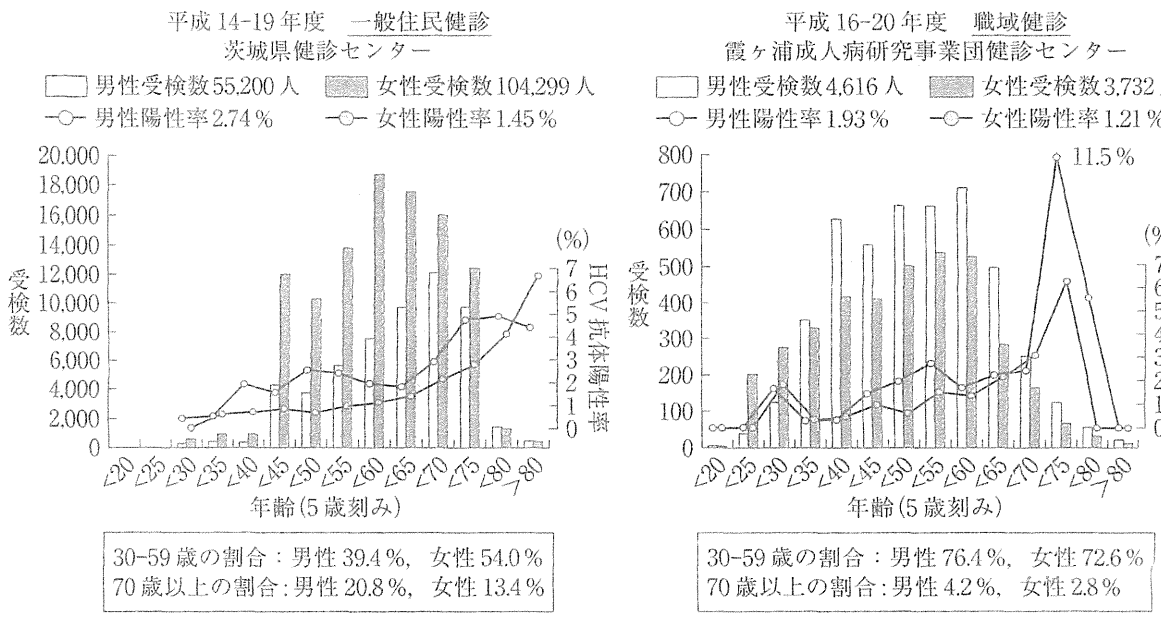

図1　茨城県における一般住民健診（肝炎節目検診事業と節目外検診[平成14-18年度]ならびに茨城県衛生研究所にて行った肝炎検査[平成19年度]）と職域健診（霞ヶ浦成人病研究事業団健診センター[平成16-20年度]）の年齢別・男女別HCV抗体検査，抗体陽性率の比較

％）．40歳節目検診は，若い世代から肝炎ウイルスキャリアを拾い上げる役割を果たしている．一方で，平成14-18年度の肝炎節目検診事業を受検しなかったキャリア（漏れ者）も多く存在する．40歳の節目以外でも，各節目年齢での検診事業を実施できれば，より効率の良い肝炎ウイルスキャリアの掘り起こしにつながるものと考えられる．

2　職域健診における拾い上げの実態と問題点

職域健診における就労年齢層を対象とした拾い上げ対策も重要である．茨城県内で実施された住民健診（平成14-19年度節目・節目外検診受検者約16万人）と職域健診（平成16-20年度，霞ヶ浦成人病研究事業団健診センターにて受検した約3.1万人[人間ドックを含む]）で併せて行われたHCV抗体検査を比較した（図1）[3]．住民健診では，HCVキャリアは男性高齢者に多い傾向があったが，職域健診では年齢層間や男女間での抗体陽性率に偏りがなかった．また，肝炎検査を受診した30-59歳の割合は，職域健診では男女とも70％以上（男性76.4％，女性72.6％）を占めていたが，HCV抗体陽性率（1.60％）は，住民健診（1.90％）と大きな差はみられなかった．職域健診でのHCV抗体検査受検率が約27％と低かったことを踏まえると，就労者の男女に多くの肝炎ウイルスキャリアが潜在していることが推測される．

この職域健診における業種別のHCV抗体検査受検率を調査した結果，医療関係（65％）が最も高かった[4]．また，公務員（58％）や独立・公益法人など（31％），自営業など（47％）においても高い受検率がみられた．一方，一般営利企業の受検率は14％と低く，派遣労働者やパートタイム就労者では1％であった．職域健診での肝炎検査は，別途，費用負担が生じるいわばオプション検査である．職種上，感染の危険性が少ない業種では，肝炎検査の必要がないと見なしている事業所が多いと考えられる．さらに，受検率が最も高い業種である医療関係でも，医療助手や歯科助手，事務職員など，直接，医療に従事しない従業員に対しては，肝炎検査を実

