A novel predictive score for hepatocellular carcinoma development in patients with chronic hepatitis C after sustained response to pegylated interferon and ribavirin combination therapy

Kuo-Chin Chang1, Chao-Hung Hung1, Sheng-Nan Lu1, Jing-Houng Wang1, Chuan-Mo Lee1, Chien-Hung Chen1, Ming-Fang Yen2, Sheng-Chieh Lin1, Yi-Hao Yen1, Ming-Chao Tsai1, Po-Lin Tseng1 and Tsung-Hui Hu1*

1Division of Hepato-Gastroenterology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan; 2School of Oral Hygiene, College of Oral Medicine, Taipei Medical University, Taipei City, Taiwan

*Corresponding author. Tel: +886-7-731-7123, ext. 8301; Fax: +886-7-732-2402; E-mail: dr.hu@msa.hinet.net

Received 15 April 2012; returned 10 May 2012; revised 14 June 2012; accepted 15 June 2012

Objectives: Antiviral therapy can prevent the development of hepatocellular carcinoma (HCC) in chronic hepatitis C (CHC) patients. However, HCC still develops in patients achieving sustained virological response (SVR). We proposed to evaluate the risk factors and derive a novel risk score for HCC (scoreHCC) by summation of products of clinical weights based on the regression coefficients in the final proportional hazards model.

Methods: From March 2002 to October 2009, we enrolled 871 patients with biopsy-proven CHC, who received combined pegylated interferon and ribavirin therapy and achieved SVR.

Results: Cox regression analysis showed that old age [hazard ratio (HR) 3.82, 95% CI 1.74–8.37, P=0.001], high α-fetoprotein levels (HR 3.15, 95% CI 1.60–6.19, P=0.001), low platelet counts (HR 2.81, 95% CI 1.22–6.44, P=0.015) and high fibrotic stage (HR 3.95, 95% CI 1.46–10.70, P=0.007) were independent risk factors. The cut-off level of risk scores was a derived value of 10 and was able to predict the HCC risk with 89.2% sensitivity and 69.5% specificity. The AUC value for the prediction was 0.848. The scoreHCC values were further categorized into three risk groups: low risk (scoreHCC ≤10), intermediate risk (scoreHCC 11–15) and high risk (scoreHCC ≥16). The proportion of HCC development increased from 1.37% (9/657) in the low-risk group to 9.14% (16/175) in the intermediate-risk group and 30.77% (12/39) in the high-risk group (P<0.001).

Conclusions: With the novel risk scores, we can estimate the chance of HCC development more exactly and practically. This approach can be used for HCC screening in CHC patients achieving SVR.

Keywords: risk score, hepatitis C, pegylated interferon, ribavirin, hepatocellular carcinoma, sustained virological response

Introduction

Infection with the hepatitis C virus (HCV) represents a major cause of chronic hepatitis and is also a well-known risk factor for the development of hepatocellular carcinoma (HCC).1–4 HCV infects ~170 million people worldwide.5 Abdominal ultrasound supplemented with α-fetoprotein (AFP) assays are two critical pillars of HCC surveillance for managing patients with chronic hepatitis.6,7 In addition, the incidence of hepatic decompensation, and possibly the development of HCC, can be reduced by HCV therapy, as meta-analyses suggest.8 Preventing progression to cirrhosis and HCC appears to be beneficial to these patients.9–13

Currently, patients with HCV infection are treated with a combination of pegylated interferon (peg-IFN) and ribavirin, and this achieves sustained virological response (SVR) in 30%–50% of HCV patients.14–16 However, patients who were able to achieve a SVR remained at risk of developing HCC.12,13 Several potential factors have been identified as being associated with a higher risk of HCC development.17–20 These include patient-related factors, including male gender or advanced age; virus-related factors, including high serum HCV levels or genotype 1b compared with non-1b;18,20 and disease-related factors, including alanine aminotransferase levels or the presence of cirrhosis.18–20 These risk factors were identified mostly by cross-sectional or cohort studies with relatively limited numbers of participants. Whether these represent independent factors for the development of HCC is still uncertain and, in addition, these various factors may interact with one another. A large-scale, longitudinal follow-up study examining all these factors could potentially help to resolve this issue.
potential risk factors for HCC is required to more accurately assess the prognosis of patients with chronic HCV infection.

