Introduction

In clinical practice, despite Hepatitis C Virus (HCV)-infected patients achieved a Sustained Viral Response (SVR) with Direct-Acting Antiviral Agents (DAAs), they remained at risk of Hepatocellular Carcinoma (HCC) development [1-3]. A recent study showed unusually

63.75% (51/80) serum negative for HCV RNA (Table 1). A er LDLT, we performed one-step reverse-transcription QPCR to generate a standard curve for the absolute quanti cation of hepatic HCV RNA. Quanti cation of relative expression (reported as arbitrary units (copy number)) was performed using the $2^{-\Delta\Delta Ct}$ relative quanti cation method. Quantitative PCR data showed that the variability coe cient of Ct was always lower than 2% of the mean values (Figure 1). A er LDLT, 85% (68/80) of native livers were positive for hepatic HCV RNA, whereas 15.0% (12/80) were negative for hepatic HCV RNA (Table 1). Among 68 recipients with positive hepatic HCV RNA, 3.75% (3/80) remained serum positive for HCV RNA (Figure 2). ere was a statistically signi cant di erence in HCV RNA identi cation between the serum HCV RNA and hepatic HCV RNA ($p<0.001$) before LT and between the serum HCV RNA and hepatic HCV RNA ($p<0.001$) a er LT (Table 1).

Among 68 recipients with positive hepatic HCV RNA, 39.7% (27/68) had been treated with DAA before LDLT, of which 51% (15/27) were positive for serum HCV RNA and 30.8% (12/27) were negative for serum HCV RNA. In total, 44% (3/68) cases remained positive for serum HCV RNA a er LDLT. All three positive serum HCV RNA recipients sustained viral response because of DAA treatment a er LDLT. ere was no signi cant di erence in hepatic HCV RNA levels between samples from patients pretreated with DAA and serum HCV RNA levels before and a er LDLT (Table 2).

Our recent study concluded that the signi cant underestimation of HCV RNA in the serum was identi ed by measuring the hepatic HCV RNA levels ($p<0.001$) followed by their comparison with the pre-LDLT serum HCV RNA and the removed native liver hepatic HCV RNA levels.

HCV core antigen (HCV Ag) in native liver of chronic hepatitis C recipients

According to guidelines from European Association for the Study of the Liver (EASL) recommended HCV core Antigen (HCV Ag) testing to be a less expensive alternative to HCV RNA for determination of HCV viraemia when HCV RNA testing is not available [14]. In 2018, Tilborg conducted a research to support the use of HCV Ag testing

Table 1: Comparison of the HCV RNA status of the native liver and serum before/after liver transplantation in 80 recipients who underwent living donor liver transplantation.

Category	Native liver		p value
	Hepatic HCV RNA (+)	Hepatic HCV RNA (-)	
	N=68 (85%)	N=12 (15.0%)	
Serum HCV RNA before LT	29 (36.3)	51 (63.7)	<0.001
Serum HCV RNA after LT	3 (3.7)	77 (96.3)	<0.001
HCV: Hepatitis C virus; LT: Liver Transplantation; Fisher's exact test			

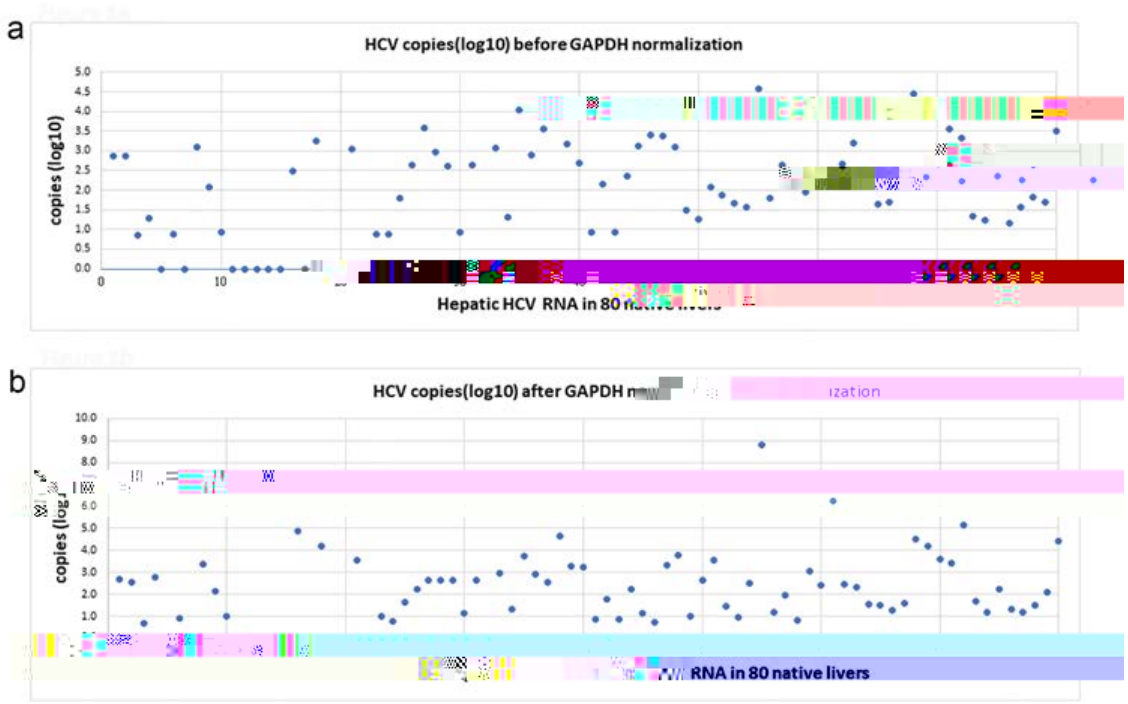

Figure 1: One step reverse-transcription QPCR for absolute quanti cation of HCV to determine copy numbers (1a) Normalized with human GAPDH expression (1b). **Abbreviations:** HCV: Hepatitis C Virus.