施していない事業所も少なくないのが現状である．

一方で，就労者自身の肝炎検査に関する情報不足や知識の低さ，また，就労者に対する広報や受検勧奨が不十分であることも，職域健診における肝炎検査受検率の低さにつながっている．広島県内の6事業所（タクシー業，ホテル業，製造業など）の定期職員健診の際に行った調査（1,409人，平均年齢48.8±14.1歳［19–80歳］）では，肝炎ウイルス検査受検率は12.0％と低かった[5]．未受検の主な理由として，「肝炎検査を知らなかった」が38.5％，「受ける機会がなかった」が41.4％であった．

就労者には，いまだ多くの肝炎ウイルスキャリアが潜在していると推測される．そのため，職域健診での肝炎検査の受検率を上げることが，就労年齢層における「拾い上げ」対策にとって大きな課題である．また，事業主と就労者の双方に対して，肝炎検査の必要性や認識度を上げる啓蒙がより必要であろう．さらに，出前検診などのシステム導入による受検しやすい環境作りの必要性も挙げられる．

3 保健所・提携医療機関による無料の肝炎ウイルス検診の状況と問題点

肝炎ウイルスキャリアの拾い上げ対策として，各都道府県，政令指定都市や都内特別区などでは，保健所，および各自治体が委託する医療機関において，無料の肝炎ウイルス検査（B型，C型肝炎ウイルス検査）を実施している．この検査では，過去に肝炎ウイルス検査を受検したことがない方を対象にしており，匿名での検査を実施している自治体もある．茨城県での無料肝炎ウイルス検査件数（平成22年4月～平成26年）は，年間平均約2,500件であった[6]．石川県でも，年間2,000件以上（平成21年度）あり，平成24年度までにHBs抗原陽性者48人，HCV抗体陽性者32人が発見されている[7]．

この保健所などによる無料肝炎ウイルス検診は，予約制としている自治体が多く，受付時間も週に1–2回の限られた数時間に限定されていることが多い．また，医療機関との提携体制がなく，保健所のみで実施している自治体も少なからずある．地域によっては，地理的に保健所へのアクセスが悪かったり，保健所に行くことに馴染まない住民が多いことも少なくない．各地域の医療機関への委託を増やし，検査の場所と時間を増やして，多くの住民に受検機会を提供することが，肝炎ウイルスキャリアをより多く拾い上げるという点で重要であろう．

一方，無料肝炎ウイルス検査が，住民に十分周知されていない問題もある．茨城県で実施された市民公開講座や肝臓病教室などにて，肝炎に関心のある参加者を対象に行ったアンケート調査であっても，保健所での無料肝炎ウイルス検査を知っている割合は36％であった[6]．さらに，委託先である連携医療機関の医師に対しても，無料肝炎ウイルス検査の周知が十分でない現状もある．住民側ならびに医師側の双方に対して受検情報をしっかりと提供することで，自治体による拾い上げ対策がより充実するものと考えられる．

4 肝炎ウイルス検査の受検勧奨方法の手段と問題点

肝炎ウイルス感染者を拾い上げる手段として，肝炎ウイルス検査受検勧奨ポスターの貼付，チラシやリーフレットの配布，新聞やテレビ，ラジオにおける広告での受検勧奨が行われている．茨城県では，平成24年と平成25年に，それぞれ異なったデザインの肝炎ウイルス検査受検勧奨ポスターを県内医療機関や自治体を中心に，各約3,000枚を貼付した[6]．その結果，県内保健所による無料肝炎ウイルス検査受検件数が，約2倍に増加した．また，肝疾患診療連携拠点病院にて，受検勧奨のチラシを約2万枚配布したところ，その地域保健所での受検件数が上昇した．アンケート調査によると，ポスターよりもチラシのほうが，肝炎検査に対するより高い認知度をもたらすことが示された．チラシやリーフレットなどの配布によって，地域を絞り情報を集中的に発信することが，効果的な勧奨方法

であると考えられる.

また，マルチメディア(テレビCMやラジオ)を活用した受検勧奨も行われている．佐賀県では，平成25年度に地元出身タレントやマスコットキャラクターを起用した肝炎検査受検勧奨CMをテレビ放映したところ，無料肝炎ウイルス検査受検件数が前年は4,031件だったのに対し，11,834件となり，飛躍的に増加した[8]．同様に，タレントを起用したテレビCMによる受検勧奨は，広島県や静岡県などでも実施されている．また，公益社団法人日本広告審査機構や製薬会社による肝炎検査受検勧奨CMも全国的にテレビ放映されている.