We therefore conducted the present large-scale longitudinal study on chronic hepatitis C (CHC) patients showing SVR to peg-IFN combination therapy, and aimed to: (i) determine the risk factors for HCC development; (ii) identify the risk factors that were independent; and (iii) test whether a projected risk estimation score for HCC development can be derived from these independent risk factors.

Patients and methods

From March 2002 to October 2009, 1271 patients with biopsy-proven CHC who received combined peg-IFN/ribavirin therapy were enrolled. The study was approved by the institutional review board of our hospital. All patients were positive for anti-HCV antibodies (AxSYM HCV 3.0; Abbott Laboratories, Chicago, IL, USA) and had detectable HCV RNA (Amplicor™; Roche Diagnostics, Branchburg, NJ, USA). The diagnosis of advanced fibrosis/cirrhosis was based on histological criteria (modified Knodell histology index). Patients underwent liver biopsies within 6 months before the start of therapy. None of the patients had a history of hepatic encephalopathy, haemorrhage from oesophageal varices or ascites. Patients with concomitant hepatitis B virus or HIV infection, alcoholism or autoimmune hepatitis were excluded. Before therapy, none of the patients had HCC or suspicious space-occupying lesions as detected by ultrasound and/or CT.

Before treatment, informed consent was obtained from each patient and the study was carried out in accordance with the provisions of the Declaration of Helsinki. Patients were treated with peginterferon-α-2a (Pegasys; Roche, Basel, Switzerland) at 135 or 180 µg/week subcutaneously or peginterferon-α-2b (Peg-Intron-A; Schering-Plough Corporation, Kenilworth, NJ, USA) at 1.5 µg/kg/week subcutaneously and oral ribavirin 800–1200 mg daily. The duration of HCC follow-up was defined from the date 24 weeks post-treatment to the date of HCC development, or to the date of last follow-up. Patient follow-up occurred every other week in the first month, every 4 weeks subsequently until the end of therapy and every 12 weeks thereafter. Serum HCV RNA levels were assessed at enrolment, at the end of antiviral therapy and 6 months later. SVR was defined as undetectable serum HCV RNA levels at 24 weeks after completion of treatment. Patients who were a relapsers or a null responder was excluded. Ultrasound examinations and serum AFP assays were carried out every 6 months. Any new space-occupying lesions raising suspicion of malignancy that were detected at the time of the examination were examined by CT, magnetic resonance imaging (MRI) and/or biopsy. The HCC diagnosis was determined according to the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Disease (AASLD) guidelines, based on imaging features of nodules >2 cm with typical arterial vascularity using two dynamic imaging methods (CT and MRI). If this diagnostic method could not be applied, the diagnosis was histologically confirmed by liver biopsy.

HCV RNA and genotyping

Qualitative detection of HCV RNA was performed by a standardized RT-PCR assay (Amplicor™; Roche Diagnostics), using biotinylated primers for the 5′-non-coding region. The lowest detection limit for this assay was 15 IU/mL. HCV genotyping was performed by a reverse hybridization assay (Inno-LiPA™ HCV II; Innogenetics N.V., Gent, Belgium) using HCV-Amplicor products.

Statistical analysis

Quantitative variables are expressed as means ± SD. Comparisons of differences in categorical data between groups were performed using χ2 analysis. Continuous variables were analysed by the t-test where appropriate. Kaplan–Meier curves were generated for the cumulative incidence of HCC. The risk factors for HCC development were evaluated by the log-rank test and multivariate Cox regression analysis. All tests were two-tailed, and a P value of <0.05 was considered statistically significant.

Risk score for HCC

The proportional hazards model was first used to identify independent risk factors for HCC. We used the summation of products of clinical weights based on the regression coefficients in the final proportional hazards model and their associated values of covariates to calculate the risk score for HCC (scoreHCC). By setting up a series of cut-off values for scoreHCC to separate predicted HCC and non-HCC groups and comparing the real data on the status of developing HCC, we can produce a series of sensitivities and specificities. Receiver operating characteristic (ROC) curves were presented in the form of a plot depicting sensitivity divided by 1 minus specificity. The area under the ROC curve was reported.

