patients in the current study should provide a better understanding of this difference in the SVR rate. We confirmed that the patients who relapse after a previous double-therapy benefit more from PI treatment than null-responders. However, we also determined that an encouraging SVR could be obtained for greater than one-third of previous null-responder cirrhotic patients; therefore, triple therapy with TPV or BOC should be offered to these patients, especially to the patients who could not be included in new drug trials<sup>[13]</sup>. The rationale for treating null-responders to PR therapy with severe liver fibrosis is that the second generation of direct-acting antiviral drugs (DAAs) would be used in the near future to obtain a higher SVR<sup>[14]</sup>.

We have shown an overall difference in the SVR of patients treated with TPV (65.9%) and BOC (44.1%). This difference was significant in the univariate analysis; however, it does not appear to be an independent value in the multivariate analysis. There were more null-responders to PR treatment in our BOC subgroup (73.5%) than in the TPV subgroup (58.6%). An analysis of the response to triple therapy (in terms of the previous response) indicated that the SVRs of relapsers in the TPV (80.6%) and BOC (77.8%) subgroups were similar. BOC tended to be less effective for the null-responders to prior PR therapy, although this difference was not significant after the multivariate analysis. Our study was not a randomized study; each clinician could choose to use TPV or BOC, although all of the patients were given the same treatment with the same doses of peg-IFN  $\alpha$ 2a + ribavirin, unlike those in the CUPIC trial<sup>[15]</sup>, which used peg-IFN  $\alpha$ 2a or  $\alpha$  b. More of our patients were treated with TPV than BOC because TPV was approved for use in France in January 2011, and BOC was approved at a later date. The possible difference between the SVR of the TPV and BOC groups should be confirmed in a randomized trial that includes more patients. However, taking into account the development of new DAAs, such a randomized study is unlikely to be completed. Two meta-analyses<sup>[16,17]</sup> compared the efficacy of TPV and BOC. Both studies determined that the SVR for TPV was superior. These meta-analyses included only a few trials on heterogeneous populations. The results could not be extrapolated to routine clinical practice, although they are compatible with our findings. In the overall population, a RVR was observed in 66.7% of individuals. A RVR was observed for 76.3% of the patients treated with TPV and for 31.6% of the patients treated with BOC. A Spanish study found that the RVR was significantly higher in the TPV patients, suggesting that the drug acted more

rapidly<sup>[18]</sup>. Close monitoring of the viral load, especially at the start of treatment, could lead to the selection of a sub-group of patients very likely to have an SVR<sup>[19-21]</sup>. This information could strongly motivate these difficult-to-treat patients on prolonged treatment for 48 wk to adhere more closely to the treatment protocol.

We found no link between the degree of liver fibrosis, Metavir F3 or Metavir F4, and the SVR. However, we demonstrated a statistically significant link between the SVR and the LS measured by Fibrosan<sup>®</sup> at inclusion. The SVR rate was significantly higher in the patients with an LS under 21.3 kPa. This LS value of 21.3 kPa is well above the value of 12.5 kPa that is typically used to diagnose cirrhosis<sup>[22]</sup>, suggesting that the population of patients with Child-Pugh class A cirrhosis is heterogeneous. The prognosis for the patients with a high LS value is likely different from the prognosis for the patients with lower LS values. A previous study<sup>[23]</sup> of patients treated with PR showed that the liver LS values were significantly lower in patients having an SVR than in non-responders. The LS value could be used to identify portal hypertension<sup>[24-27]</sup>. Various studies have given thresholds from 13.6 kPa to 48 kPa for portal hypertension. One study on patients with various liver diseases observed that a threshold of 21 kPa is useful for predicting significant portal hypertension<sup>[25]</sup>. Other studies specifically included patients with hepatitis C for diagnosing non-invasive portal hypertension. A LS threshold of 19.8 kPa<sup>[26]</sup> in one study and of 21.5 kPa in two others<sup>[22,27]</sup> enabled to accurately predict the presence of esophageal varices in this population. These threshold values assessed in studies on heterogeneous populations are similar to the thresholds that we identified as predictive of an SVR. Our finding of a link between the LS value and the SVR suggests that there is a subgroup of patients with severe liver fibrosis who have high LS values and a diminished response to triple therapy with PI. The threshold identified in this study should be confirmed by studies on larger populations of patients.

One of our secondary objectives was to assess how well PI treatment was tolerated by difficult-to-treat patients in the context of routine clinical practice. Because the great majority of patients suffered from at least one side effect, we focused on the development of severe side effects and the factors predicting their development. We determined that 50.8% of patients treated with PI developed these severe adverse reactions, causing 8.9% of the patients to abandon their treatment early. The preliminary results of the CUPIC trial<sup>[15]</sup> demonstrated that 40% of patients suffered an SAE after 16 wk, and 14.7% of these patients abandoned treatment prematurely. Published studies indicate that 57% of patients on TPV<sup>[18,9]</sup> suffered an SAE, as did 40.4% of those on BOC<sup>[28]</sup>. In phase III trials<sup>[28]</sup>, SAEs were observed in only 16% of patients with severe liver fibrosis, which was a much lower percentage than in our findings. This type of difference between initial studies and routine clinical practice is not uncommon; 24% of our patients had at least one criterion that would have excluded them from a phase III trial. Our patients were also older than the patients included in



phase III trials and were likely more fragile, making them more susceptible to an SAE. None of our patients died during follow-up, unlike the patients in the CUPIC trial.

We had to administer EPO to 52% of the patients in this study and had to reduce the ribavirin dose for 38.7% of the patients. In addition, 13.6% of the patients required a blood transfusion. Reducing the ribavirin dose had no effect on the SVR for our patients. Anemia was treated in the SPRINT-2 trial<sup>[7]</sup>, similarly to our study, with a reduction in the ribavirin dose for only 8% of patients; EPO was used for 38% of patients, and a combination of the reducing the ribavirin dose and EPO was used for 44% of patients. TPV appeared to cause anemia more frequently than BOC among our patients; however, this difference was not significant. TPV treatment was not an independent factor suggesting the development of SAEs in our study. We have confirmed that patients with severe liver fibrosis, whether Metavir F3 or F4, do not readily tolerate triple therapy with PI and suffer from a high incidence of SAEs. We determined that two factors were independently associated with the development of an SAE. One factor was the platelet count at inclusion, which was significantly lower in the patients who developed an SAE. The threshold for development of an SAE was 150000 platelets/mm<sup>3</sup>. Data from the CUPIC cohort<sup>[15]</sup> indicate that a threshold of 100000/mm<sup>3</sup> was predictive of death and severe complications. Some of our patients had Metavir F3 or F4 scores, whereas the CUPIC cohort<sup>[15]</sup> included only patients with cirrhosis; this difference could account for the lower SVR rate in the CUPIC study. Female gender is the second factor independently associated with the development of an SAE. The PI dose should not be scaled to the patient's body weight; however, further studies on men and women are needed to define any differences in body mass index in men and women to assess whether this factor influences tolerance.

In conclusion, we studied a large cohort of patients with genotype 1 HCV infection and severe liver fibrosis (Metavir F3 or F4) who had failed to respond to an earlier PR treatment. Our data obtained in routine clinical practice confirm the satisfactory efficacy of PI triple therapy. We have also demonstrated that a threshold value of 21.3 kPa of LS is associated with an SVR. However, triple therapy with PI is rather poorly tolerated. We should use better methods to select patients for PI treatment and should be able to offer the patients at the greatest risk of treatment failure (as well as those with an intolerance for other therapies) a second generation of new DAAs.

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## COMMENTS

### Background

The addition of telaprevir (TPV) or boceprevir (BOC) in the treatment of geno-

type 1 hepatitis C virus (HCV) patients has significantly increased the sustained virologic response rate of pegylated-interferon (peg-IFN)  $\alpha$ 2a + ribavirin (PR). Data on these new therapeutic options are limited in the setting of very difficult-to-treat patients, although these patients are in the highest priority for achieving viral clearance.

### Research frontiers

The research goal is to assess the efficacy and safety of telaprevir- and boceprevir-based triple therapies in a multicentric cohort of previously treated HCV-genotype 1 patients with severe liver fibrosis.

### Innovations and breakthroughs

In the clinical setting, the efficacy of TPV and BOC-based triple therapies in previously treated HCV-genotype 1 patients with severe liver fibrosis is similar or even better than the results obtained in controlled trials [overall sustained virological response (SVR) 59.8%]. The SVR is inversely correlated to liver stiffness, as assessed by elastography with a cut-off of 21.3 kPa, which is predictive of a poor response rate. These treatments are poorly tolerated, and half of all patients experience at least one grade 3-4 adverse event.

### Applications

This study suggests that telaprevir and boceprevir-based triple therapies could be used in clinical practice in the subset of very difficult-to-treat patients; these triple therapies resulted in a viral clearance rate similar to or even better than the rates obtained in controlled trials. An SVR was achieved even in cirrhosis patients. However, patients with the most advanced stage of fibrosis should be considered for other treatments because these treatments are significantly less efficient when the liver stiffness is higher than 21.3 kPa and are significantly less tolerated in the presence of biological markers of advanced liver fibrosis (platelet count < 150 × 10<sup>3</sup>/mm<sup>3</sup>).

### Terminology

SVR: undetectable levels of viral RNA at 24 wk following treatment completion; Rapid virological response (RVR): undetectable levels of viral RNA at week 4 or week 8 after initiation of telaprevir- or boceprevir-based triple therapies, respectively.

### Peer review

This manuscript by Bonnet *et al* described the efficacy and safety of Telaprevir or Boceprevir therapy for treatment experienced patients with advanced fibrosis. The majority of patients were cirrhosis (F4 72%) and null-responder to prior therapy (63%), thus reflecting most difficult-to-treat patients. The results are encouraging showing high SVR rate in prior relapsers (80%) and even in null-responders (47%) with low rate of premature discontinuation due to SAE (8%) and no death. This information in real-life setting may be of value for physicians treating hepatitis C.

## REFERENCES

- 1 Dienstag JL, Ghany MG, Morgan TR, Di Bisceglie AM, Bonkovsky HL, Kim HY, Seeff LB, Szabo G, Wright EC, Sterling RK, Everson GT, Lindsay KL, Lee WM, Lok AS, Morishima C, Stoddard AM, Everhart JE. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. *Hepatology* 2011; **54**: 396-405 [PMID: 21520194 DOI: 10.1002/hep.24370.24370]
- 2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 123244553]
- 3 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749]
- 4 Cammà C, Cabibbo G, Bronte F, Enea M, Licata A, Attanasio M, Andriulli A, Craxi A. Retreatment with pegylated interferon plus ribavirin of chronic hepatitis C non-responders to interferon plus ribavirin: a meta-analysis. *J Hepatol* 2009; **51**: 675-681 [PMID: 19665247 DOI: 10.1016/j.jhep.2009.06.018]
- 5 Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N,

- Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716 [PMID: 20692693 DOI: 10.1016/S0140-6736(10)60934-8]
- 6 Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839-1850 [PMID: 19403903 DOI: 10.1056/NEJMoa0807650]
- 7 Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783]
- 8 Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]
- 9 Liu J, Jadhav PR, Amur S, Fleischer R, Hammerstrom T, Lewis L, Naeger L, O'Rear J, Pacanowski M, Robertson S, Seo S, Soon G, Birnkrant D. Response-guided telaprevir therapy in prior relapsers? The role of bridging data from treatment-naïve and experienced subjects. *Hepatology* 2013; **57**: 897-902 [PMID: 22487907 DOI: 10.1002/hep.25764]
- 10 Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]
- 11 Wedemeyer H, Jensen DM, Godofsky E, Mani N, Pawlotsky JM, Miller V. Recommendations for standardized nomenclature and definitions of viral response in trials of hepatitis C virus investigational agents. *Hepatology* 2012; **56**: 2398-2403 [PMID: 22707382 DOI: 10.1002/hep.25888]
- 12 Leroy V, Serfaty L, Bourlière M, Bronowicki JP, Delasalle P, Pariente A, Pol S, Zoulim F, Pageaux GP. Protease inhibitor-based triple therapy in chronic hepatitis C: guidelines by the French Association for the Study of the Liver. *Liver Int* 2012; **32**: 1477-1492 [PMID: 22891751 DOI: 10.1111/j.1478-3231.2012.02856.x]
- 13 Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. *N Engl J Med* 2013; **368**: 1907-1917 [PMID: 23675659 DOI: 10.1056/NEJMra1213651]
- 14 Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR, Jacobson IM, Kowdley KV, Nyberg L, Subramanian GM, Hyland RH, Arterburn S, Jiang D, McNally J, Brainard D, Symonds WT, McHutchison JG, Sheikh AM, Younossi Z, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**: 1878-1887 [PMID: 23607594 DOI: 10.1056/NEJMoa1214853]
- 15 Hézode C, Fontaine H, Dorival C, Larrey D, Zoulim F, Canva V, de Ledinghen V, Poynard T, Samuel D, Bourlière M, Zarski JP, Raabe JJ, Alric L, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Métivier S, Tran A, Serfaty L, Abergel A, Causse X, Di Martino V, Guyader D, Lucidarme D, Grando-Lemaire V, Hillon P, Feray C, Dao T, Cacoub P, Rosa I, Attali P, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP. Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC) - NCT01514890. *J Hepatol* 2013; **59**: 434-441 [PMID: 23669289 DOI: 10.1016/j.jhep.2013.04.035]
- 16 Sitole M, Silva M, Spooner L, Comee MK, Malloy M. Telaprevir versus boceprevir in chronic hepatitis C: a meta-analysis of data from phase II and III trials. *Clin Ther* 2013; **35**: 190-197 [PMID: 23369368 DOI: 10.1016/j.clinthera.2012.12.017]
- 17 Cure S, Diels J, Gavart S, Bianic F, Jones E. Efficacy of telaprevir and boceprevir in treatment-naïve and treatment-experienced genotype 1 chronic hepatitis C patients: an indirect comparison using Bayesian network meta-analysis. *Curr Med Res Opin* 2012; **28**: 1841-1856 [PMID: 23016967 DOI: 10.1185/03007995.2012.734798]
- 18 Benito JM, Sánchez-Parra C, Maida I, Aguilera A, Rallón NI, Rick F, Labarga P, Fernández-Montero JV, Barreiro P, Soriano V. Triple combination therapy for hepatitis C with telaprevir exhibits greater early antiviral activity than with boceprevir. *Antivir Ther* 2013; **18**: 709-715 [PMID: 23645335 DOI: 10.3851/IMP261]
- 19 Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, Fried MW, Adler M, Reesink HW, Martin M, Sankoh AJ, Adda N, Kauffman RS, George S, Wright CL, Poordad F. Response-guided telaprevir combination treatment for hepatitis C virus infection. *N Engl J Med* 2011; **365**: 1014-1024 [PMID: 21916639 DOI: 10.1056/NEJMoa1014463]
- 20 Foster GR, Zeuzem S, Andreone P, Pol S, Lawitz EJ, Diago M, Roberts S, Pockros PJ, Younossi Z, Lonjon-Domanec I, De Meyer S, Luo D, George S, Beumont M, Picchio G. Sustained virologic response rates with telaprevir by response after 4 weeks of lead-in therapy in patients with prior treatment failure. *J Hepatol* 2013; **58**: 488-494 [PMID: 23183521 DOI: 10.1016/j.jhep.2012.11.013]
- 21 Poordad F, Bronowicki JP, Gordon SC, Zeuzem S, Jacobson IM, Sulkowski MS, Poynard T, Morgan TR, Molony C, Pedicone LD, Sings HL, Burroughs MH, Sniukiene V, Boparai N, Goteti VS, Brass CA, Albrecht JK, Bacon BR. Factors that predict response of patients with hepatitis C virus infection to boceprevir. *Gastroenterology* 2012; **143**: 608-618.e1-5 [PMID: 22626609 DOI: 10.1053/j.gastro.2012.05.011]
- 22 Castéra L, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Ledinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68 [PMID: 19013661 DOI: 10.1016/j.jhep.2008.08.018]
- 23 Patel K, Friedrich-Rust M, Lurie Y, Grigorescu M, Stanciu C, Lee CM, Schiff ER, Häussinger D, Manns MP, Gerken G, Colle I, Torbenson M, Pulkstenis E, Subramanian GM, McHutchison JG, Zeuzem S. FibroSURE and FibroScan in relation to treatment response in chronic hepatitis C virus. *World J Gastroenterol* 2011; **17**: 4581-4589 [PMID: 22147963 DOI: 10.3748/wjg.v17.i41.4581]
- 24 Forestier N, Gaus A, Herrmann E, Sarrazin C, Bojunga J, Poynard T, Albert J, Gerber L, Schneider MD, Dultz G, Zeuzem S, Friedrich-Rust M. Acoustic radiation force impulse imaging for evaluation of antiviral treatment response in chronic hepatitis C. *J Gastrointest Liver Dis* 2012; **21**: 367-373 [PMID: 23256119]
- 25 Bureau C, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268 [PMID: 18397389 DOI: 10.1111/j.1365-2036.2008.03701.x]
- 26 Pritchett S, Cardenas A, Manning D, Curry M, Afdhal NH. The optimal cut-off for predicting large oesophageal varices using transient elastography is disease specific. *J Viral Hepat* 2011; **18**: e75-e80 [PMID: 21040236 DOI: 10.1111/

- j.1365-2893.2010.01375.x]
- 27 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971]
- 28 **Bruno S**, Vierling JM, Esteban R, Nyberg LM, Tanno H, Goodman Z, Poordad F, Bacon B, Gottesdiener K, Pedicone LD, Albrecht JK, Brass CA, Thompson S, Burroughs MH. Efficacy and safety of boceprevir plus peginterferon-ribavirin in patients with HCV G1 infection and advanced fibrosis/cirrhosis. *J Hepatol* 2013; **58**: 479-487 [PMID: 23183529 DOI: 10.1016/j.jhep.2012.11.020]

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## Skin toxicity predicts efficacy to sorafenib in patients with advanced hepatocellular carcinoma

Masako Shomura, Tatehiro Kagawa, Koichi Shiraishi, Shunji Hirose, Yoshitaka Arase, Jun Koizumi, Tetsuya Mine

Masako Shomura, Department of Nursing, Tokai University School of Health Sciences, Isehara, Kanagawa 2591193, Japan  
Tatehiro Kagawa, Koichi Shiraishi, Shunji Hirose, Yoshitaka Arase, Tetsuya Mine, Division of Gastroenterology, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa 2591193, Japan

Jun Koizumi, Department of Radiology, Tokai University School of Medicine, Isehara, Kanagawa 2591193, Japan

Author contributions: Shomura M and Kagawa T contributed equally to this study; Shomura M, Kagawa T, Shiraishi K, Hirose S and Arase Y performed the majority of sorafenib therapy; Koizumi J and Kagawa T provided evaluation the efficacy of therapy; Shomura M and Kagawa T provided the collection of all the data in addition to performing a statistical analysis and were also involved in writing and editing the manuscript; Shomura M provided financial support for this work; Mine T designed and coordinated the study.

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Correspondence to: Masako Shomura, RN, PhD, Associate Professor, Department of Nursing, Tokai University School of Health Sciences, 143 Shimokasuya, Isehara, Kanagawa 2591193, Japan. rocky36j@is.icc.u-tokai.ac.jp

Telephone: +81-463-902035 Fax: +81-463-902035

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follow-up was provided once in 1-2 wk.

**RESULTS:** A total of 37 patients were enrolled in the study, comprising 30 males (81%) with a median age of 71 years. The disease control rate at 3 mo was 41%, and the median OS and treatment duration were 259 and 108 d, respectively. Nursing intervention was given to 24 patients (65%). Every patient exhibited some kinds of AEs, but no patients experienced G4 AEs. Frequently observed AEs > G2 included anorexia (57%), skin toxicity (57%), and fatigue (54%). Factors significantly associated with longer OS in multivariate analysis demonstrated that age  $\leq 70$  years, presence of > G2 skin toxicity, and absence of > G2 hypoalbuminemia. The disease control rate in patients with > G2 skin toxicity was 13/20 (65%), which was significantly higher compared with that in patients with no or G1 skin toxicity. Multivariate analysis revealed that nursing intervention and > G2 skin toxicity were independent significant predictors for longer treatment duration.

**CONCLUSION:** Skin toxicity was associated with favorable outcomes with sorafenib therapy for advanced HCC. Nursing intervention contributed to better adherence, which may improve the efficacy of sorafenib.

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### Abstract

**AIM:** To study the relationship between adverse events (AEs), efficacy, and nursing intervention for sorafenib therapy in patients with hepatocellular carcinoma (HCC).

**METHODS:** We enrolled 37 consecutive patients with advanced HCC who received sorafenib therapy. Relationships among baseline characteristics as well as AE occurrence and tumor response, overall survival (OS), and treatment duration were analyzed. The nursing intervention program consisted of education regarding self-monitoring and AEs management, and telephone

**Key words:** Hepatocellular carcinoma; Molecular targeted therapy; Drug toxicity; Surrogate marker; Nursing intervention

**Core tip:** Sorafenib therapy for advanced hepatocellular carcinoma (HCC) often causes adverse events (AEs), subsequently leading to dose reduction or discontinuation. Conversely, few studies have associated serious AEs with a favorable response to sorafenib. We aimed to elucidate the relationship between AEs occurrence, therapeutic efficacy, and the impact of nursing intervention on adherence to therapy. We observed that



skin toxicity was associated with favorable outcomes in sorafenib therapy for advanced HCC. Furthermore nursing intervention contributed to better adherence, which may improve the efficacy of sorafenib therapy.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide<sup>[1,2]</sup>; in addition, it is one of the intractable cancers, considering its high rate of recurrence even after curative therapies<sup>[3]</sup>. In particular, vascular invasion and extrahepatic metastasis greatly decrease survival rates<sup>[4-8]</sup>.

Sorafenib, an oral inhibitor, is currently used as a standard therapeutic option for advanced HCC<sup>[9-11]</sup>. This drug occasionally causes severe adverse events (AEs), which include hand-foot skin reaction (HFSR), hypertension, diarrhea, anorexia, fatigue, weight loss, and so on. Although most AEs are reversible, they can significantly impact a patient's quality of life and occasionally result in dose reduction or discontinuation of therapy<sup>[12]</sup>. On the other hand, recent studies of sorafenib therapy for HCC have reported that the occurrence of any grade (G) hypertension<sup>[13]</sup> or > G2 diarrhea<sup>[14]</sup> was associated with longer overall survival (OS); in addition, skin toxicity resulted in preferable outcomes as well<sup>[15,16]</sup>. However, studies investigating the relationship between AEs occurrence and efficacy of this drug remain insufficient.

The increase of available oral anticancer drugs has introduced a shift in responsibility from clinicians to patients and their families for self-administration of these drugs and AEs management. Reduced adherence leads to poor clinical outcomes and subsequent increase in healthcare costs<sup>[17,18]</sup>. Several studies have suggested that an adequate intervention by nurses and pharmacists may improve treatment adherence<sup>[19-22]</sup>. However, the contribution of nursing intervention to treatment adherence remains elusive.

This study aimed to elucidate the relationship between AEs occurrence and the efficacy of sorafenib therapy for patients with advanced HCC. In addition, we evaluated the impact of nursing intervention on the adherence to this drug therapy.

## MATERIALS AND METHODS

### Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by the Insti-

tutional Review Board of Tokai University (NO.10R-046). All patients provided informed written consents.

### Sorafenib therapy

We enrolled consecutive patients with advanced HCC who received sorafenib therapy from August 2009 to December 2012 at Tokai University Hospital. Eligibility criteria were as follows: (1) unresectable advanced HCC; (2) resistance to or no indication of transcatheter arterial chemoembolization (TACE); (3) Child-Pugh class A or B; and (4) Eastern Cooperative Oncology Group Performance Status of 0 or 1. Patients received 800 mg sorafenib as an initial daily dose. However, lower doses were occasionally selected by doctors, particularly when patients were aged > 70 years or had liver function of Child-Pugh class B. The HCC stage was classified according to the tumor-node-metastasis criteria of the Liver Cancer Study Group of Japan<sup>[23]</sup>.

### Nursing intervention

The nursing intervention program consisted of education regarding self-monitoring and AEs management, and telephone follow-up was provided once in 1-2 wk<sup>[24,25]</sup>. One nurse who experienced and trained specialized care with liver cancer patients provided the nursing intervention.

### Clinical evaluation

Efficacy was evaluated according to the modified Response Evaluation Criteria in Solid Tumors<sup>[26]</sup> 3 mo after the initiation of therapy. Thereafter, dynamic computed tomography (CT) scan or magnetic resonance imaging (MRI) was performed every 3 mo. The disease control rate was defined as the percentage of patients with complete response (CR), partial response (PR), and stable disease (SD). AEs were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Effects, version 4.0<sup>[27]</sup>. Skin toxicity included HFSR and any kind of rash. Patients were followed up until January 7, 2013 or death.

### Statistical analysis

Relationships of efficacy to baseline patient characteristics and AEs occurrence were evaluated using Fisher's exact probability test or multiple logistic regression analysis. Further, OS and treatment duration were analyzed using the log-rank test or Cox proportional hazards regression model. Multivariate analyses were performed using the stepwise (step-up) procedure (likelihood ratio). All variables with *P* values < 0.15 in univariate analysis were included for multivariate analysis. *P* values < 0.05 were considered to indicate statistical significance. Statistical analysis was performed using the statistical package IBM® SPSS® Statistics version 21 for Windows (1989, Somers, NY).

## RESULTS

### Baseline patient characteristics (Table 1)

A total of 37 patients were enrolled in the study, com-

**Table 1** Baseline patient characteristics *n* (%)

Variables	Number of patients	
Gender	Male	30 (81)
	Female	7 (19)
Age (yr)	> 70	19 (51)
	≤ 70	18 (49)
Child-pugh class	A	33 (89)
	B	4 (11)
Etiology	HCV	20 (54)
	HBV	11 (30)
	Others	6 (16)
TNM stage	III	16 (43)
	IVa	8 (22)
	IVb	13 (35)
Previous therapies	Yes	31 (84)
	No	6 (16)
AFP (ng/mL)	> 100	20 (54)
	≤ 100	17 (46)
DCP (mAU/mL) <sup>a</sup>	> 1000	20 (59)
	≤ 1000	14 (41)
Initial dose of sorafenib (mg/d)	800	21 (57)
	< 800	16 (43)
Nursing intervention	Yes	24 (65)
	No	13 (35)

<sup>a</sup>DCP values were not available for 3 cases; TNM: Tumor-Node-Metastasis staging system; AFP: α-fetoprotein; DCP: Des-gamma-carboxy prothrombin; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

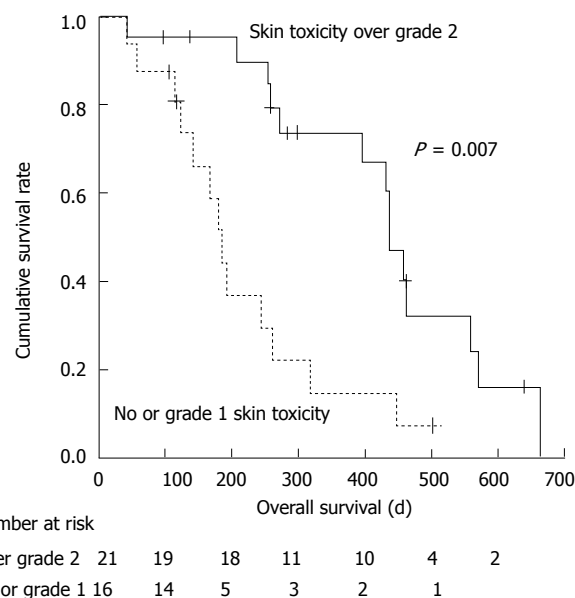
prising 30 males (81%) with a median age of 71 years (range, 36-83 years). More than half of the patients (54%) were infected with hepatitis C virus. Most patients (84%) had received other treatment for HCC before sorafenib therapy, including surgical resection, TACE, and radiofrequency ablation. Sixteen patients (43%) received < 800 mg sorafenib as an initial daily dose. Nursing intervention was given to 24 patients (65%).