芸能人やマスコットキャラクターなどを起用したマルチメディアによる広告は，注目を集めやすく，非常に効果的な勧奨方法である．しかし，放映期間が終了すると，検診受検者数は徐々に減少し，効果は長続きしない．茨城県では芸能人を起用したポスターの貼付やチラシの配布に加え，肝疾患診療連携拠点病院内の待合室でのデジタルサイネージを用いて肝炎検診受検勧奨を行った．その結果，県内の保健所による肝炎検査受検件数が増加したものの，ポスター貼付やチラシ配布，デジタルサイネージ放映が終了するに従い，受検件数は減少した(図2)．このようなマルチメディアを利用した広告による受検勧奨では，芸能人などの起用やテレビCM放映にかかる費用などが経済的に負担となるため，継続性の課題がある.

5 保健師と地域肝炎治療コーディネーターによる受検勧奨

各自治体の保健師による肝炎検査の受検勧奨も，肝炎ウイルスキャリア拾い上げに大きな役割をもつ．佐賀大学医学部附属病院肝疾患センター実施のアンケート調査によると，保健師による受検勧奨が，テレビCMとともに受検動機に影響する最大の因子であることが示された[9]．また，かかりつけ医による勧奨も受検への大きな動機づけとなっている．医療従事者による肝炎検査受検勧奨は，住民に直に接して行え，影響も大きいため，効果が得られやすい．しかし，保健師などの勧奨を直接行える医療関係者には限りがある．現在，各自治体では，地域肝炎治療コーディネーターを育成している．保健師に加え，看護師や薬剤師，ソーシャルワーカーなどが地域肝炎治療コーディネーターとして認定を受け，肝炎検査の受検勧奨や治療導入などの活動を行っている．医療関係者による住民への直接的な働きかけによって，住民や地域の状況に沿った肝炎ウイルスキャリアの拾い上げにつながることが期待される.

おわりに

肝炎ウイルスに感染していることを知らないまま社会に潜在しているキャリアが多くおり，2005年の集計(日本赤十字センター平成7-12年の初回供血者集団の成績と節目検診事業での成績)によると，HCVキャリアが約80万人，HBVキャリアが約90万人も存在していると推計されている[10]．これら肝炎ウイルスキャリアを効率良く拾い上げ，早期治療に結びつけることが，肝がん撲滅の観点から非常に重要である．行政や医療機関が行っている肝炎対策を広く正しく住民に周知し，肝炎検査受検率を上げ，住民が自身の検査結果をしっかりと把握して正しい対応ができるように啓発することが重要であろう．住民と行政，医療機関が，正しい知識や情報を周知・共有し，連携した総合的な肝炎ウイルスキャリア拾い上げ対策の推進が望まれる.