Results

Patient characteristics

The demographic, virological and clinical characteristics of the 1271 patients who received peg-IFN and ribavirin combination therapy for CHC are summarized in Table 1. The mean age was 55.4 ± 9.4 years (range 20–83 years). There were 661 males and 610 females (sex ratio 1.1:1). Among the patients, 46.4% were infected with HCV genotype 1, the majority (72.1%) had chronic hepatitis rather than liver cirrhosis (27.9%) and 262 (20.6%) had diabetes mellitus. The median follow-up period was 41.3 months (range 3.5–113.9 months).

Table 1. Demographic, virological and clinical characteristics of the 1271 patients

| Characteristic | Mean ± SD (range) |
|---------------|------------------|
| Mean age (years) | 55.4 ± 9.4 (range 20–83) |
| Gender (male/female) | 661/610 |
| HCV genotype 1/non-1 | 590/599 |
| Median BMI (kg/m²) | 24.6 (range 15.6–39.9) |
| Clinical state, n (%) | |
| chronic hepatitis | 916 (72.1) |
| liver cirrhosis | 355 (27.9) |
| Diabetes mellitus, n (%) | 262 (20.6) |
| SVR, n (%) | 871 (68.5) |
| Mean serum value | |
| AST (IU/L) | 109.6 ± 66.3 |
| ALT (IU/L) | 181.4 ± 214.2 |
| bilirubin (mg/dL) | 1.0 ± 0.6 |
| platelets (<10⁵/µL) | 16.6 ± 7.0 |
| AFP (ng/mL) | 17.8 ± 55 |
| Median follow-up (months) | 41.3 (range 3.5–113.9) |
**Cumulative incidence of HCC**

Of the 1271 chronic HCV patients who received the peg-IFN and ribavirin combination treatment, 871 (68.5%) CHC patients achieved SVR. Among the SVR patients, 37 of 871 developed HCC within a median follow-up period of 41.3 months. At the first, third and fifth years of follow-up, the cumulative incidence of HCC for all patients was 0.8%, 1.2% and 3.0%, respectively (Figure 1).

**Risk factors for HCC development in SVR patients**

Table 2 compares patients with and without HCC in the SVR group. Patients with HCC were older (mean age 66.0 ± 6.0 versus 56.6 ± 11.2, P < 0.001), had lower pre-treatment platelet counts (P < 0.001), higher pre-treatment AFP levels (≥20 ng/mL) (P < 0.001), higher fibrotic stage (P < 0.001) and a higher incidence of diabetes mellitus (P = 0.029). There were no significant differences between the two groups regarding alanine aminotransferase, total bilirubin, total peg-IFN dose, daily ribavirin dose, viral load, genotype and body mass index.

**Factors associated with the development of HCC**

Figure 2 shows the comparisons of cumulative incidences of HCC development by the Kaplan–Meier method and the log-rank test. Compared with patients without HCC, those who developed HCC tended to be older (age ≥60 years) (P < 0.001), had a higher AFP level (≥20 ng/mL) (P < 0.001), a higher fibrotic stage (P = 0.001) and a higher incidence of diabetes mellitus (P = 0.029). There were no significant differences between the two groups regarding alanine aminotransferase, total bilirubin, total peg-IFN dose, daily ribavirin dose, viral load, genotype and body mass index.

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**Table 2. Risk factors for HCC development in patients with SVR**