### Treatment efficacy

Disease control was obtained in 15 patients (41%) comprising 1 (3%) with CR, 3 (8%) with PR, and 11 (30%) with SD at 3 mo after the initiation of sorafenib therapy. The patient who achieved CR was a 69-year-old male patient, a noteworthy case that has also been reported elsewhere<sup>[28]</sup>. He received sorafenib at a dose of 800 mg for HCC metastasis to a portal lymph node metastasis that appeared 3 years after surgical resection for primary HCC. However, he discontinued sorafenib administration after 11 d because of G3 HFSR. Despite treatment termination, the portal lymph node metastasis disappeared along with the normalization of serum (des-gamma-carboxy prothrombin) DCP (and α-fetoprotein) AFP levels. A total of 27 patients (73%) died. One patient was lost to follow-up. The median OS period was 259 d (range, 41-664 d).

### AEs (Table 2)

Every patient exhibited some kind of AEs, but no patient experienced G4 AEs. Frequently observed > G2 AEs included anorexia (57%), skin toxicity (57%), fatigue (54%), hypoalbuminemia (41%), and hypertension (30%).



**Figure 1** Overall survival and skin toxicity. Kaplan-Meier curve shows that patients with over grade 2 skin toxicity could live longer. Thus more severe skin toxicity is associated with longer survival.

Sorafenib therapy was discontinued in 5 patients (14%) because of skin toxicity (*n* = 4) and anorexia (*n* = 1). Fifteen patients (41%) required dose reduction because of skin toxicity (*n* = 8), anorexia (*n* = 4), hyperbilirubinemia (*n* = 2), and hypertension (*n* = 1).

### Factors associated with OS (Table 3)

Factors significantly associated with longer OS in univariate analysis included the presence of previous therapy, serum DCP levels ≤ 1000 mAU/mL, 800 mg initial sorafenib dose, absence of > G2 anorexia, fatigue or hypoalbuminemia, and presence of > G2 skin toxicity. Multivariate analysis demonstrated that age ≤ 70 years (HR = 0.354, 95%CI: 0.135-0.933; *P* = 0.036), presence of > G2 skin toxicity (Figure 1, HR = 0.267, 95%CI: 0.102-0.701; *P* = 0.007), and absence of > G2 hypoalbuminemia (HR = 0.221, 95%CI: 0.085-0.575; *P* = 0.002) were significant predictors for longer OS. The disease control rate in patients with > G2 skin toxicity was 13/20 (65%), which was significantly higher compared with that in patients with no or G1 skin toxicity [2/17 (12%); *P* = 0.002].

### Nursing intervention and treatment duration (Table 4)

The median duration of medication was 108 d (range, 4-462 d). A total of 33 patients (89%) discontinued sorafenib: 18 (55%) for deterioration of general status, 10 (30%) for the lack of beneficial effects of sorafenib, and 5 (15%) for G3 AEs.

We provided face-to-face counseling 1-2 times per mo and telephone follow-up once in 1-2 wk to manage AEs by supporting patient's self-monitoring and self-care<sup>[24,25]</sup>. Multivariate analysis revealed that nursing intervention (HR = 0.398, 95%CI: 0.181-0.874; *P* = 0.022) and > G2 skin toxicity (HR = 0.225, 95%CI: 0.095-0.534; *P* =

**Table 2** Sorafenib-related adverse events *n* (%)

Adverse events	Any Grade	Grade 1	Grade 2	Grade 3
Anorexia	29 (78)	8 (22)	10 (27)	11 (30)
Skin toxicity <sup>a</sup>	27 (73)	6 (16)	8 (22)	13 (35)
Fatigue	23 (62)	3 (8)	11 (30)	9 (24)
Diarrhea	20 (54)	9 (24)	8 (21)	3 (8)
Hypoalbuminemia	19 (51)	4 (11)	14 (38)	1 (3)
Weight loss	17 (46)	7 (19)	9 (24)	1 (3)
Hyperbilirubinemia	16 (43)	10 (27)	5 (14)	1 (3)
Decreased platelet count	14 (38)	3 (8)	9 (24)	2 (5)
Hypertension	13 (35)	2 (5)	6 (16)	5 (14)
Alopecia	13 (35)	5 (14)	8 (22)	0 (0)
Anemia	9 (24)	4 (11)	4 (11)	1 (3)

<sup>a</sup>Skin toxicity includes hand-foot skin reaction and any kind of rash.

**Table 3** Variables associated with overall survival

Variables	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Gender, male ( <i>vs</i> female)	0.384 (0.147-1.005)	0.051		
Age, ≤ 70 yr ( <i>vs</i> > 70 yr)	0.491 (0.225-1.071)	0.074	0.354 (0.135-0.933)	0.036
Previous therapy yes ( <i>vs</i> no)	0.035 (0.128-0.961)	0.042		
DCP, ≤ 1000 mAU/mL ( <i>vs</i> > 1000 mAU/mL)	0.416 (0.178-0.974)	0.043		
Initial dose of sorafenib, 800 mg ( <i>vs</i> < 800 mg)	0.405 (0.185-0.888)	0.024		
Adverse events > grade 2 Anorexia - ( <i>vs</i> +)	0.374 (0.158-0.888)	0.026		
Skin toxicity <sup>a</sup> + ( <i>vs</i> -)	0.278 (0.122-0.635)	0.002	0.267 (0.102-0.701)	0.007
Fatigue - ( <i>vs</i> +)	0.404 (0.176-0.924)	0.032		
Hypoalbuminemia - ( <i>vs</i> +)	0.379 (0.170-0.842)	0.017	0.221 (0.085-0.575)	0.002

<sup>a</sup>Skin toxicity includes hand-foot skin reaction and any kind of rash. HR: Hazard ratio; DCP: Des-gamma-carboxy prothrombin. This table only includes variables with *P* values < 0.15 in univariate analyses.

0.001) were independent significant predictors for longer treatment duration. Median treatment durations were 122 and 36 d in patients with and without nursing intervention, respectively. However, nursing intervention was not associated with OS, with the median OS being 258 and 274 d for patients with and without nursing intervention, respectively.

## DISCUSSION

In this study, the median OS was 8.6 mo, which is comparable to previous studies: 10.7, 6.5, and 9.3 mo in the SHARP trial<sup>[9]</sup>, the Asia-Pacific study<sup>[10]</sup>, and the Global Infectious Diseases and Epidemiology Network study<sup>[29]</sup>, respectively. The disease control rate was 41%, which was lower compared with that in the abovementioned studies, ranging from 57%<sup>[10]</sup> to 73%<sup>[9]</sup>. This difference may be attributable to the timing of tumor evaluation; CT or

**Table 4** Variables associated with treatment duration

Variables	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Age ≤ 70 yr ( <i>vs</i> > 70 yr)	0.543 (0.257-1.147)	0.110		
Other etiologies ( <i>vs</i> HCV infection)	0.411 (0.191-0.886)	0.023		
DCP ≤ 1000 mAU/mL ( <i>vs</i> > 1000 mAU/mL)	0.402 (0.190-0.851)	0.017		
Nursing intervention yes ( <i>vs</i> no)	0.577 (0.278-1.198)	0.140	0.398 (0.181-0.874)	0.022
Efficacy, disease control ( <i>vs</i> PD)	0.431 (0.206-0.903)	0.026		
Adverse events				
> grade 2 + ( <i>vs</i> -)				
Skin toxicity <sup>a</sup>	0.306 (0.139-0.675)	0.003	0.225 (0.095-0.534)	0.001
Diarrhea	0.352 (0.156-0.796)	0.012		
Weight loss	0.555 (0.254-1.213)	0.140		
Alopecia	0.236 (0.081-0.686)	0.008		

<sup>a</sup>Skin toxicity includes hand-foot skin reaction and any kind of rash. HR: Hazard ratio; HCV: Hepatitis C virus; DCP: Des-gamma-carboxy prothrombin. This table only includes variables with *P* values < 0.15 in univariate analyses.

MRI was performed 3 and 1.5 mo after the initiation of sorafenib in the present and abovementioned studies, respectively. AEs were observed in all patients. Similar to previous studies, frequently observed AEs included anorexia, skin toxicity, fatigue, and diarrhea<sup>[9-11,29,30]</sup>.

Multivariate analysis indicated that age ≤ 70 years, presence of > G2 skin toxicity, and absence of > G2 hypoalbuminemia were significant predictors of longer OS. HR of patients aged ≤ 70 years against older patients was 0.35. The Asia-Pacific study reported that a beneficial effect of sorafenib was obtained only in patients aged < 65 years<sup>[10]</sup>. Therefore, an elderly patient aged > 70 years with advanced HCC may not be a good candidate for this therapy.

We demonstrated that the occurrence of skin toxicity was associated with a higher disease control rate (65% *vs* 12%; *P* = 0.002) and longer OS (HR = 0.267). These results are in concordance with those of previous studies<sup>[15,16]</sup>. Sorafenib exerts anticancer effects by inhibiting the serine-threonine kinases Raf-1 (c-Raf) and B-Raf, and the receptor tyrosine kinase activity of the vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3 and platelet-derived growth factor receptor α<sup>[31]</sup>. Nevertheless, mechanisms underlying skin toxicity induced by this drug remain largely unknown. Recent studies demonstrated that genetic polymorphisms of VEGFR<sup>[32]</sup> and VEGFR2<sup>[33]</sup> were related to the occurrence of HFSR, suggesting the involvement of VEGF signaling. Patients with a genetic predisposition to HFSR may be more sensitive to the antitumor effects of sorafenib. Further research regarding the contribution of genetic variation to skin toxicity and efficacy in this therapy is clearly required.

Other studies reported that the occurrence of hypertension<sup>[13]</sup> or diarrhea<sup>[14]</sup> was related to favorable clinical outcomes. However, we could not confirm these results in our study.

In our study, hypoalbuminemia was related to poor prognosis; this can be interpreted as a sign of progression of liver disease. In previous sorafenib studies, patients with lower pretreatment serum albumin levels had a greater risk of treatment discontinuation<sup>[28]</sup> and poor prognosis<sup>[29,34]</sup>.

Because skin toxicity was associated with better prognosis, controlling this AE potentially offers benefit to patients. Moisturizers, sunscreen creams, steroid ointments, and oral antibiotics such as doxycycline can effectively prevent skin toxicity<sup>[35,36]</sup>. In particular, applying moisturizers before initiation of sorafenib therapy and avoiding stimulation to palms and soles are important<sup>[36]</sup>.

In this study, we observed that nursing intervention significantly extended the treatment duration. Our nursing intervention program consisted of education on self-monitoring and AEs management and telephone follow-up. Improved management of AEs or removal of anxiety by telephone follow-up may contribute to these results. The importance of patient and family education and continuity of care along with the increasing use of oral anticancer drugs has been reaffirmed in previous studies<sup>[25]</sup>. However, the impact of nursing intervention on adherence or AEs management remains elusive. Nurse-led telephone follow-up for patients receiving oral capecitabine resulted in decreased occurrence of AEs compared with historical data<sup>[37]</sup>. On the other hand, a randomized controlled trial evaluating the role of nursing intervention in symptom management and treatment adherence for patients, who were prescribed oral chemotherapy agents, could not verify its efficacy<sup>[22]</sup>. Therefore, further studies in this regard are warranted. This study has limitations. This was a retrospective study, with a relatively small number of patients from a single institution.

In conclusion, our study revealed that skin toxicity may be a surrogate marker for preferable effects of sorafenib in the management of advanced HCC. Moreover, nursing intervention significantly contributed to treatment adherence. Establishment of better nursing intervention programs that can maintain adherence by controlling serious AEs is important in maximizing the efficacy of this oral anticancer drug.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Sorafenib therapy for advanced hepatocellular carcinoma (HCC) often causes adverse events (AEs), subsequently leading to dose reduction or discontinu-

ation. Conversely, a few studies have associated serious AEs with a favorable response. Contributions of nursing intervention on treatment adherence to therapy were unclear.

### Research frontiers

In this study, the authors revealed that skin toxicity may be a surrogate marker for preferable effects of sorafenib in the management of advanced HCC. Moreover, nursing intervention significantly contributed to treatment adherence. Establishment of better nursing intervention programs that can maintain adherence by controlling serious AEs is important in maximizing the efficacy of this oral anticancer drug.

### Innovations and breakthroughs

The authors demonstrated that the occurrence of skin toxicity was associated with a higher disease control rate (65% vs 12%;  $P = 0.002$ ) and longer overall survival [hazard ratio: 0.267]. These results are in concordance with those of previous studies. In this study, hypoalbuminemia was related to poor prognosis; this can be interpreted as a sign of progression of liver disease. In this study, hypoalbuminemia was related to poor prognosis; this can be interpreted as a sign of progression of liver disease. In previous sorafenib studies, patients with lower pretreatment serum albumin levels had a greater risk of treatment discontinuation and poor prognosis. This nursing intervention program consisted of education on self-monitoring and AEs management and telephone follow-up. Improved management of AEs or removal of anxiety by telephone follow-up may contribute to these results.