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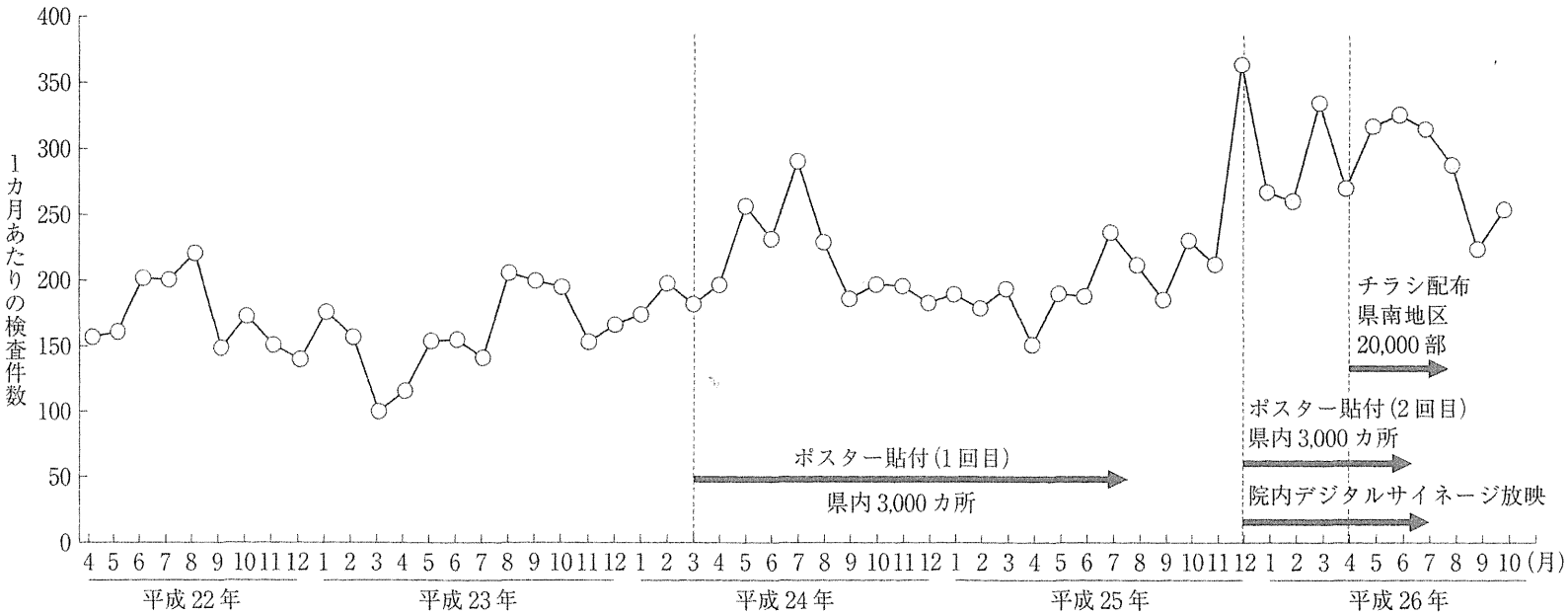

図2　茨城県における肝炎検診受診勧奨ポスター貼付やチラシ配布，肝疾患診療拠点病院（県南地区）内デジタルサイネージ放映による県内保健所無料肝炎ウイルス検査受検件数の推移

値は，1カ月あたりの各種肝炎検査件数の合計（HBs抗原，HCV抗体検出，HCV抗体検査，HCV抗原検査，HCV核酸増幅）を示す．

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ORIGINAL ARTICLE

Viral eradication reduces all-cause mortality in patients with chronic hepatitis C virus infection: a propensity score analysis

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Abstract

Background & Aims: Eradication of hepatitis C virus (HCV) by interferon (IFN)-based therapy has been reported to reduce all-cause mortality rates in patients with chronic HCV infection. However, the impact of HCV eradication on non–liver-related mortality including the causes of death has not been sufficiently investigated in patients with chronic HCV infection. *Methods:* We enrolled 2743 patients with chronic HCV infection. Causes of death, incidence of hepatocellular carcinoma (HCC), and all-cause mortality including non–liver-related diseases, were analysed. *Results:* Of these 2743 patients, 587 achieved sustained virological response (SVR) (eradication of HCV) by IFN-based therapy (IFN-SVR), 475 did not (without HCV eradication) (IFN-non-SVR), or 1681 did not receive IFN-based therapy (non-IFN patients) (Cohort 1); of these, 309 were selected from IFN-SVR and non-IFN groups using propensity score matching (Cohort 2).The median follow-up duration was 11.4 years. In Cohort 1 patients, mortality rates from non–liver-related diseases were 71.0% (22/31) in IFN-SVR patients, 34.9% (37/106) in IFN-non-SVR patients and 50.0% (248/496) in non-IFN patients respectively. In Cohort 2 patients, mortality rates from non–liver-related diseases were 72.2% (13/18) in IFN-SVR patients and 46.8% (29/62) in non-IFN patients respectively. The eradication of HCV reduced all-cause mortality (hazard ratio (HR), 0.265; 95% confidence interval (CI), 0.058–0.380) including non–liver-related mortality (HR, 0.439; 95% CI, 0.231–0.834) and the incidence of HCC (HR, 0.275; 95% CI, 0.156–0.448). *Conclusions:* Eradication of HCV reduced not only liver-related mortality but also non–liver-related mortality in patients with chronic HCV.