|                       | SVR patients, n = 871 (68.5%) |
|-----------------------|--------------------------------|
|                       | Total patients treated, n = 1271 | Non-HCC, n=834 (65.6%) | HCC, n=37 (2.9%) | P value |
| Age (years)           |                               | 55.4 ± 9.4                 | 56.6 ± 11.2       | 66.0 ± 6.0       | <0.001 |
| BMI (kg/m²)           |                               | 24.6 ± 3.4                 | 24.7 ± 4.6        | 24.9 ± 2.9       | 0.875  |
| Sex                   |                               |                            |                   |                   | 0.093  |
| male                  |                               | 661 (52.0%)                | 441 (52.9%)       | 25 (67.6%)       |        |
| female                |                               | 610 (48.0%)                | 393 (47.1%)       | 32 (32.4%)       |        |
| AST (IU/L)            |                               | 109.6 ± 66.3               | 105.1 ± 62.5      | 125.1 ± 74.2     | 0.060  |
| ALT (IU/L)            |                               | 181.4 ± 214.2              | 188.3 ± 234.5     | 236.9 ± 435.3    | 0.256  |
| bilirubin (mg/dL)     |                               | 1.0 ± 0.6                  | 0.9 ± 0.6         | 1.1 ± 0.5        | 0.045  |
| platelets (10⁹/L)     |                               | 16.6 ± 7.0                 | 17.5 ± 6.2        | 13.1 ± 5.8       | <0.001 |
| AFP (ng/mL)           | <20                            | 1036 (81.5%)               | 729 (87.4%)       | 20 (54.1%)       | <0.001 |
|                       | ≥20                            | 235 (18.5%)                | 105 (12.6%)       | 17 (45.9%)       |        |
| total peg-IFN (µg)    |                               | 3132.13 ± 2095.0           | 3489.78 ± 1920.8  | 2961.3 ± 1433.2  | 0.133  |
| ribavirin (mg/kg/day) |                               | 13.1 ± 5.7                 | 13.3 ± 3.9        | 11.1 ± 5.9       | 0.115  |
| HCV genotype          | non-1                          | 599 (47.1%)                | 474 (56.8%)       | 18 (48.6%)       | 0.291  |
|                       | 1                              | 590 (46.4%)                | 304 (36.5%)       | 17 (45.9%)       |        |
| Viral load            | <4 × 10⁷ IU/mL                 | 432 (34.0%)                | 286 (34.3%)       | 24 (64.9%)       | 0.228  |
|                       | ≥4 × 10⁷ IU/mL                 | 190 (14.9%)                | 84 (10.1%)        | 3 (8.1%)         | <0.001 |
| Fibrosis stage        | F0–2                           | 672 (52.9%)                | 527 (63.2%)       | 5 (13.5%)        |        |
|                       | F3–4                           | 599 (47.1%)                | 307 (36.8%)       | 32 (86.5%)       | 0.029  |
| Diabetes mellitus     | yes                            | 262 (20.6%)                | 146 (17.5%)       | 12 (32.4%)       |        |
|                       | no                             | 1009 (79.4%)               | 688 (82.5%)       | 25 (67.6%)       |        |

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
fibrosis stage (P<0.001), lower platelet counts (<150×10^9/L) (P<0.001), higher AFP levels (≥20 ng/mL) (P<0.001) and had diabetes mellitus (P<0.001). Using Cox regression analysis, the development of HCC by univariate analysis was significantly higher in patients with an older age (≥60 years) (P<0.001), platelet count <150×10^9/L (P<0.001), AFP ≥20 ng/mL (P<0.001), higher fibrotic stage (P<0.001) and diabetes mellitus (P=0.04). Further multivariate analysis showed that old age
Table 3. Univariate and multivariate analyses for factors associated with the development of HCC in patients with SVR

| Comparison                          | Univariate |          |          |          |          |          |          |          |
|-------------------------------------|------------|----------|----------|----------|----------|----------|----------|----------|
|                                     | HR         | 95% CI   | P value  | HR       | 95% CI   | P value  |          |          |
| Age (years) ≥60 versus <60          | 4.93       | 2.25–10.80 | <0.001   | 3.82     | 1.74–8.37 | 0.001    |          |          |
| Sex male versus female              | 1.82       | 0.91–3.65 | 0.09     | 1.92     | 0.96–3.88 | 0.066    |          |          |
| Platelets (10^9/L) <150 versus ≥150 | 6.26       | 2.85–13.74 | <0.001   | 2.81     | 1.22–6.44 | 0.015    |          |          |
| AFP (ng/mL) <20 versus ≥20          | 6.10       | 3.15–11.81 | <0.001   | 3.15     | 1.60–6.19 | 0.001    |          |          |
| Fibrosis 0–2 versus 3–4             | 8.75       | 3.40–22.52 | <0.001   | 3.95     | 1.46–10.70 | 0.007    |          |          |
| Diabetes mellitus yes versus no     | 2.03       | 1.02–4.04 | 0.04     | 1.26     | 0.62–2.57 | 0.526    |          |          |

**Figure 3.** ROC curve with simplified risk score to predict HCC development in 871 sustained responders to peg-IFN and ribavirin.

[hazard ratio (HR) 3.82, 95% CI 1.74–8.37, P = 0.001], AFP ≥20 ng/mL (HR 3.15, 95% CI 1.60–6.19, P = 0.001), low platelet count (<150 x 10^9/L) (HR 2.81, 95% CI 1.22–6.44, P = 0.015) and high fibrotic stage (F3–F4) (HR 3.95, 95% CI 1.46–10.70, P = 0.007) were independent risk factors for HCC (Table 3).