### Applications

This study revealed that skin toxicity may be a surrogate marker for preferable effects of sorafenib in the management of advanced HCC. Moreover, nursing intervention significantly contributed to treatment adherence. Establishment of better nursing intervention programs that can maintain adherence by controlling serious AEs is important in maximizing the efficacy of this oral anticancer drug.

### Terminology

Sorafenib, an oral multikinase inhibitor, is currently used as a standard therapeutic option for advanced HCC. Sorafenib exerts anticancer effects by inhibiting the serine-threonine kinases Raf-1 (c-Raf) and B-Raf, and the receptor tyrosine kinase activity of the vascular endothelial growth factor receptors 1, 2, and 3 and platelet-derived growth factor receptor  $\alpha$ .

### Peer review

In this study, the authors reported that significant skin toxicity (> grade 2), young age (< 70 years), and absence of hypoalbuminemia were associated with better overall survival. Significant skin toxicity and nursing intervention were associated with longer treatment duration. This paper refers to skin toxicity as predictor of efficacy to sorafenib in patients with advanced HCC. The paper is of interest since it gives important clues for better selection of patients who may benefit at best from treatment with sorafenib.

## REFERENCES

- 1 **Gomaa AI**, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World J Gastroenterol* 2008; **14**: 4300-4308 [PMID: 18666317 DOI: 10.3748/wjg.14.4300]
- 2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
- 3 **Ochiai T**, Ikoma H, Okamoto K, Kokuba Y, Sonoyama T, Otsuji E. Clinicopathologic features and risk factors for extrahepatic recurrences of hepatocellular carcinoma after curative resection. *World J Surg* 2012; **36**: 136-143 [PMID: 22051887 DOI: 10.1007/s00268-011-1317-y]
- 4 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
- 5 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojima M, Makuuchi M. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339-364 [PMID: 21829027 DOI: 10.1159/000327577]
- 6 **Kokudo N**, Nakajima J, Hatano E, Numata K. Current status of hepatocellular carcinoma treatment in Japan: practical use



- of sorafenib (Nexavar®). *Clin Drug Investig* 2012; **32** Suppl 2: 25-35 [PMID: 22873625 DOI: 10.2165/1163023-S0-000000000-00000]
- 7 **European Association for Study of Liver; European Organisation for Research and Treatment of Cancer.** EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 599-641 [PMID: 22424278 DOI: 10.1016/j.ejca.2011.12.021]
  - 8 **Verslype C, Rosmorduc O, Rougier P.** Hepatocellular carcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii41-vii48 [PMID: 22997453 DOI: 10.1093/annonc/mds225]
  - 9 **Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J.** Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
  - 10 **Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z.** Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
  - 11 **Nakano M, Tanaka M, Kuromatsu N, Nagamatsu H, Sakata K, Matsugaki S, Kajiwara M, Fukuizumi K, Tajiri N, Matsukuma N, Sakai T, Ono N, Yano Y, Koga H, Kurogi J, Takata A, Sumie S, Satani M, Yamada S, Niizeki T, Aino H, Iwamoto H, Torimura T, Sata M.** Efficacy, safety, and survival factors for sorafenib treatment in Japanese patients with advanced hepatocellular carcinoma. *Oncology* 2013; **84**: 108-114 [PMID: 23147476 DOI: 10.1159/000342650]
  - 12 **Zhang X, Yang XR, Huang XW, Wang WM, Shi RY, Xu Y, Wang Z, Qiu SJ, Fan J, Zhou J.** Sorafenib in treatment of patients with advanced hepatocellular carcinoma: a systematic review. *Hepatobiliary Pancreat Dis Int* 2012; **11**: 458-466 [PMID: 23060390 DOI: 10.1016/S1499-3872(12)60209-4]
  - 13 **Estfan B, Byrne M, Kim R.** Sorafenib in advanced hepatocellular carcinoma: hypertension as a potential surrogate marker for efficacy. *Am J Clin Oncol* 2013; **36**: 319-324 [PMID: 22547010 DOI: 10.1097/COC.0b013e3182468039]
  - 14 **Koschny R, Gotthardt D, Koehler C, Jaeger D, Stremmel W, Ganten TM.** Diarrhea is a positive outcome predictor for sorafenib treatment of advanced hepatocellular carcinoma. *Oncology* 2013; **84**: 6-13 [PMID: 23075905 DOI: 10.1159/000342425]
  - 15 **Vincenzi B, Santini D, Russo A, Addeo R, Giuliani F, Montella L, Rizzo S, Venditti O, Frezza AM, Caraglia M, Colucci G, Del Prete S, Tonini G.** Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist* 2010; **15**: 85-92 [PMID: 20051477 DOI: 10.1634/theoncologist.2009-0143]
  - 16 **Otsuka T, Eguchi Y, Kawazoe S, Yanagita K, Ario K, Kitahara K, Kawasoe H, Kato H, Mizuta T.** Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. *Hepatol Res* 2012; **42**: 879-886 [PMID: 22469363 DOI: 10.1111/j.1872-034X.2012.00991.x]
  - 17 **Senst BL, Achusim LE, Genest RP, Cosentino LA, Ford CC, Little JA, Raybon SJ, Bates DW.** Practical approach to determining costs and frequency of adverse drug events in a health care network. *Am J Health Syst Pharm* 2001; **58**: 1126-1132 [PMID: 11449856]
  - 18 **McDonnell PJ, Jacobs MR.** Hospital admissions resulting from preventable adverse drug reactions. *Ann Pharmacother* 2002; **36**: 1331-1336 [PMID: 12196047 DOI: 10.1345/aph.1A333]
  - 19 **Haynes RB, Ackloo E, Sahota N, McDonald HP, Yao X.** Interventions for enhancing medication adherence. *Cochrane Database Syst Rev* 2008; **(2)**: CD000011 [PMID: 18425859 DOI: 10.1002/14651858.CD000011]
  - 20 **Decker V, Spoelstra S, Miezio E, Bremer R, You M, Given C, Given B.** A pilot study of an automated voice response system and nursing intervention to monitor adherence to oral chemotherapy agents. *Cancer Nurs* 2009; **32**: E20-E29 [PMID: 19816160 DOI: 10.1097/NCC.0b013e3181b31114]
  - 21 **Given BA, Spoelstra SL, Grant M.** The challenges of oral agents as antineoplastic treatments. *Semin Oncol Nurs* 2011; **27**: 93-103 [PMID: 21514479 DOI: 10.1016/j.soncn.2011.02.003]
  - 22 **Spoelstra SL, Given BA, Given CW, Grant M, Sikorskii A, You M, Decker V.** An intervention to improve adherence and management of symptoms for patients prescribed oral chemotherapy agents: an exploratory study. *Cancer Nurs* 2013; **36**: 18-28 [PMID: 23235499 DOI: 10.1097/NCC.0b013e3182551587]
  - 23 **Minagawa M, Ikai I, Matsuyama Y, Yamaoka Y, Makuuchi M.** Staging of hepatocellular carcinoma: assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13,772 patients in Japan. *Ann Surg* 2007; **245**: 909-922 [PMID: 17522517 DOI: 10.1097/01.sla.0000254368.65878.da]
  - 24 **Hartigan K.** Patient education: the cornerstone of successful oral chemotherapy treatment. *Clin J Oncol Nurs* 2003; **7**: 21-24 [PMID: 14705496 DOI: 10.1188/03.CJON.S6.21-24]
  - 25 **Neuss MN, Polovich M, McNiff K, Esper P, Gilmore TR, LeFebvre KB, Schulmeister L, Jacobson JO.** 2013 updated American Society of Clinical Oncology/Oncology Nursing Society chemotherapy administration safety standards including standards for the safe administration and management of oral chemotherapy. *Oncol Nurs Forum* 2013; **40**: 225-233 [PMID: 23619103 DOI: 10.1188/13.ONF.40-03AP2]
  - 26 **Lencioni R, Llovet JM.** Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]
  - 27 **Japan Clinical Oncology Group.** Common Terminology Criteria for Adverse Events (CTCAE) v4.0-Japan Clinical Oncology Group. Available from: URL: <http://www.jcog.jp/doctor/tool/ctcae4.html>, 2013 (accessed Jul 29, 2013)
  - 28 **Mizukami H, Kagawa T, Arase Y, Nakahara F, Tsuruya K, Anzai K, Hirose S, Shiraishi K, Shomura M, Koizumi J, Tobita K, Mine T.** Complete response after short-term sorafenib treatment in a patient with lymph node metastasis of hepatocellular carcinoma. *Case Rep Oncol* 2012; **5**: 380-384 [PMID: 23525021 DOI: 10.1159/000341259]
  - 29 **Lencioni R, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JF, Ladrón de Guevara L, Papandreou C, Sanyal AJ, Takayama T, Yoon SK, Nakajima K, Cihon F, Heldner S, Marrero JA.** First interim analysis of the GIDEON (Global Investigation of therapeutic decisions in hepatocellular carcinoma and of its treatment with sorafenib) non-interventional study. *Int J Clin Pract* 2012; **66**: 675-683 [PMID: 22698419 DOI: 10.1111/j.1742-1241.2012.02940.x]
  - 30 **Iavarone M, Cabibbo G, Piscaglia F, Zavaglia C, Grieco A, Villa E, Cammà C, Colombo M.** Field-practice study of sorafenib therapy for hepatocellular carcinoma: a prospective multicenter study in Italy. *Hepatology* 2011; **54**: 2055-2063 [PMID: 21898496 DOI: 10.1002/hep.24644]
  - 31 **Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M.** Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther* 2008; **7**: 3129-3140 [PMID: 18852116 DOI: 10.1158/1535-7163.MCT-08-0013]
  - 32 **Lee JH, Chung YH, Kim JA, Shim JH, Lee D, Lee HC, Shin ES, Yoon JH, Kim BI, Bae SH, Koh KC, Park NH.** Genetic predisposition of hand-foot skin reaction after sorafenib therapy in patients with hepatocellular carcinoma. *Cancer* 2013; **119**: 136-142 [PMID: 22736425 DOI: 10.1002/cncr.27705]
  - 33 **Jain L, Sissung TM, Danesi R, Kohn EC, Dahut WL, Kummars S, Venzon D, Liewehr D, English BC, Baum CE, Yarchoan R, Giaccone G, Venitz J, Price DK, Figg WD.** Hypertension

- and hand-foot skin reactions related to VEGFR2 genotype and improved clinical outcome following bevacizumab and sorafenib. *J Exp Clin Cancer Res* 2010; **29**: 95 [PMID: 20630084 DOI: 10.1186/1756-9966-29-95]
- 34 **Morimoto M**, Numata K, Moriya S, Kondo M, Nozaki A, Morioka Y, Maeda S, Tanaka K. Inflammation-based prognostic score for hepatocellular carcinoma patients on sorafenib treatment. *Anticancer Res* 2012; **32**: 619-623 [PMID: 22287754]
  - 35 **Lacouture ME**, Mitchell EP, Piperdi B, Pillai MV, Shearer H, Iannotti N, Xu F, Yassine M. Skin toxicity evaluation protocol with panitumumab (STEPP), a phase II, open-label, randomized trial evaluating the impact of a pre-Emptive Skin treatment regimen on skin toxicities and quality of life in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 1351-1357 [PMID: 20142600 DOI: 10.1200/JCO.2008.21.7828]
  - 36 **Balagula Y**, Garbe C, Myskowski PL, Hauschild A, Rapoport BL, Boers-Doets CB, Lacouture ME. Clinical presentation and management of dermatological toxicities of epidermal growth factor receptor inhibitors. *Int J Dermatol* 2011; **50**: 129-146 [PMID: 21244375 DOI: 10.1111/j.1365-4632.2010.04791.x]
  - 37 **Craven O**, Hughes CA, Burton A, Saunders MP, Molassiotis A. Is a nurse-led telephone intervention a viable alternative to nurse-led home care and standard care for patients receiving oral capecitabine? Results from a large prospective audit in patients with colorectal cancer. *Eur J Cancer Care (Engl)* 2013; **22**: 413-419 [PMID: 23527965 DOI: 10.1111/ecc.12047]

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## Insulin resistance and steatosis in HBV-HCV co-infected patients: Role of PNPLA3 polymorphisms and impact on liver fibrosis progression

Rosa Zampino, Nicola Coppola, Grazia Cirillo, Adriana Boemio, Carmine Minichini, Aldo Marrone, Maria Stanzione, Mario Starace, Emanuele Durante-Mangoni, Evangelista Sagnelli, Luciano Restivo, Giovanna Salzillo, Maria Chiara Fascione, Riccardo Nevola, Emanuele Miraglia del Giudice, Luigi Elio Adinolfi

Rosa Zampino, Adriana Boemio, Aldo Marrone, Luciano Restivo, Maria Chiara Fascione, Riccardo Nevola, Luigi Elio Adinolfi, Department of Medical, Surgical, Neurological, Metabolic, and Geriatric Sciences, Second University of Naples, 80100 Naples, Italy

Nicola Coppola, Carmine Minichini, Mario Starace, Evangelista Sagnelli, Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, 80100 Naples, Italy

Grazia Cirillo, Emanuele Miraglia del Giudice, Department of Pediatrics, Second University of Naples, 80100 Naples, Italy

Maria Stanzione, Department of Clinical and Experimental Medicine and Surgery, "F. Magrassi e A. Lanzara", Second University of Naples, 80100 Naples, Italy

Emanuele Durante-Mangoni, Internal Medicine Monaldi Hospital, Second University of 80100 Naples, Italy

Luciano Restivo, Giovanna Salzillo, Luigi Elio Adinolfi, Clinical Hospital of Marcianise, ASL Caserta, 81025 Marcianise (CE), Italy

**Author contributions:** Zampino R and Coppola N conceived and drafted the manuscript; Cirillo G, Boemio A, Minichini C, Starace M and Salzillo G carried out laboratory work; Marrone A, Stanzione M, Durante-Mangoni E, Restivo L and Fascione MC co-operated in patients enrollment; Sagnelli E and del Giudice EM critically reviewed the manuscript; Adinolfi LE conceived, draft and critically reviewed the manuscript; all authors approved the final version of the manuscript.