Keywords
causes of death – eradication of HCV – hepatitis C – non–liver-related mortality – survival

Chronic infection with hepatitis C virus (HCV) is associated with several extrahepatic manifestations, including sicca syndrome, lichen planus, type 2 diabetes and non-Hodgkin lymphoma. Overall 15–35% of patients with chronic HCV infection have circulating cryoglobulins. Of these, between 5% and 25% will develop clinical conditions such as mixed essential cryoglobulinaemia, systemic vasculitis, peripheral neuropathy, Raynaud's phenomenon and membranoproliferative glomerulonephritis. The risk of developing non-Hodgkin B-cell lymphoma is increased, likely as a consequence of long-term HCV stimulation of B cells (1, 2). HCV is also known to influence several metabolic pathways, increasing the risk of insulin resistance, type 2 diabetes and vascular disease (3). Therefore, extrahepatic manifestations of HCV are being discussed to explain or explore non–

Abbreviations
AFP, α-foetoprotein; ALT, alanine aminotransferase; APRI, aspartate aminotransferase/platelet ratio index; AST, aspartate aminotransferase; AUC, area under the curve; CI, confidence interval; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; ICD, International Statistical Classification of Diseases and Related Health Problems; IFN, interferon; ROC, receiver operating characteristic SVR, sustained virological response.

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Key points

- Impact of HCV eradication on non–liver-related mortality has not been sufficiently investigated.
- 70% of HCV eradicated and 50% of chronic HCV patients died as a result of non–liver-related diseases.
- IFN-SVR reduced significantly non–liver-related mortality compared with IFN-non-SVR.
- Eradication of HCV reduced not only liver-related mortality but also non–liver-related mortality.

liver-related morbidity and mortality associated with HCV.

Interferon (IFN)-based therapy has been used to treat patients with chronic HCV. Many investigators have reported that IFN-based therapy in chronic HCV patients effectively reduces serum levels of alanine aminotransferase (ALT), eliminates circulating HCV RNA and decreases the degree of liver fibrosis (4). Eradication of HCV has also been reported to reduce the incidence of hepatocellular carcinoma (HCC) (5). Taken together, IFN-based therapy clearly reduces the rate of liver disease-related mortality and improves life expectancy (6). In addition, eradication of HCV has been found to resolve extrahepatic immune complex disease, reduce the incidence of lymphoma, decrease the risk of type 2 diabetes and its associated complications and improve patients' overall quality of life (7, 8). Recently, there were several reports for the relationship between the HCV eradication and the reduction in all-cause mortality (9, 10). However, the impact of HCV eradication on non–liver-related mortality including the causes of death has not been sufficiently investigated in patients with chronic HCV infection.

In this study, we conducted long-term follow-up of patients with chronic HCV and analysed the impact of HCV eradication on prognosis, including non–liver-related mortality. In addition, we compared the causes of death between patients in whom HCV was eradicated and those with chronic HCV infection. We used propensity score matching to reduce biases associated with the selection of study patients (11).

Materials and methods

Patients

Ogaki Municipal Hospital is the only general hospital located in a region of 400 000 inhabitants, employing approximately 200 specialists, including more than 15 gastroenterologists. Therefore, a large number of patients with chronic HCV who require HCC surveillance visit the hospital regularly as outpatients. There is also close contact between family medicine clinics, community hospitals and our hospital, including the sharing of patient mortality data. That is, if the patient died other than our hospital, there is the information of their death from the doctor of these institutions.

A total of 8954 consecutive HCV antibody by second- or third-generation enzyme-linked immunosorbent assay-positive patients visited the Department of Gastroenterology at Ogaki Municipal Hospital, Japan, between October 1994 and September 2014. Of these, 2743 met the following inclusion criteria: (i) follow-up duration >3 years; (ii) HCV RNA detectable for longer than 6 months; (iii) HCC surveillance performed during the follow-up period; (iv) absence of human immunodeficiency virus or hepatitis B infection; (v) no other causes of chronic liver disease (alcohol consumption >80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, haemochromatosis and Wilson's disease) and (vi) no evidence of malignancies, including HCC, for at least 1 year from the start of the follow-up period.

We first compared the three groups (achieved sustained virological response (SVR) (IFN-SVR), IFN-non-SVR, and non-IFN) of 2743 patients in terms of HCC incidence, survival from all-cause mortality and mortality from liver-related and non–liver-related diseases (Cohort 1). We then compared the propensity score-matched IFN-SVR and non-IFN (control) patients (Cohort 2) in terms of HCC incidence, survival from all-cause mortality, and mortality from liver-related and non–liver-related diseases (Fig. 1).

In this study, the clinical data for analyses were collected at the time of follow-up start (regarding the IFN-based therapy patients, the analysed clinical data were obtained prior to the treatment). The date of the first visit of all patients including IFN groups was defined as the start of follow-up. The end of follow-up was defined as the date of the final visit for patients who have not died, and as the date of death for patients who died during follow-up.

Hepatitis C virus genotype and RNA were determined using PCR. Amplicor 2 (Roche Diagnostics, Tokyo, Japan) was used prior to 1 January 2008 and COBAS TaqMan HCV (Roche Diagnostics) was used thereafter.