**Predictive risk score for the development of HCC**

Based on the estimated results and the fact that the proportions of the regression coefficients for age, low platelet levels, elevated AFP and higher score for fibrosis were around 5:4:4:6, we developed a predictive model with the following formula:

\[
\text{score}_{\text{HCC}} = 5 \times (\text{aged} \geq 60 \text{ years}) + 4 \times (\text{platelet number} < 150 \times 10^9/\text{L}) + 4 \times (\text{AFP} \geq 20 \text{ ng/mL}) + 6 \times (\text{fibrosis} F3–F4)
\]

As the weight for each covariate was integral, the calculation for \( \text{score}_{\text{HCC}} \) was easy to use. Figure 3 shows the ROC curve for this scoring. The AUC was 84.8%. The optimal cut-off value of 10, the point that was close to the upper-left corner of the ROC curve, resulted in 89.2% sensitivity and 69.5% specificity.

The predicted survival curves for the three groups, based on our predictive model, are shown in Figure 4. Patients could be further categorized, based on \( \text{score}_{\text{HCC}} \), into three risk groups: low risk (\( \text{score}_{\text{HCC}} \leq 10 \)), intermediate risk (\( \text{score}_{\text{HCC}} 11–15 \)) and high risk (\( \text{score}_{\text{HCC}} \geq 16 \)). The proportion of HCC that occurred increased from 1.37% (9/657) in the low-risk group to 9.14% (16/175) in the intermediate-risk group and 30.77% (12/39) in the high-risk group. There was an increasing cumulative risk of HCC for patients with low-, intermediate- and high-risk scores (0.3%, 22.1% and 66.7%, respectively; \( P < 0.001 \) for the trend).

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 75.7%, 77.7%, 13.1%, 98.6% and 77.6%, respectively.

**Discussion**

Peg-IFN combination therapy is already a widely used therapeutic modality for CHC.\(^\text{14–16}\) It is generally assumed that HCV eradication by peg-IFN halts disease progression and prevents clinical complications, including HCC development.\(^\text{14–16}\) However, there have been reports of several patients who developed HCC despite successful IFN-based therapy.\(^\text{17–20}\) The HCC incidence and risk factors in these patients were not fully elucidated. This was due to the rarity of HCC development in sustained responders to IFN-based therapy.

Of the 871 sustained responders to peg-IFN therapy, 37 developed HCC within a median period of 41.3 months. During the first, third and fifth years of follow-up, the cumulative HCC incidence in all patients was 0.8%, 1.2% and 3.0%, respectively.
This result was consistent with previous studies involving small numbers of sustained responders (1.4%–7%) to IFN-based therapy who developed HCC.17–20 It was obvious that IFN-based therapy decreased the risk of HCC in sustained responders. However, the incidence of HCC gradually increased over a period of at least 8 years after terminating the peg-IFN combination therapy (Figure 1). This suggested that the risks of developing HCC were not completely eliminated in patients who achieved a sustained response to the peg-IFN combination therapy, even up to 8 years following treatment cessation.