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**Correspondence to:** Luigi Elio Adinolfi, MD, Professor, Clinical Hospital of Marcianise, ASL Caserta, Rione Santella, 81025 Marcianise (CE), Italy. [luigi.elinio.adinolfi@unina2.it](mailto:luigi.elinio.adinolfi@unina2.it)

Telephone: +39-0823-690642 Fax: +39-0823-690642

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and patatin-like phospholipase domain-containing 3 (PNPLA3) and their relation to disease progression in hepatitis B and C viruses (HCV-HBV) co-infected patients.

**METHODS:** Three hundred and thirty patients with biopsy proven chronic hepatitis were enrolled: 66 had HBV-HCV, 66 HBV and 198 HCV infection. Prevalence of steatosis, IR and PNPLA3 polymorphisms and their relation to anthropometric, biochemical, virological and histological parameters were evaluated.

**RESULTS:** Prevalence of steatosis in group HBV-HCV was similar to that in HCV (47.0% *vs* 49.5%, respectively); group HBV showed the lowest steatosis (33.3%). Group HBV-HCV had a lesser degree of steatosis than HCV ( $P = 0.016$ ), lower HCV RNA levels ( $P = 0.025$ ) and lower prevalence and degree of IR ( $P = 0.01$ ). PNPLA3 polymorphisms were associated with steatosis. Group HBV-HCV showed higher levels of liver fibrosis than group HCV ( $P = 0.001$ ), but similar to that observed in HBV group. In HBV-HCV group, liver fibrosis was not associated with steatosis, IR or PNPLA3. HBV infection was the independent predictor of advanced liver fibrosis.

**CONCLUSION:** HBV-HCV co-infected patients have lower degree of hepatic steatosis, IR and HCV RNA than HCV mono-infected; co-infected patients showed a more rapid liver fibrosis progression that seems to be due to the double infection and/or HBV dominance.

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### Abstract

**AIM:** To evaluate steatosis, insulin resistance (IR)

**Key words:** Steatosis; Insulin resistance; Hepatitis B and C viruses co-infection; Patatin-like phospholipase domain-containing 3; Liver fibrosis

**Core tip:** We evaluated the prevalence and role of steatosis, insulin resistance and patatin-like phospholipase domain-containing 3 (PNPLA3) polymorphisms on disease progression in hepatitis B and C viruses (HCV-HBV) co-infected patients. The data showed that HBV-HCV patients have lower levels of liver steatosis and insulin resistance than HCV mono-infected patients. HBV seems to interact with HCV reducing HCV replication and HCV-related metabolic features. Thus, the influence of HCV-related steatosis and insulin resistance as well as PNPLA3 polymorphism do not significantly impact liver fibrosis progression in HBV-HCV patients. The more rapid progression of liver fibrosis observed in HBV-HCV co-infected patients seems to be mostly associated with HBV infection.

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## INTRODUCTION

Liver steatosis is a feature of chronic hepatitis C virus (HCV) infection<sup>[1-3]</sup>. HCV genotype 3 directly induces the highest degree and prevalence of steatosis (up to 80%), whereas HCV-related steatosis in non-3 genotypes is mainly associated with metabolic conditions<sup>[4]</sup>. A close association between steatosis and insulin resistance (IR) has been reported in HCV non-3 genotype-infected patients, but, normally, insulin resistance is not a feature of genotype-3 infection<sup>[2]</sup>. In HCV infection, IR precedes the development of steatosis and modulates fatty liver deposition through several, non-mutually exclusive, mechanisms; the appearance of steatosis, in turn, worsens IR<sup>[2,5]</sup>. Furthermore, it has been reported that in genotype-1 infection, IR correlates with the serum level of HCV RNA<sup>[6-7]</sup>. Both steatosis and IR are associated with a more rapid progression of liver fibrosis<sup>[1,8]</sup>. In chronic hepatitis B virus (HBV) infection, hepatic steatosis has been reported with a lower prevalence<sup>[9,10]</sup> than that observed in HCV infection, although one report<sup>[11]</sup> showed a high prevalence of steatosis in HBV-infected patients. Furthermore, in HBV infection, steatosis seems to be related to metabolic factors and does not seem to correlate with histological hepatic damage<sup>[9,12-14]</sup>. Recently, the single nucleotide polymorphism (SNP) of the patatin-like phospholipase domain-containing 3 (PNPLA3) gene, involved in the lipid metabolism, has been associated with liver steatosis in chronic hepatitis<sup>[15-20]</sup>.

Chronic HBV-HCV co-infection is infrequent, but it is associated with a more severe clinical presentation<sup>[21-25]</sup> and with a more rapid progression to liver cirrhosis and

hepatocellular carcinoma<sup>[26-28]</sup>. There are no direct data on liver steatosis and IR in patients with HBV-HCV co-infection, nor on their impact on the progression of the liver disease. During HBV-HCV co-infection, a reciprocal inhibition of the viral genomes has been reported<sup>[29-31]</sup> and this condition, especially in HCV-genotype-1 infection, could influence the development of IR and steatosis. Thus, at present, it remains unclear whether HBV infection affects the prevalence and level of steatosis and IR in HBV-HCV co-infected patients and their impact on liver disease progression.

Accordingly, the aim of this study was to evaluate the prevalence and degree of liver steatosis and IR and their role in the progression of liver disease in a cohort of HBV-HCV co-infected patients as compared with a cohort of HBV and HCV mono-infected patients. The role of the viral and host metabolic and genetic factors, such as PNPLA3 polymorphisms, was also evaluated.

## MATERIALS AND METHODS

### Patients

Three hundred and thirty Caucasian patients with histology proven chronic hepatitis were enrolled in the study. Sixty-six were HBV-HCV co-infected patients, 66 HBV mono-infected (ratio 1:1) and 198 HCV mono-infected (ratio 1:3). HBV-HCV co-infected patients were age-, gender-, and HCV genotype-matched with control mono-infected groups. The study was conducted from 2009 to 2013. However, considering the low prevalence of HBV-HCV co-infected patients, all HBV-HCV co-infected patients recorded in the data base from 2006 were enrolled if there was a serum sample stored at -30 °C at the time of the liver biopsy and if there was a sample available for genetic purposes.

Patients were recruited from four Liver Units (see author's affiliations) of the Second University of Naples, Italy. The patients were considered co-infected and enrolled in the study if they were HBs Ag positive/HCV-Ab positive/HBV-DNA and HCV-RNA positive; all patients HBV and HCV mono-infected were HBV-DNA positive and HCV-RNA positive, respectively. All patients included were anti-HIV- and anti-HDV-negative, naive for antiviral therapy and reported no active intravenous drug addiction or daily alcohol intake over 30 g. The possible source of infection was identified only in the minority of the enrolled patients; in fact, anamnestic blood transfusion was present in 8%, previous surgery in 4%, a family history of hepatitis infection in 4%, and a past history of drug abuse in 1.8%. All patients underwent complete physical examination, full liver function tests, fasting glucose, triglycerides, cholesterol, blood cell counts, viral markers (HBV, HCV, HDV, HIV) and liver ultrasound scan. The body mass index (BMI: kg/m<sup>2</sup>) and waist circumference were recorded for all patients. Visceral obesity was defined as waist circumference > 102 cm in male and > 88 in female. An anamnestic estimation of possible duration of infection was made. All laboratory data presented in this study refer to the values at the time



of the liver biopsy. All blood samples were withdrawn at the time of liver biopsy and serum were stored at -30 °C within two hours from collection.

### **Serum insulin and homeostasis model assessment-insulin resistance**

Serum insulin was evaluated using human insulin immunoassay (Insulin Cobas, Roche Diagnostics, Indianapolis, IN, United States). IR was determined by homeostasis model assessment-insulin resistance (HOMA-IR) using the following formula: fasting plasma glucose (mmol/dL) x fasting serum insulin (IU/mL)]/22.5. To establish the cut-off level of IR in our population, HOMA-IR was evaluated in 130 healthy subjects and the cut-off value was set at the 75<sup>th</sup> percentile of the HOMA-IR value in our mono-infected control groups<sup>[32]</sup> that was 2.60. In the three groups evaluated the determination of levels of insulinemia and glycaemia were done at the same time using the stored serum and samples from the three groups were analysed in parallel.

### **Liver histology**

All patients gave their informed consent for liver biopsy. Liver specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin and Masson's trichrome stain and evaluated in a blinded way by the pathologist. The Ishak scores to grade necro-inflammation and fibrosis were used<sup>[33]</sup>. Steatosis was scored as follows: 0, if less than 5% of hepatocytes had fatty deposition; (1) from 5% to 29%; (2) from 30% to 59%; and (3) > 60%.

All the evaluations were conducted in accordance with good clinical practice and with the Helsinki Declaration. The local Ethics Committee approved the study.

### **Serological determinations**

Serum markers for HBV, HCV, HDV and HIV infection were sought in serum using commercially available immune-enzymatic assays (Abbott Laboratories, North Chicago, IL and Ortho Diagnostic Systems, Raritan, NJ).

### **HBV and HCV genotypes and viral load**

Hepatitis B virus genotypes were determined by phylogenetic analysis of sequences of 400 nt of the S region, as previously described<sup>[34]</sup>. HCV genotypes were determined using immunoblotting HCV genotype assay Lipa (VER-SANT HCV Genotype 2.0 Assay (LIPA), Siemens, Erlangen, Germany) following the manufacturer's instructions. HBV DNA and HCV RNA viral load were assessed by real-time PCR using commercial kits (COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0, COBAS® TaqMan® HCV Test v2.0; Roche diagnostics, S.p.A. Monza, Italy).

### **PNPLA3 polymorphism study**

Genomic DNA was extracted from whole blood by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and analyzed for the PNPLA3 polymorphism. All patients were genotyped for the PNPLA3 rs738409 C to G variant underlying the I148M substitution. The following prim-

ers were used, F: 5'-GCCCTGCTCACTTGGAGAAA-3' and R: 5'-TGAAAGGCAGTGAGGCATGG-3'. The FokI restriction enzyme, as previously described, was used to identify the variant, since the G allele eliminates a FokI restriction site. Random samples were confirmed by direct genotyping, which provided concordant results in all cases<sup>[35]</sup>.

### **Statistical analysis**

The data are expressed as mean or median. Differences between the groups were evaluated by Student's *t* test for parametric data and by the Mann-Whitney *U* test for non-parametric data. Spearman's correlation test was used to identify factors significantly associated. The Chi-square test was used to evaluate differences in the prevalence. The analysis of variance was used to evaluate the different distribution of steatosis in the genetic polymorphisms. Logistic regression analysis was used to evaluate the independent factors associated with advanced liver fibrosis. The data were analysed using SPSS 13.5, and *P* < 0.05 was assumed to denote significance.

## **RESULTS**

### **General characteristics of patients**

The general characteristics of the study population are shown in Table 1. The three groups were comparable for demographic and anthropometric parameters. The approximate duration of the disease and the lipid profile were similar in the three groups. Waist circumference was similar in groups HCV-HBV and HCV ( $91.7 \pm 9.8$  and  $90.2 \pm 10$ , respectively). The serum glucose levels were lower, albeit not significantly, in group HBV-HCV than in group HCV (*P* = 0.09). The AST values were significantly lower in the co-infected patients than in the HBV mono-infected (*P* = 0.02), while the ALT values were significantly lower in the co-infected than in the HBV and HCV mono-infected (*P* = 0.001).

### **Virological characteristics of patients**

The median values of the HBV DNA and HCV RNA levels are shown in Table 1. HBV-HCV co-infected patients showed lower levels of HBV DNA and HCV RNA than those observed in the HBV and HCV mono-infected patients (*P* = 0.0001 and *P* = 0.025, respectively). The majority (93%) of the HCV patients were infected by genotype non-3 (Table 1) and 98% of HBV patients had genotype D.