In our hospital, the decision to offer IFN-based therapy to patients with chronic HCV infection was determined according to the guidelines of the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver or the Asian Pacific Association for the Study of the Liver (12–14). In the present study, 1681 patients who did not receive IFN-based therapy for chronic HCV infection had no indications for IFN-based therapy or declined the treatment. If a patient with an indication for IFN-based therapy declined after being provided with sufficient information about the treatment, they received hepatoprotective medications (e.g. ursodeoxycholic acid) as an alternative therapy with the goal of decreasing ALT levels. Patients receiving IFN-based therapy also received hepatoprotective medications until their next antiviral therapy if non-SVR status was confirmed.

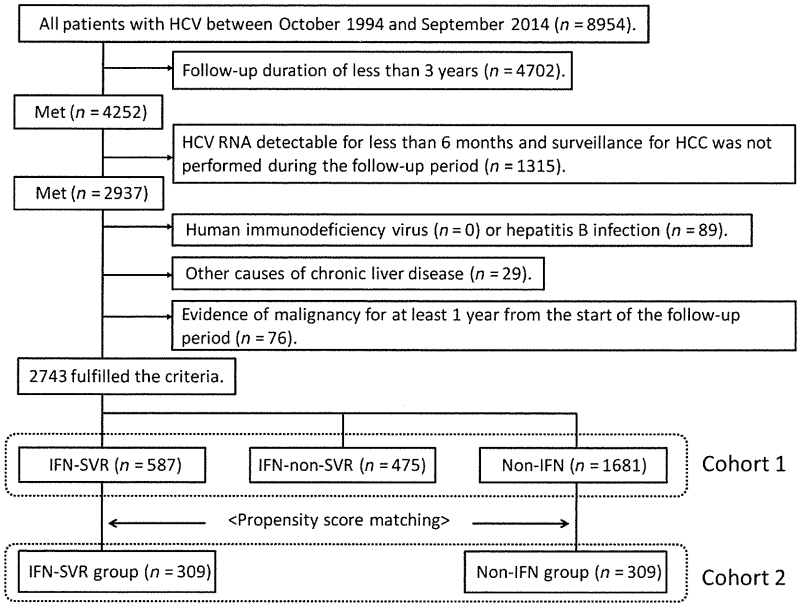

Fig. 1. Flow chart of the patient selection process. HCV, hepatitis C virus; HCC, hepatocellular carcinoma; SVR, sustained virological response IFN, interferon.

In this study, there were no sufficient liver biopsy data for the analysis. Therefore, we used the aspartate aminotransferase (AST)/platelet ratio index (APRI) as an index of liver fibrosis. This index was calculated as (AST [IU/L]/upper limit of normal AST [IU/L]) × 100/platelet count [10^9/L] and previously reported to have utility in liver fibrosis diagnosis (15).

The study protocol was approved by the institutional review board of Ogaki Municipal Hospital and conducted in compliance with the Helsinki Declaration. Informed consent was obtained from each patient for analysing patient records.

Surveillance, diagnosis and causes of death

In IFN-SVR, IFN-non-SVR and non-IFN patients, HCC surveillance was conducted every 3–6 months via ultrasonography and blood tests, including measurement of the tumour marker α-foetoprotein (AFP) according to the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan (16). If a nodular lesion was detected by ultrasonography or a tumour marker was found to be elevated, additional imaging studies (computed tomography, magnetic resonance imaging, or both) were performed. The diagnosis of HCC was based on imaging characteristics specified by the guidelines of the American Association for the Study of Liver Diseases (17).

Diseases other than HCC were initially detected based on clinical symptoms, abnormal surveillance data, medical check-ups (in the community or workplace) or physician assessments. These conditions were then diagnosed and treated by the appropriate specialists in our hospital based on disease-specific criteria and guidelines. Causes of death were categorized by these specialists using International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-9 codes for deaths occurring prior to 1 January 2003, and ICD-10 codes thereafter) (18). All determinations of causes of death were performed retrospectively by collecting and analysing data from all patient medical records in our hospital (including the information of death from the doctor of other than our hospital).

Statistical analysis

Continuous variables are expressed as medians (interquartile range). The Mann–Whitney *U* test (between the two groups) and the Kruskal–Wallis test (among the three groups) were used for continuous variables, and the chi-square test with Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of HCC and survival from all-cause mortality were performed using the Kaplan–Meier method, and differences were tested using the log-rank test (the Holm correction was used in adjustment for multiple comparisons testing among the three groups). Cox proportional hazards models were used for the assessment of hazard ratios (HRs) for the incidence of HCC and overall survival. Actuarial analysis of cumulative mortality from liver-related or non–liver-related diseases was performed using the cumulative incidence with the competing risks method, and

differences were tested using the Gray test (the Holm correction was used in adjustment for multiple comparisons testing among the three groups). Fine and Gray proportional hazards models (19) were used for the assessment of HRs for liver-related and non–liver-related mortality.