HCV in liver cells. e new liver gra may become a target organ for HCV infection a er DAA treatment. In our recent studies, we showed that HCV enhanced expression of micro-RNA 122, micro-RNA 92b and PNPLA3 variants, as well as increased the risk of developing HCC; these form the clinical evidence of the recurrence of HCC [20-22]. In addition to environmental factors such as alcohol and tobacco [23,24], hepatic HCV RNA has been reported to play a role in the severity of HCV infection and the response to antiviral treatment [25]. We suggest that the chronic liver injury concomitant residual hepatic HCV-RNA and long-term outcomes with DAA therapy could still remain an issue a er SVR [7].

HCV-positive donors to HCV-negative recipients

Liver transplantation has been hampered by the shortage of available donors in relation to the number of people on the waiting list. To decrease the number of deaths on the waiting list and overcome the problems of lack of donors, many transplant centers aggressively use marginal donors or even HCV seropositive, non-aviremic donors to HCV-seronegative recipients [26]. Recent research from Kapila showed a real-world experience of the transplantation of HCV-aviremic organs into aviremic recipients and they stated that in carefully selected patients, the use of HCV-aviremic gra s in the DAA era appears to be e cacious and well tolerated [27].

However, based on our study, hepatic HCV RNA might still be found a er LDLT even in patients with pre-transplant negative serum HCV RNA. Serum HCV-RNA load might represent an underrated estimation to entire HCV viral loads in patients receiving anti-viral therapy. Nowadays, there is no strong evidence published yet to correlate high , , HCC occurrence or recurrence a er LT in hepatic HCV RNA patients, but given the constant exposure to immunosuppressant in this organ-transplant cohort in the real-world setting, the oncologic safety of aviremic/viraemia HCV-infected organ donor may need longer duration of observation, even in the DAA era.

Conclusion

A complete eradication of HCV in patients treated with antiviral agents a er clinical diagnosis of SVR seems unlikely despite of substantially improved anti-HCV e cacy. Based on the results of our study, the importance of pre-transplant antiviral therapy to attain negative HCV RNA needs to be emphasized here; it has proven to be far more bene cial than post-transplant antiviral therapy.

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Authors Contributions

Study concept and design: King-Wah Chiu and Chih-Chi Wang; data collection: Shu-Hsien Lin and Chih-Che Lin; data analysis and interpretation: King-Wah Chiu and Chih-Chi Wang; performed experiments: Kuang-Den Chen, Kuang-Tzu Huang and Li-Wen Hsu; and manuscript dra ing and critical revisions: Shu-Hsien Lin, King-Wah Chiu.

Con icts of Interest

e authors declare that they have no competing interests.

References

1. Singal AG, Lim JK, Kanwal F (2019) AGA Clinical practice update on interaction between oral direct-acting antivirals for chronic hepatitis c infection and hepatocellular carcinoma: Expert review. Gastroenterology 156: 2149-2157.
2. European Association for the Study of the Liver (2018) EASL clinical practice guidelines: Management of hepatocellular carcinoma. J Hepatol 69: 182-236.
3. Ghany MG, Morgan TR (2020) Hepatitis C guidance 2019 update: American association for the study of liver diseases-infectious diseases society of america recommendations for testing, managing, and treating hepatitis c virus infection. Hepatology 71: 686-721.
4. Ravi S, Axley P, Jones D, Kodali S, Simpson H, et al. (2017) Unusually high rates of hepatocellular carcinoma after treatment with direct-acting antiviral therapy for hepatitis c related cirrhosis. Gastroenterology 152: 911-912.
5. Zuo T, Liu Q, Zhang F, GCY Lui, Tso EYK, et al. (2021) Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut 70: 276-284.
6. Cheung CCL, Goh D, Lim X, Tien TZ, Lim JCT, et al. (2021) Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from fve recovered patients with COVID-19. Gut.
7. W

microenvironment mediated by suppression of autophagic flux drives liver malignancy. Biomed J 41: 163-168.

23. Hou W, Bukong TN, Kodys K, Szabo G (2013) Alcohol facilitates HCV RNA replication *via* up-regulation of miR-122 expression and inhibition of cyclin G1 in human hepatoma cells. Alcohol Clin Exp Res 37: 599-608.

24. Zhao L, Li F, Taylor EW (2013) Can tobacco use promote HCV-induced *miR-122* hijacking and hepatocarcinogenesis? Med Hypotheses 80: 131-133.

25. Maylin S, Laouénan C, Peignoux MM, Panhard X, Lapalus M, et al. (2012) Role of hepatic HCV-RNA level on the severity of chronic hepatitis c and response to antiviral therapy. J Clin Virol 53: 43-47.

26. Hughes CB, Humar A(2021) Liver transplantation: Current and future. Abdom Radiol 46: 2-8.

27. Kapila N, Menon KVN, Al-Khallouf K, Vanatta JM, Murgas C, et al. (2020) Hepatitis C Virus NAT-positive solid organ allografts transplanted into hepatitis c virus-negative recipients: A Real-world experience. Hepatology 72: 32-41.