### **Steatosis and IR**

Table 2 shows the prevalence and degree of steatosis in the three groups. There was no difference in the prevalence of liver steatosis between group HBV-HCV and group HCV (47.0% *vs* 49.5%), but a lower prevalence of steatosis was observed in group HBV (34%). An analysis of the degree of steatosis showed higher levels (scores 2-3) in group HCV than in group HBV-HCV (*P* = 0.016, Table 2). The above results did not change when the

**Table 1** General characteristics of the 330 patients include in the study

	HBV-HCV group	HBV group	HCV group	P
No. of patients	66	66	198	
Median age (range)	48.5 (25-67)	47 (23-65)	50 (22-65)	NS
Males	60.60%	63.60%	55.50%	NS
Disease duration (yr $\pm$ SD)	22.3 $\pm$ 9.7	21 $\pm$ 7.6	23.2 $\pm$ 8.4	NS
BMI (mean $\pm$ SD)	25.7 $\pm$ 3	26 $\pm$ 4.5	26.7 $\pm$ 4	NS
Glycaemia (mean $\pm$ SD) mg/dL	90 $\pm$ 13.4	85.8 $\pm$ 14.4 <sup>a</sup>	95 $\pm$ 20 <sup>b</sup>	0.09 (a vs b)
HOMA	2.48 $\pm$ 2.65 <sup>c</sup>	2.0 $\pm$ 1.17	3.63 $\pm$ 4.5 <sup>d</sup>	0.042 (c vs d)
Cholesterol (mean $\pm$ SD) mg/dL	182 $\pm$ 34	182 $\pm$ 31	182 $\pm$ 41	NS
Triglycerides (mean $\pm$ SD) mg/dL	109 $\pm$ 55	85 $\pm$ 29	103 $\pm$ 53	NS
AST (mean $\pm$ SD) IU/L	55 $\pm$ 39 <sup>e</sup>	83 $\pm$ 84 <sup>f</sup>	65 $\pm$ 52	0.02 (e vs f)
ALT (mean $\pm$ SD), IU/L	44 $\pm$ 62.5 <sup>g</sup>	124.95 $\pm$ 92 <sup>h</sup>	90 $\pm$ 74 <sup>i</sup>	0.001 (g vs h) 0.001 (g vs i)
Median HBV DNA (range) IU/mL	1.9 $\times$ 10 <sup>3</sup> (1500-10 $\times$ 10 <sup>7</sup> )	2 $\times$ 10 <sup>5</sup> (3000-1 $\times$ 10 <sup>8</sup> )		0.0001
Median HCV RNA (range) IU/mL	1.15 $\times$ 10 <sup>5</sup> (120- 6.4 $\times$ 10 <sup>5</sup> )		6.98 $\times$ 10 <sup>5</sup> (2818-8 $\times$ 10 <sup>6</sup> )	0.025
HCV genotype:				
3	7%		8.7%	NS
Non-3	93%		91.3%	
HAI score (mean $\pm$ SD)	5.9 $\pm$ 2.9	6.2 $\pm$ 3.4	6.3 $\pm$ 3.6	NS
Fibrosis score (mean $\pm$ SD)	3.32 $\pm$ 0.45 <sup>l</sup>	3.46 $\pm$ 0.48 <sup>m</sup>	2.9 $\pm$ 0.30 <sup>n</sup>	0.001 (l vs n)
				0.001 (m vs n)

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HOMA: Homeostasis model assessment; AST: Aspartate transferase; ALT: Alanine transferase; NS: No significant.

**Table 2** Steatosis prevalence and distribution in the different groups

	Steatosis prevalence	Steatosis grade 1	Steatosis grade 2-3
HBV-HCV group (n = 66)	47.0%	43.6%	3.4%
HBV group (n = 66)	33.3%	21.2%	12.1%
HCV group (n = 198)	49.5%	23.2%	26.3% <sup>a</sup>

<sup>a</sup>P = 0.016 vs group HBV-HCV. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

analysis was done excluding patients with HCV genotype 3, but considering the low number of patients with genotype 3 the results deserve further evaluation. In group HBV, a higher degree of steatosis (score 2-3) was closely associated with obesity (BMI > 30).

Figure 1 shows the mean serum levels of HOMA-IR in the three groups studied. HBV-HCV co-infected patients showed an intermediate value of HOMA-IR, *i.e.*, between the highest level in group HCV and the lowest in group HBV, however, such value was significantly lower than that observed in HCV but not significantly higher than that observed in group HBV. Similarly, the prevalence of IR (HOMA-IR cut-off > 2.60) in the HBV-HCV co-infected patients was lower than that observed in group HCV (21% vs 54%, P = 0.005), but not significantly different from that observed in group HBV (23%).

The relation between IR and steatosis was evaluated and, as expected, in group HCV a correlation between the levels of IR and steatosis was observed ( $r = 0.27$ ; P = 0.006), whereas, such a correlation was not seen in the HBV-HCV co-infected group (data not shown).

### PNPLA3 polymorphisms and steatosis

Table 3 shows the distribution of the PNPLA3 polymor-

**Table 3** Distribution of patatin-like phospholipase domain-containing 3 polymorphisms and their relation to steatosis n (%)

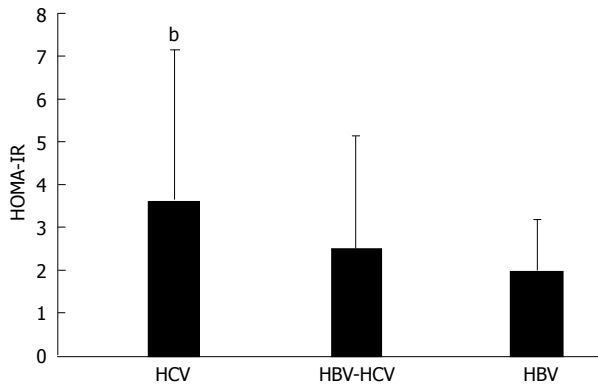
PNPLA3	Steatosis no	Steatosis yes	Steatosis score 1	Steatosis score 2-3
Overall				
p.148I/I	83 (55)	65 (36)	44%	23%
p.148I/M	62 (41)	82 (46)	49%	40%
p.148M/M	6 (4)	32 (18) <sup>b</sup>	7%	37%
HBV/HBV-HCV groups				
p.148I/I	32 (61)	36 (45.6)	55%	12.5%
p.148I/M	20 (38)	33 (41.8)	42%	37.5%
p.148M/M	1 (1)	10 (12.6) <sup>d</sup>	3%	50.0%
HCV group				
p.148I/I	53 (54)	44 (44)	60%	17%
p.148I/M	43 (44)	31 (31)	30%	33%
p.148M/M	2 (2)	25 (25) <sup>f</sup>	10%	50%

<sup>b</sup>P = 0.0001; <sup>d</sup>P = 0.0001; <sup>f</sup>P = 0.0001 vs Steatosis no group. PNPLA3: Patatin-like phospholipase domain-containing 3; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

phisms. In accordance with our recently published data<sup>[19]</sup>, the results of the present study showed that the PNPLA3 I148M polymorphism was associated with a more severe degree of steatosis both in groups HCV and HBV-HCV (P = 0.003). An analysis of the overall study population confirmed that the PNPLA3 I148M polymorphism caused a predisposition to liver steatosis (P = 0.001). In Table 3, the data have been showed aggregate (HBV and HBV-HCV groups) considering that similar results have been obtained.

### Liver fibrosis progression

The data given in Table 1 show that HBV-HCV co-infected patients had similar levels of liver fibrosis to those observed in group HBV, but significantly higher than those observed in group HCV (P = 0.001). This higher



**Figure 1** Homeostasis model assessment-insulin resistance in the three groups of patients. <sup>b</sup> $P < 0.001$ , HCV vs HBV-HCV and HBV groups. HOMA-IR: Homeostasis model assessment-insulin resistance; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

degree of fibrosis in HBV-HCV group was independent of necro-inflammatory activity, because the HAI was similar in the three groups (Table 1), and, in addition, was not independently associated with liver steatosis, IR or PNPLA3 polymorphisms.

#### Factors associated with liver fibrosis

An overall evaluation, including all groups, on the factors associated with advanced liver fibrosis showed that the presence of HBV ( $P = 0.0001$ ), age ( $P = 0.031$ ), liver necro-inflammation ( $P = 0.02$ ), and liver steatosis ( $P = 0.047$ ) were the factors associated at univariate analysis with liver fibrosis. Regression analysis showed that HBV was the only independent factor associated with advanced fibrosis (coefficient B, 0.214; standard error of B, 0.055; 95%CI, lower: 0.104 - higher: 0.323;  $P = 0.0001$ ).

## DISCUSSION

In the present study, we explored the prevalence and possible role of liver steatosis and IR on liver disease progression in patients with chronic HBV-HCV co-infection. The data show that HBV-HCV co-infection does not influence the well-known capacity of HCV to induce steatosis; HBV-HCV co-infected patients showed a lesser amount of liver fat accumulation in comparison with HCV-infected patients. In addition, the results of this study demonstrate that HBV-HCV co-infected patients had lower serum levels of HCV RNA, a lower prevalence and degree of IR, and despite a similar duration of the disease, HBV-HCV patients showed higher levels of liver fibrosis than those observed in HCV mono-infected patients. The data suggest that HBV may interact with HCV and change some HCV metabolic characteristics. The mechanisms implicated in such interaction are not known, but some hypotheses can be made based on the results of this study.

Hepatic steatosis in chronic HCV infection is associated with alterations in the lipid and glucose metabolism<sup>[36,37]</sup>. IR in HCV infection has been reported in up to 80% of cases<sup>[38]</sup>. A close association between steatosis

and IR has been observed in HCV genotype non-3-infected patients, but IR is not generally a feature of genotype-3 infection<sup>[39,40]</sup>. HCV genotype-1-infected patients have higher prevalence of impaired glucose metabolism, and IR is correlated with the level of viral replication<sup>[6,7]</sup>. In HCV infection, IR precedes the development of steatosis and modulates fatty liver deposition<sup>[41,42]</sup>. The data of the present study show that HBV-HCV co-infected patients had lower levels of HCV RNA, IR and glucose. A fluctuating virological profile related to mutual HBV-HCV interference and the effect of this biological process on the clinical presentation and treatment strategy have been described<sup>[29,43]</sup>. It is possible that viral interference between HBV and HCV in hepatocytes might control or modulate the interaction between HCV and the lipid and glucose metabolism. However, a recent *in vitro* study<sup>[44]</sup> supports the hypothesis that HBV and HCV can replicate in the same cell without evidence of direct interference and that the *in vivo* effects may depend on the host immune response. However, the extensive virological and molecular interactions between the two viruses in co-infected patients are not well understood. Evidence seem to indicate that an inverse relationship occurs in the replication levels of the two viruses, suggesting direct or indirect viral interference<sup>[44,45]</sup>. Studies *in vitro* showed that the HCV core protein suppresses HBV replication<sup>[29,46,47]</sup>. On the other hand, an inhibition of HCV replication in patients with chronic hepatitis C who were super-infected with HBV have also been demonstrated<sup>[21,48]</sup>. Thus, the type of interaction between these two viruses in patients who are co-infected may be influenced by which virus infection is experienced first<sup>[24]</sup>. On these bases, our results seem to confirm that HBV “interference” induces lower levels of HCV replication, which may not support a significant development of IR and, in turn, not favor high amounts of liver fat deposition. Future experimental studies analyzing the effects of HBV replication on the development of IR and steatosis in HBV-HCV co-infected cells could produce interesting results.

It is well known that metabolic factors, in particular high levels of steatosis and IR are associated with a decreased likelihood of achieving a sustained virological response with interferon-based treatment<sup>[49-51]</sup>, but little information is available for protease-inhibitor regimens<sup>[52]</sup>. Thus, determining the metabolic profile in HBV-HCV patients could prove useful to predict the outcome of treatment for these patients, but specific studies are necessary.

In accordance with the data available on the correlation between the PNPLA3 I148M variant and liver steatosis in NAFLD and in chronic HCV and HBV infection<sup>[15-20]</sup>, the data of this study confirm the independent role of the PNPLA3 polymorphisms in inducing high degree of steatosis.

It has been well established that in chronic HCV infection, IR, a high degree of steatosis (greater than 20%-30%) and higher levels of glucose are associated with a more rapid progression of liver fibrosis<sup>[1,39]</sup>. Although the data from this study showed that HBV-HCV

co-infected patients had a more “favorable” anti-fibrotic metabolic profile, these patients had higher levels of liver fibrosis than those observed in HCV-infected patients. These data seem to indicate a prominent “direct” viral effect of the two viruses, rather than HCV-related metabolic factors, in the progression of liver fibrosis. Alternatively, considering that the levels of fibrosis in HBV-HCV co-infected patients are similar to those observed in the HBV mono-infected, and that HBV is the independent factor associated with advanced fibrosis, it is possible that HBV infection plays a dominant role in the progression of liver fibrosis.

It is necessary to underline that this study has some limitations; first, it is a cross-sectional study conducted in one geographic area; second, the very low number of HCV genotype 3 enrolled do not permit to draw conclusion about the role of genotype; third, due to the very low frequency of occurrence of double infection, a relative low number of patients have been included in the HBV-HCV co-infected group. However, despite these limitations, this study represents the essential basis for a future larger multicenter study evaluating the interaction between HBV and HCV infection.

In conclusion, the results of this study demonstrate that in HBV-HCV co-infected patients a high degree of liver steatosis is uncommon, possibly due to reciprocal viral interference causing lower levels of HCV replication and subsequently lower levels of IR. However, despite the “anti-fibrotic” metabolic profile observed, HBV-HCV co-infected patients had a higher degree of fibrosis, probably due to the dual infection and/or HBV dominance. Thus, in the unstandardized complex therapeutic managements of HBV-HCV co-infected patients an early control of HBV infection could be of importance to avoid the rapid progression of liver fibrosis.

## COMMENTS

### Background

Liver steatosis and insulin resistance (IR) are closely associated with chronic hepatitis C infection. The pathogenic link between steatosis, IR and chronic hepatitis C virus (HCV) infection is complex and it is associated with both viral and host factors. A host genetic factor, such as the polymorphism of the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene, involved in the lipid metabolism, is associated with liver steatosis in chronic hepatitis of different etiology. Both liver steatosis and IR are associated with a more rapid progression to liver cirrhosis. In chronic hepatitis B virus (HBV) infection, hepatic steatosis and IR have been reported with a lower prevalence than that observed in HCV infection. Chronic HBV-HCV co-infection is associated with a more rapid progression to liver cirrhosis. During HBV-HCV co-infection, a reciprocal inhibition of the viral genomes has been reported that could influence both steatosis and IR. There are no direct data on prevalence and pathogenic role of liver steatosis and IR in patients with HBV-HCV co-infection.

### Research frontiers

At present, it remains unclear whether HBV infection affects the prevalence and level of steatosis and IR as well as the role of *PNPLA3* in HBV-HCV co-infected patients and their impact on liver disease progression. The role of insulin resistance as promoting factor for liver steatosis and of this latter in promoting liver fibrosis has been extensively demonstrated in non-alcoholic fatty liver disease and in HCV related chronic hepatitis.