To reduce the confounding effects of covariates, we used propensity scores to match IFN-SVR patients to unique non-IFN patients. The following 10 covariates that are related to the prognosis of patients with chronic HCV infection were taken into account at the start of follow-up: age, sex, AST level, ALT level, albumin level, total bilirubin level, prothrombin time, platelet count, AFP level and HCV genotype. The calculated propensity scores of the IFN-SVR and non-IFN patients were 0.25324–0.62539 (interquartile range) (median, 0.46524) and 0.25346–0.62589 (median, 0.46505) respectively; these scores were then rounded to two decimal places. We conducted one-to-one patient matching based on these propensity scores. Propensity score matching resulted in the selection of 618 patients (IFN-SVR group, 309 patients; non-IFN group, 309 patients) (Cohort 2) (Fig. 1). Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test. The Hosmer–Lemeshow test compares model performance (observed vs. expected) across deciles of risk to test whether the model is biased. A non-significant value for the Hosmer–Lemeshow test suggests the absence of such bias. The *P*-value of the calculated propensity scores based on the Hosmer–Lemeshow test was 0.583. Discrimination of the propensity score model was assessed using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, with higher values indicating better discrimination. The AUC-calculated propensity score was 0.912 (95% confidence interval (CI), 0.900–0.924).

Statistical significance was defined as $P < 0.05$. Propensity score analysis was performed with SPSS, version 18.0 for Windows (IBM Japan, Tokyo, Japan). Remaining statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (20). More precisely, it is modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Patient characteristics and causes of death

The characteristics of all 2743 patients are summarized in Table 1. The patients consisted of 1297 women (47.2%) and 1446 men (52.6%) with a median (interquartile range) age of 60.0 (51.0–66.0) years old. There was no patient with HCV genotype 3. Of the 2743 patients, 633 died during follow-up; causes of death are also shown in Table 1. Mortality was caused by liver-related diseases in 51.5% (326/633) of patients, 80.4% (262/326) of whom died of HCC. In contrast, the causes of death in the 48.5% (307/633) of patients who died of non–liver-related diseases included a variety of malignancies other than HCC, including haematological malignancies. There were no patterns observed regarding the cause of death in non–liver-related, non-malignant diseases.

Patient characteristics stratified by the IFN-based therapy and SVR status (Cohort 1)

Of these 2743 patients, 1062 received IFN-based therapy, while the other 1681 did not receive IFN-based therapy (non-IFN). Of these 1062 patients who received IFN-based therapy, 587 achieved SVR (IFN-SVR) and the remaining 475 did not (relapse, 265 patients; non-response, 210 patients) (IFN-non-SVR). Together the IFN-SVR (HCV eradication), IFN-non-SVR (without HCV eradication) and non-IFN (chronic HCV infection) groups comprised Cohort 1. The baseline characteristics of the 2743 study patients stratified by the IFN-based therapy and SVR status are summarized in Table 1. There were significant differences in age, ALT, albumin, total bilirubin, prothrombin time, platelet count, AFP, APRI, HCV genotype, HCV RNA and follow-up duration among the three groups.

The respective mortality rates from liver-related and non–liver-related diseases were 29.0% (9/31) and 71.0% (22/31) in IFN-SVR patients, 65.1% (69/106) and 34.9% (37/106) in IFN-non-SVR patients, and 50.0% (248/496) and 50.0% (248/496) in non-IFN patients ($P < 0.001$).

Cumulative incidence of HCC, survival from all-cause mortality, and mortality from liver-related and non–liver-related diseases in Cohort 1 patients

Figure 2a shows the curves for the incidence of HCC in Cohort 1 patients, stratified by the IFN-based therapy and SVR status. The respective 5-, 10-, 15- and 20-year cumulative incidences of HCC were 1.0%, 4.4%, 5.7% and 8.0% in the IFN-SVR group, 5.9%, 14.7%, 26.4% and 37.1% in the IFN-non-SVR group, and 8.7%, 21.3%, 30.9% and 43.1% in the non-IFN group. The incidence of HCC differed significantly between IFN-SVR group and IFN-non-SVR group ($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$) and IFN-non-SVR group and non-IFN group ($P = 0.015$), respectively, after Holm correction.