The mechanisms involved in the development of HCC among these patients are not well understood.24 Although, peg-IFN combination therapy reduces the rate of fibrosis progression and may result in the regression of fibrosis.4 However, accelerated hepatocyte cycling may result in the development of monoclonal populations of dysplastic hepatocytes with malignant potential.25,26 The increase in hepatic regeneration and hepatocyte cycling that occurs after antiviral therapy may activate cellular pathways leading to dysplasia and hence increase the risk of hepatocarcinogenesis. HCCs can be detected in patients with advanced fibrotic stages. As patients age, they may suffer from longer periods of HCV infection, and this may increase the severity of the liver fibrosis.20 According to the previous study, the risk of HCC development depended on the age at the primary biopsy and increased >15-fold after age 65. Even when stratified by the stage of fibrosis, the cumulative and annual incidence values for HCC were significantly higher in older than in younger patients at the same stage of fibrosis. The impact of viral eradication on HCC prevention was less significant in older compared with younger patients.11,27,28 Serum platelet counts were also an independent risk factor in the present study. Prior reports noted that decreased platelet counts represented an important risk factor for the development of HCC in patients with CHC.29–31 The risk seems to be sustained after antiviral eradication. This may be explained by the fact that progression of liver fibrosis is associated with decreased thrombopoietin production by hepatocytes and progressive hypersplenism with worsening portal hypertension, resulting in reduced platelet production and increased platelet destruction. Therefore, decreased platelet counts may reflect more progressive liver fibrosis and vice versa. However, we cannot explain the independence between the risks of fibrosis and platelet counts in this study, although it has been found in previous studies.29–31 HCC even developed in a patient who only had mild liver fibrosis (stage F1) after peg-IFN therapy. Therefore, single risk factor notification may not be enough for all patients with HCC. Identification of an overall risk for the development of HCC in sustained responders is important. After identifying these four independent risk factors for the development of HCC, the present study derived a novel HCC scoring system that was able to identify patients who were at risk of developing HCC if the score was greater than or equal to the optimal cut-off value of 10. This score was validated by stringent statistical analysis with high sensitivity and specificity (89.2% and 69.5%, respectively) for predicting HCC development after SVR with peg-IFN and ribavirin combination therapy (Figure 3). In this study, we also developed a simple score composed of routinely available clinical and laboratory parameters to predict the future risk of HCC in CHC patients after eradication of virus. This prediction score is accurate and reproducible. Patients with predictive scores of <10, 11–15 and ≥16 had distinctly different risks of developing HCC. In this study, more than half of the patients belonged to the low-risk category with a low incidence of HCC (1.37% at 8 years), whereas ~40% of the patients had a graduated risk of HCC development across the intermediate–to high-risk groups.

Using these risk scores, one can calculate the prognosis of patients on presentation, which is important for a clinician when devising each individual patient's management. One can also identify high-risk patients (e.g. there is a high risk of HCC development if the score is >16). In clinical practice, we are not aware of any predictive scores for the development of HCC in CHC patients that integrate all possible independent factors. Our novel score may provide further insight for clinicians to identify high-risk patients who should be treated preferentially and who should undergo intensive screening for HCC after eradicating the virus.

Prospective screening of patients at high risk of developing HCC increases the proportion of those who are diagnosed with potentially curable disease. As for the time interval between surveillance tests, both the AASLD31 and the Asian Pacific Association of the Study of Liver (APASL)32 recommend serum AFP level measurements, combined with liver ultrasound, for HCC surveillance at 6-month intervals for HBV carriers and patients with chronic hepatitis. Meta-regression analysis demonstrated a significantly higher sensitivity for early HCC with ultrasound every 6 months than with annual surveillance. A stringent programme with a 3–4-month surveillance interval was proposed for super high-risk patients by a consensus guideline from the Japanese Society of Hepatology (JSH).33 However, no studies were conducted to determine the ideal surveillance interval for patients achieving SVR under various guidelines. Patients with SVR of HCV therapy reduced the risk of HCC than patients without SVR or without treatment. Therefore, the ideal surveillance interval for SVR patients should be different, and may range from 3 to 12 months. Based on the results of our study, we propose that surveillance of high-risk patients should be performed 3–6 months after achieving SVR. However, a 12-month interval for surveillance may be enough for low-risk patients. Liver CT and MRI have not been validated and are therefore not currently recommended for screening.

In conclusion, CHC patients who respond to peg-IFN combination therapy should be followed even after HCV eradication, and special attention should focus on those who have severe liver fibrosis (F3 or F4), those with low pre-treatment platelet levels (<150×10^9/L) and those who are aged ≥60 years, to detect potentially treatable HCC. A novel HCC score has been formulated. It is of great clinical use to identify CHC patients who are high risk of the development of HCC after SVR.

Funding
This study was supported by a grant from Chang Gung Memorial Hospital (CMRPD8A0401) (to T.-H. H.).

Transparency declarations
None to declare.


Author contributions
Conception and design: T.-H. H. Manuscript writing: K.-C. C. Collection and assembly of data: K.-C. C., C.-M. L., S.-N. L., J.-H. W., C.-H. H., C.-H. C., S.-C. L., Y.-H. Y., M.-C. T., P.-L. T. and T.-H. H. Data analysis and interpretation: K.-C. C., M.-F. Y. and T.-H. H. Final approval of manuscript: all authors.

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