### Innovations and breakthroughs

The study explores the unknown area of interaction between HBV with HCV on

development of IR and liver steatosis, the role of *PNPLA3* gene polymorphisms, and their impact on the progression of liver disease. The results seem to indicate that HBV interacts with HCV reducing HCV replication and HCV-related metabolic features. Thus, steatosis and IR as well as *PNPLA3* polymorphism do not significantly impact liver fibrosis progression in HBV-HCV patients. The more rapid progression of liver fibrosis observed in HBV-HCV co-infected patients seems to be mostly associated with HBV infection.

### Applications

The knowledge of factors that influence the liver disease progression can improve therapeutic strategy in HBV-HCV co-infected patients.

### Terminology

Liver steatosis is considered as a burden greater than 5% of triglycerides and other fats inside liver cells; it is the hepatic manifestation of the metabolic syndrome and contributes to progression of liver disease. Insulin resistance is a reduced ability of body tissues to respond to insulin, thus larger quantities of insulin are needed to maintain normal blood levels of glucose. It contributes to serious health problems including type 2 diabetes and metabolic syndrome. The *PNPLA3* is a gene, involved in the lipid metabolism and has been associated with liver steatosis.

### Peer review

The authors reported that a close association between steatosis and IR has been reported in HCV non-3 genotype-infected patients. The pathogenetic link between IR and chronic HCV infection is complex and is associated with HCV genotype.

## REFERENCES

- 1 Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364 [PMID: 11391523]
- 2 Adinolfi LE, Restivo L, Marrone A. The predictive value of steatosis in hepatitis C virus infection. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 205-213 [PMID: 23445230 DOI: 10.1586/egh.13.7]
- 3 Powell EE, Jonsson JR, Clouston AD. Steatosis: co-factor in other liver diseases. *Hepatology* 2005; **42**: 5-13 [PMID: 15962320]
- 4 Loria A, Loria P, Adinolfi LE, Carulli N, Ruggiero G. Hepatitis C and steatosis: a reappraisal. *J Viral Hepat* 2006; **13**: 73-80 [PMID: 16436124 DOI: 10.1111/j.1365-2893.2005.00669.x]
- 5 Vidali M, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, Sutti S, Marrone A, Ruggiero G, Albano E, Adinolfi LE. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. *J Hepatol* 2008; **48**: 399-406 [PMID: 18164507 DOI: 10.1016/j.jhep.2007.10.011]
- 6 Adinolfi LE, Zampino R, Restivo L, Guerrera B, Santoro A, Cierro A, Rinaldi L, Scialdone VR, Ferrara M, Ruggiero G. HCV infection impairs glucose metabolism which promotes a more aggressive course of liver disease and metabolic syndrome. *J Hepatol* 2010; **52** (Suppl 1): S401-S402
- 7 Dai CY, Yeh ML, Huang CF, Hou CH, Hsieh MY, Huang JF, Lin IL, Lin ZY, Chen SC, Wang LY, Chuang WL, Yu ML, Tung HD. Chronic hepatitis C infection is associated with insulin resistance and lipid profiles. *J Gastroenterol Hepatol* 2013 Jun 28; Epub ahead of print [PMID: 23808794 DOI: 10.1111/jgh.12313]
- 8 Petta S, Cammà C, Di Marco V, Alessi N, Cabibi D, Caldarello R, Licata A, Massenti F, Tarantino G, Marchesini G, Craxi A. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *Am J Gastroenterol* 2008; **103**: 1136-1144 [PMID: 18477344 DOI: 10.1111/j.1572-0241.2008.01813.x]
- 9 Machado MV, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. *J Gastroenterol Hepatol* 2011; **26**: 1361-1367 [PMID: 21649726]



- DOI: 10.1111/j.1440-1746.2011.06801.x]
- 10 **Bugianesi E**, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? *J Hepatol* 2012; **56** Suppl 1: S56-S65 [PMID: 22300466 DOI: 10.1016/S0168-8278(12)60007-5]
  - 11 **Petta S**, Tripodo C, Grimaudo S, Cabibi D, Cammà C, Di Cristina A, Di Marco V, Di Vita G, Ingraio S, Mazzola A, Marchesini G, Pipitone R, Craxi A. High liver RBP4 protein content is associated with histological features in patients with genotype 1 chronic hepatitis C and with nonalcoholic steatohepatitis. *Dig Liver Dis* 2011; **43**: 404-410 [PMID: 21324757 DOI: 10.1016/j.dld.2010.12.013]
  - 12 **Wong VW**, Chu WC, Wong GL, Chan RS, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Woo J, Chan FK, Chan HL. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut* 2012; **61**: 409-415 [PMID: 21846782 DOI: 10.1136/gutjnl-2011-300342]
  - 13 **Rastogi A**, Sakhuja P, Kumar A, Hissar S, Jain A, Gondal R, Sarin SK. Steatosis in chronic hepatitis B: prevalence and correlation with biochemical, histologic, viral, and metabolic parameters. *Indian J Pathol Microbiol* 2011; **54**: 454-459 [PMID: 21934202 DOI: 10.4103/0377-4929.85074]
  - 14 **Zheng RD**, Chen JN, Zhuang QY, Lu YH, Chen J, Chen BF. Clinical and virological characteristics of chronic hepatitis B patients with hepatic steatosis. *Int J Med Sci* 2013; **10**: 641-646 [PMID: 23569427 DOI: 10.7150/ijms.5649]
  - 15 **Valenti L**, Nobili V, Al-Serri A, Rametta R, Leathart JB, Zappa MA, Dongiovanni P, Fracanzani AL, Alterio A, Roviara G, Daly AK, Fargion S, Day CP. The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. *J Hepatol* 2011; **55**: 1409-1414 [PMID: 21777557 DOI: 10.1016/j.jhep.2011.03.035]
  - 16 **Valenti L**, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, Dongiovanni P, Maggioni M, Fracanzani AL, Rametta R, Colombo M, Fargion S. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011; **53**: 791-799 [PMID: 21319195 DOI: 10.1002/hep.24123]
  - 17 **Trépo E**, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, Lemmers A, Berthillon P, Amininejad L, Chevallier M, Schlué J, Kreipe H, Devière J, Manns M, Trépo C, Sninsky J, Wedemeyer H, Franchimont D, Moreno C. Impact of patatin-like phospholipase-3 (rs738409 C > G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011; **54**: 60-69 [PMID: 21488075 DOI: 10.1002/hep.24350]
  - 18 **Müller T**, Buch S, Berg T, Hampe J, Stickel F. Distinct, alcohol-modulated effects of PNPLA3 genotype on progression of chronic hepatitis C. *J Hepatol* 2011; **55**: 732-733 [PMID: 21316406 DOI: 10.1016/j.jhep.2011.01.025]
  - 19 **Zampino R**, Coppola N, Cirillo G, Boemio A, Pisaturo M, Marrone A, Macera M, Sagnelli E, Perrone L, Adinolfi LE, Miraglia del Giudice E. Abdominal fat interacts with PNPLA3 I148M, but not with the APOC3 variant in the pathogenesis of liver steatosis in chronic hepatitis C. *J Viral Hepat* 2013; **20**: 517-523 [PMID: 23808989 DOI: 10.1111/jvh.12053]
  - 20 **Viganò M**, Valenti L, Lampertico P, Facchetti F, Motta BM, D' Ambrosio R, Romagnoli S, Dongiovanni P, Donati B, Fargion S, Colombo M. Patatin-like phospholipase domain-containing 3 I148M affects liver steatosis in patients with chronic hepatitis B. *Hepatology* 2013; **58**: 1245-1252 [PMID: 23564580 DOI: 10.1002/hep.26445]
  - 21 **Sagnelli E**, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, Santantonio T, Gentile A, Piccinino F. Influence of chronic coinfection with hepatitis B and C virus on liver histology. *Infection* 2004; **32**: 144-148 [PMID: 15188073]
  - 22 **Coppola N**, Pisapia R, Tonziello G, Martini S, Imparato M, Piai G, Stanzione M, Sagnelli C, Filippini P, Piccinino F, Sagnelli E. Virological pattern in plasma, peripheral blood mononuclear cells and liver tissue and clinical outcome in chronic hepatitis B and C virus coinfection. *Antivir Ther* 2008; **13**: 307-318 [PMID: 18505182]
  - 23 **Sagnelli E**, Coppola N, Pisaturo M, Masiello A, Tonziello G, Sagnelli C, Messina V, Filippini P. HBV superinfection in HCV chronic carriers: a disease that is frequently severe but associated with the eradication of HCV. *Hepatology* 2009; **49**: 1090-1097 [PMID: 19263473 DOI: 10.1002/hep.22794]
  - 24 **Coppola N**, Stanzione M, Messina V, Pisaturo M, De Pascalis S, Macera M, Tonziello G, Fiore M, Sagnelli C, Pasquale G, Sagnelli E. Current Concepts of HBV/HCV Coinfection: Coexistence, but Not Necessarily in Harmony. *Curr Hepat Rep* 2010; **9**: 260-269 [PMID: 21258658]
  - 25 **Coppola N**, Stanzione M, Messina V, Pisaturo M, De Pascalis S, Macera M, Tonziello G, Fiore M, Sagnelli C, Pasquale G, Sagnelli E. Tolerability and efficacy of anti-HBV nucleos(t)ide analogues in HBV-DNA-positive cirrhotic patients with HBV/HCV dual infection. *J Viral Hepat* 2012; **19**: 890-896 [PMID: 23121368 DOI: 10.1111/j.1365-2893.2012.01627.x]
  - 26 **Donato F**, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int J Cancer* 1998; **75**: 347-354 [PMID: 9455792]
  - 27 **Kew MC**, Yu MC, Kedda MA, Coppin A, Sarkin A, Hodgkinson J. The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in southern African blacks. *Gastroenterology* 1997; **112**: 184-187 [PMID: 8978357]
  - 28 **Amin J**, Law MG, Bartlett M, Kaldor JM, Dore GJ. Causes of death after diagnosis of hepatitis B or hepatitis C infection: a large community-based linkage study. *Lancet* 2006; **368**: 938-945 [PMID: 16962883]
  - 29 **Raimondo G**, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C, Squadrito G, Tono N. Longitudinal evaluation reveals a complex spectrum of virological profiles in hepatitis B virus/hepatitis C virus-coinfected patients. *Hepatology* 2006; **43**: 100-107 [PMID: 16323213]
  - 30 **Squadrito G**, Orlando ME, Pollicino T, Raffa G, Restuccia T, Cacciola I, Di Marco V, Picciotto A, Colucci G, Craxi A, Raimondo G. Virological profiles in patients with chronic hepatitis C and overt or occult HBV infection. *Am J Gastroenterol* 2002; **97**: 1518-1523 [PMID: 12094876]
  - 31 **Coppola N**, Marrone A, Pisaturo M, Starace M, Signoriello G, Gentile I, Adinolfi LE, Sagnelli E, Zampino R. Role of interleukin 28-B in the spontaneous and treatment-related clearance of HCV infection in patients with chronic HBV/HCV dual infection. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 559-567 [PMID: 24081499 DOI: 10.1007/s10096-013-1985-7]
  - 32 **Gayoso-Diz P**, Otero-González A, Rodríguez-Alvarez MX, Gude F, García F, De Francisco A, Quintela AG. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 2013; **13**: 47 [PMID: 24131857 DOI: 10.1186/1472-6823-13-47]
  - 33 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864]
  - 34 **Coppola N**, Potenza N, Pisaturo M, Mosca N, Tonziello G, Signoriello G, Messina V, Sagnelli C, Russo A, Sagnelli E. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. *PLoS One* 2013; **8**: e65336 [PMID: 23843939 DOI: 10.1371/journal.pone.0065336]
  - 35 **Giudice EM**, Grandone A, Cirillo G, Santoro N, Amato A, Brienza C, Savarese P, Marzuillo P, Perrone L. The association of PNPLA3 variants with liver enzymes in childhood

- obesity is driven by the interaction with abdominal fat. *PLoS One* 2011; **6**: e27933 [PMID: 22140488 DOI: 10.1371/journal.pone.0027933]
- 36 **Syed GH**, Amako Y, Siddiqui A. Hepatitis C virus hijacks host lipid metabolism. *Trends Endocrinol Metab* 2010; **21**: 33-40 [PMID: 19854061 DOI: 10.1016/j.tem.2009.07.005]
  - 37 **Felmlee DJ**, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins--impact for the viral life cycle and pathogenesis of liver disease. *Viruses* 2013; **5**: 1292-1324 [PMID: 23698400]
  - 38 **Adinolfi LE**, Restivo L, Zampino R, Lonardo A, Loria P. Metabolic alterations and chronic hepatitis C: treatment strategies. *Expert Opin Pharmacother* 2011; **12**: 2215-2234 [PMID: 21883025 DOI: 10.1517/14656566.2011.597742]
  - 39 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704 [PMID: 14724822]
  - 40 **Hwang SJ**, Lee SD. Hepatic steatosis and hepatitis C: Still unhappy bedfellows? *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 96-101 [PMID: 21199519 DOI: 10.1111/j.1440-1746.2010.06542.x]
  - 41 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838]
  - 42 **Fartoux L**, Poujol-Robert A, Guécho J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005; **54**: 1003-1008 [PMID: 15951550]
  - 43 **Zarski JP**, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Betzeaux F, Tran van Nhieu J, Seigneurin JM, Buffet C, Dhumeaux D. Characteristics of patients with dual infection by hepatitis B and C viruses. *J Hepatol* 1998; **28**: 27-33 [PMID: 9537860]
  - 44 **Bellocave P**, Gouttenoire J, Gajer M, Brass V, Koutsoudakis G, Blum HE, Bartenschlager R, Nassal M, Moradpour D. Hepatitis B and C virus coinfection: a novel model system reveals the absence of direct viral interference. *Hepatology* 2009; **50**: 46-55 [PMID: 19333911 DOI: 10.1002/hep.22951]
  - 45 **Raimondo G**, Saitta C. Treatment of the hepatitis B virus and hepatitis C virus co-infection: still a challenge for the hepatologist. *J Hepatol* 2008; **49**: 677-679 [PMID: 18804888 DOI: 10.1016/j.jhep.2008.08.003]
  - 46 **Schüttler CG**, Fiedler N, Schmidt K, Repp R, Gerlich WH, Schaefer S. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein. *J Hepatol* 2002; **37**: 855-862 [PMID: 12445429]
  - 47 **Chen SY**, Kao CF, Chen CM, Shih CM, Hsu MJ, Chao CH, Wang SH, You LR, Lee YH. Mechanisms for inhibition of hepatitis B virus gene expression and replication by hepatitis C virus core protein. *J Biol Chem* 2003; **278**: 591-607 [PMID: 12401801]
  - 48 **Sagnelli E**, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; **36**: 1285-1291 [PMID: 12395342]
  - 49 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]
  - 50 **Romero-Gómez M**, Del Mar Vioria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636-641 [PMID: 15765399]
  - 51 **Grasso A**, Malfatti F, De Leo P, Martines H, Fabris P, Toscanini F, Anselmo M, Menardo G. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. *J Hepatol* 2009; **51**: 984-990 [PMID: 19695729 DOI: 10.1016/j.jhep.2009.07.008]
  - 52 **Serfaty L**, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, Drenth JP, Lonjon-Domanec I, DeMasi R, Picchio G, Beumont M, Marcellin P. Insulin resistance and response to telaprevir plus peginterferon  $\alpha$  and ribavirin in treatment-naïve patients infected with HCV genotype 1. *Gut* 2012; **61**: 1473-1480 [PMID: 22387529 DOI: 10.1136/gutjnl-2011-300749]

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## Liver fibrosis in primary intestinal lymphangiectasia: An undervalued topic

Raffaele Licinio, Mariabeatrice Principi, Enzo Ierardi, Alfredo Di Leo

Raffaele Licinio, Mariabeatrice Principi, Enzo Ierardi, Alfredo Di Leo, Gastroenterology Unit, Department of Emergency and Organ Transplantation, University of Bari, Cesare, 70124 Bari, Italy

Author contributions: Ierardi E, Di Leo A, Principi M and Licinio R followed the patient, reviewed the literature, wrote the manuscript and approved the final version.

Correspondence to: Alfredo Di Leo, Professor, Gastroenterology Unit, Department of Emergency and Organ Transplantation, University of Bari, Piazza G, Cesare, 70124 Bari, Italy. [alfredo.dileo@uniba.it](mailto:alfredo.dileo@uniba.it)

Telephone: +39-08-05592577 Fax: +39-08-05593088

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order. The fibrosis outcome after a low-fat diet in the patient described in this report is in contrast with other literature reports. We emphasize the need for systematic monitoring of liver fibrosis in primary intestinal lymphangiectasia.

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### Abstract

The relationship between primary intestinal lymphangiectasia (PIL) and liver fibrosis is an emerging topic with many obscure aspects due to the rarity of the disorder. A recent paper reported that a six-month low-fat diet improved liver fibrosis. We report the case of a 17-year-old girl affected by PIL whose hepatic fibrosis progressively worsened within one year, despite dietetic support. This and the previous case report describe extraordinary events, which do not allow clear-cut clinical aspects to be established. Nevertheless, both cases suggest that in patients with PIL, it is necessary to closely monitor liver morphology with in-depth investigations including not only ultrasonography, but also elastography.

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**Key words:** Hepatic transient elastography; Liver fibrosis; Low-fat diet; Primary intestinal lymphangiectasia

**Core tip:** The relationship between primary intestinal lymphangiectasia and liver fibrosis is an emerging topic with many obscure aspects due to the rarity of the dis-

### INTRODUCTION

Primary intestinal lymphangiectasia (PIL), featuring a dilatation of intestinal lymphatic vessels and malabsorption, is a rare condition often requiring nutritional enteral/parenteral support<sup>[1]</sup>. Enteral nutrition is based on a hyperproteic, low-fat diet with vitamin and medium-chain triglyceride supplementation<sup>[2]</sup>. The association between PIL and primary liver fibrosis is uncommon<sup>[3]</sup>, however, Milazzo *et al*<sup>[4]</sup> recently reported a case of associated PIL and liver fibrosis characterized by high stiffness at elastography. The authors reported that a six-month low-fat diet combined with medium-chain triglyceride supplementation improved liver alterations by reducing fibrosis. The authors attributed the fibrosis onset to lymphatic stasis, as occurs in cardiac congestive liver. However, since fibrosis reversibility has not been previously described in this condition, the hypothesis may be purely speculative. In this scenario, we believe our case of PIL featuring progressively worsening hepatic fibrosis, despite dietetic support, may be of interest.

### CASE REPORT

A 17-year-old female patient was admitted to our unit for peripheral and facial edema, ascites and intestinal malab-

sorption (hypovitaminosis, low serum magnesium, severe hypoproteinemia with hypoalbuminemia, lymphocytopenia). The symptoms had developed four years before and progressively worsened. Her body mass index was 16.4.

Upper endoscopy and colonoscopy were performed, with biopsy samples showing only a microscopic dilatation of lymphatic vessels. Video-capsule endoscopy showed hyperemia, edema and several mucosal elevations, which was suggestive of PIL. Therefore, we evaluated intestinal protein loss by fecal alpha-1-antitrypsin clearance, which was found to be > 24 mL/d, confirming our clinical suspicion. The final diagnosis was made with technetium-labeled human serum albumin scintigraphy, which highlighted patchily distributed areas of protein dispersion in the small intestine at the level of the jejunum and ileum. During her hospital stay, ultrasonography revealed splenomegaly and hepatomegaly with inhomogeneous echogenicity, whilst transient elastography (FibroScan; Echosens, Paris, France) demonstrated hepatic fibrosis (10 kPa, interquartile range: 1.5 kPa; success rate, 100%; F3). Laboratory examinations displayed slightly increased amino transferase and gamma-glutamyl transferase (twice the normal upper limit), leading us to exclude all known causes of chronic liver disease: negative hepatitis B virus-DNA, hepatitis C virus-RNA (excluding chronic viral hepatitis); normal cupremia and ceruloplasmin (excluding Wilson's disease); normal serum iron, ferritin and transferrin saturation (excluding hemochromatosis and hemosiderosis); negative anti-nuclear, anti-smooth muscle, anti-mitochondria and anti-liver-kidney microsome antibodies (excluding autoimmune hepatitis, primary biliary cirrhosis). There was no history of alcohol or potential hepatotoxic drug use. Cardiac failure was ruled out by echocardiography.

At discharge, the patient began a hyperproteic diet (2.1 g/kg per day of amino acids), with low-fat intake and medium-chain triglycerides and vitamin supplementation<sup>[2]</sup>. Six months later, peripheral edema and ascites had improved, as well as nutritional parameters, with normalization of amino transferase and gamma-glutamyl transferase values. The decreased values were presumably due to improved nutritional conditions, and reducing the hepatic cytolysis and cholestasis that characterize malnutrition-induced liver steatosis. Indeed, these cannot be considered as markers of liver fibrosis. Paradoxically, this condition may decrease amino transferase values by reducing the hepatocyte mass.

Despite the clinical improvement, the liver stiffness value had doubled by one year later (20 kPa, interquartile range: 2.9 kPa; success rate, 100%; F4). Liver biopsy showed pericellular and periportal fibrosis. The framework was interpreted as "congenital liver fibrosis", excluding other possible causes of chronic liver diseases such as primary biliary cirrhosis and Caroli's disease.

## DISCUSSION

The rarity of PIL and the extraordinary events surrounding its uncommon association with liver fibrosis are

exhibited by the present case, thus preventing the establishment of clear-cut clinical characteristics. Indeed, this report demonstrates that liver fibrosis may not improve after nutritional therapy. Nevertheless, this and a previous case<sup>[4]</sup> suggest that in patients with PIL, it is necessary to closely monitor liver function, with in-depth investigations including not only ultrasonography, but also elastography<sup>[5]</sup>. Early detection of liver involvement in PIL is important in order to promote regression and prevent progression towards portal hypertension and recurrent cholangitis.

## COMMENTS

### Case characteristics

Main symptoms: facial edema, abdominal swelling, weight loss.

### Clinical diagnosis

Physical examination: edema, ascites, reduced body mass index (16.4), hepatomegaly.

### Differential diagnosis

Malabsorption syndrome causes and chronic liver disorders were investigated.

### Laboratory diagnosis

Main findings: hypovitaminosis, low serum magnesium, severe hypoproteinemia with hypoalbuminemia, lymphocytopenia, increased amino transferase and gamma-glutamyl transferase (twice the normal upper limit), negative hepatitis B-DNA and hepatitis C-RNA, normal cupremia and ceruloplasmin, normal serum iron, ferritin and transferrin, negative anti-nuclear, anti-smooth muscle, anti-mitochondria and anti-liver-kidney microsome antibodies; alpha-1-antitrypsin clearance > 24 mL/d.

### Imaging diagnosis

Video-capsule endoscopy showed hyperemia, edema and several mucosal elevations, suggestive of primary intestinal lymphangiectasia. Technetium-labeled human serum albumin scintigraphy highlighted patchily distributed areas of protein dispersion in the small intestine at the level of the jejunum and ileum. Ultrasonography revealed splenomegaly and hepatomegaly with inhomogeneous echogenicity, whilst transient elastography demonstrated hepatic fibrosis (10 kPa, interquartile range: 1.5 kPa; success rate 100%; F3).

### Pathological diagnosis

Microscopic dilatation of lymphatic vessels in duodenal biopsy specimens.

### Treatment

Hyper-proteic diet (2.1 g/kg per day of amino acids), with low-fat intake and medium-chain triglycerides and vitamin supplementation.

### Related reports

A recent case report shows an association between primary intestinal lymphangiectasia and liver fibrosis, which was improved by a 6-mo low-fat diet combined with medium-chain triglyceride supplementation.

### Experiences and lessons

In patients with primary intestinal lymphangiectasia, it is necessary to closely monitor liver function with in-depth investigations including not only ultrasonography, but also elastography.

### Peer review

This is a case report written in the format of a Letter to the Editor. The case report is on a 17-year old female who was diagnosed to suffer from primary intestinal lymphangiectasia. In spite of enteral nutrition which was based on hyper-proteic, vitamin, low fat and medium-chain triglyceride supplementation, the patient's liver fibrosis doubled on elastography.

## REFERENCES

- 1 Vignes S, Bellanger J. Primary intestinal lymphangiectasia (Waldmann's disease). *Orphanet J Rare Dis* 2008; 3: 5 [PMID: 18294365 DOI: 10.1186/1750-1172-3-5]
- 2 Desai AP, Guvenc BH, Carachi R. Evidence for medium chain triglycerides in the treatment of primary intestinal lymphangiectasia. *Eur J Pediatr Surg* 2009; 19: 241-245 [PMID: 19254365 DOI: 10.1007/s00381-009-0000-0]



- 19449286 DOI: 10.1055/s-0029-1216389]
- 3 **Chagnon JP**, Barge J, Hay JM, Devars du Mayne JF, Ricahrd JP, Hardouin JP. Congenital hepatic fibrosis, multiple renal cysts and primary intestinal lymphangiectasia (author's transl). *Gastroenterol Clin Biol* 1982; **6**: 326-332 [PMID: 7084582]
- 4 **Milazzo L**, Peri AM, Lodi L, Gubertini G, Ridolfo AL, Antinori S. Intestinal lymphangiectasia and reversible high liver stiffness. *Hepatology* 2014; **60**: 759-761 [PMID: 24449480 DOI: 10.1002/hep.27025]
- 5 **Colli A**, Fraquelli M, Casazza G, Conte D, Nikolova D, Duca P, Thorlund K, Gluud C. The architecture of diagnostic research: from bench to bedside--research guidelines using liver stiffness as an example. *Hepatology* 2014; **60**: 408-418 [PMID: 24277656 DOI: 10.1002/hep.26948]

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Institute of Molecular Biology and Pathology, Rome 00161, Italy

**Wan-Long Chuang, MD, PhD, Doctor, Professor,** Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

### Editorial office

Jin-Lei Wang, Director  
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*World Journal of Hepatology*  
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- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

*Volume with supplement*

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache Relat Res* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

*Issue with no volume*

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

*No volume or issue*

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

### Books

*Personal author(s)*

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

*Chapter in a book (list all authors)*

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

*Author(s) and editor(s)*

- 12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

*Conference proceedings*

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

*Conference paper*

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

**Electronic journal** (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

**Patent** (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

### Statistical data

Write as mean  $\pm$  SD or mean  $\pm$  SE.

### Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as  $\chi^2$  (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

### Units

Use SI units. For example: body mass,  $m$  (B) = 78 kg; blood pressure,  $p$  (B) = 16.2/12.3 kPa; incubation time,  $t$  (incubation) = 96 h; blood glucose concentration,  $c$  (glucose)  $6.4 \pm 2.1$  mmol/L; blood CEA mass concentration,  $p$  (CEA) = 8.6–24.5  $\mu\text{g/L}$ ;  $\text{CO}_2$  volume fraction, 50 mL/L  $\text{CO}_2$ , not 5%  $\text{CO}_2$ ; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

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Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

### Italics

Quantities:  $t$  time or temperature,  $c$  concentration,  $A$  area,  $l$  length,  $m$  mass,  $V$  volume.

Genotypes: *gyrA*, *arg 1*, *c myc*, *c fos*, *etc.*

Restriction enzymes: *EcoRI*, *HindIII*, *BamHI*, *Kho I*, *Kpn I*, *etc.*

Biology: *H. pylori*, *E. coli*, *etc.*

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