Figure 2b shows the curves for survival from all-cause mortality in Cohort 1 patients stratified by the IFN-based therapy and SVR status. The respective 5-, 10-, 15- and 20-year cumulative survival rates were 99.1%, 97.7%, 95.6% and 91.8% in the IFN-SVR group, 99.4%, 93.3%, 82.2% and 68.6% in the IFN-non-SVR group, and 94.9%, 79.2%, 63.3% and 47.7% in the non-IFN group. The survival from all-cause mortality differed significantly between IFN-SVR group and IFN-non-SVR group

Table 1. Characteristics of the Cohort 1 patients

		Stratified by the IFN-based therapy			
	Overall (n = 2743)	IFN-SVR (n = 587)	IFN-non-SVR (n = 475)	Non-IFN (n = 1681)	P-value
Age (years)*	60.0 (51.0–66.0)	50.0 (42.0–57.0)	54.0 (48.0–60.0)	64.0 (57.0–69.0)	<0.001**
Sex (female/male)	1297/1446	263/324	209/266	825/856	0.059***
AST (IU/L)*	44 (29–73)	44 (29–76)	49 (31–85)	42 (28–70)	0.073**
ALT (IU/L)*	49 (29–88)	62 (35–119)	64 (38–107)	42 (26–74)	<0.001**
Albumin (g/dl)*	4.1 (3.9–4.3)	4.2 (4.0–4.4)	4.2 (3.9–4.4)	4.0 (3.8–4.3)	<0.001**
Total bilirubin (mg/dl)*	0.6 (0.4–0.8)	0.6 (0.4–0.8)	0.6 (0.5–0.8)	0.6 (0.4–0.8)	0.001**
Prothrombin time (%)*	97.0 (86.0–105.0)	100.0 (92.8–107.0)	100.0 (90.0–102.0)	94.0 (82.0–105.0)	<0.001**
Platelet count ($\times 10^4/mm^3$)*	16.7 (12.7–21.1)	18.8 (15.0–23.1)	16.4 (12.7–20.8)	16.2 (11.7–20.4)	<0.001**
AFP (ng/ml)*	3.6 (2.2–7.6)	2.7 (1.6–4.4)	4.1 (2.5–9.5)	4.2 (2.3–8.0)	<0.001**
APRI*	0.69 (0.37–1.36)	0.61 (0.36–1.15)	0.79 (0.4–1.52)	0.71 (0.37–1.43)	0.001**
HCV genotype (1/2/unknown)	1476/789/478	256/309/22	348/117/10	872/363/446	<0.001***
HCV RNA (log_{10} IU/ml)*	5.6 (4.5–6.1)	5.3 (4.1–6.0)	6.0 (5.2–6.5)	5.6 (4.4–6.0)	<0.001**
Follow-up duration (years)*	11.8 (7.5–17.0)	14.0 (8.6–20.0)	14.2 (9.8–20.0)	10.5 (6.6–14.9)	<0.001**
Development of HCC	574	31	131	412	
Deaths	633	31	106	496	
Causes					
Liver-related diseases	326/633 (51.5%)	9/31 (29.0%)	69/106 (65.1%)	248/496 (50.0%)	<0.001***
HCC	262/326 (80.4%)	8	60	194	
Liver failure	64/326 (19.6%)	1	9	54	
Non–liver-related diseases	307/633 (48.5%)	22/31 (71.0%)	37/106 (34.9%)	248/496 (50.0%)	
Malignancies	119/307 (38.8%)	14/22 (63.6%)	18/37 (48.6%)	87/248 (35.1%)	
Digestive malignancies	64	10	10	44	
Respiratory malignancies	26	1	3	22	
Haematological malignancies	16	1	3	12	
Other	13	2	2	9	
Non-malignant diseases	188/307 (61.2%)	8/22 (36.4%)	19/37 (51.4%)	161/248 (64.9%)	
Digestive diseases	7	0	0	7	
Respiratory diseases	52	1	4	47	
Cardiovascular diseases	51	1	5	45	
Renal diseases	14	0	2	12	
Cerebrovascular diseases	41	5	5	31	
Injury	13	1	1	11	
Other	10	0	2	8	
Propensity score		0.64719 (0.40867–0.82733)		0.04069 (0.00681–0.18379)	<0.001****

*Values are expressed as medians (interquartile range).

**Kruskal–Wallis test.

***Chi-square test.

****Mann–Whitney U test.

IFN, interferon; SVR, sustained virological response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-foetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; HCV, hepatitis C virus; HCC, hepatocellular carcino.

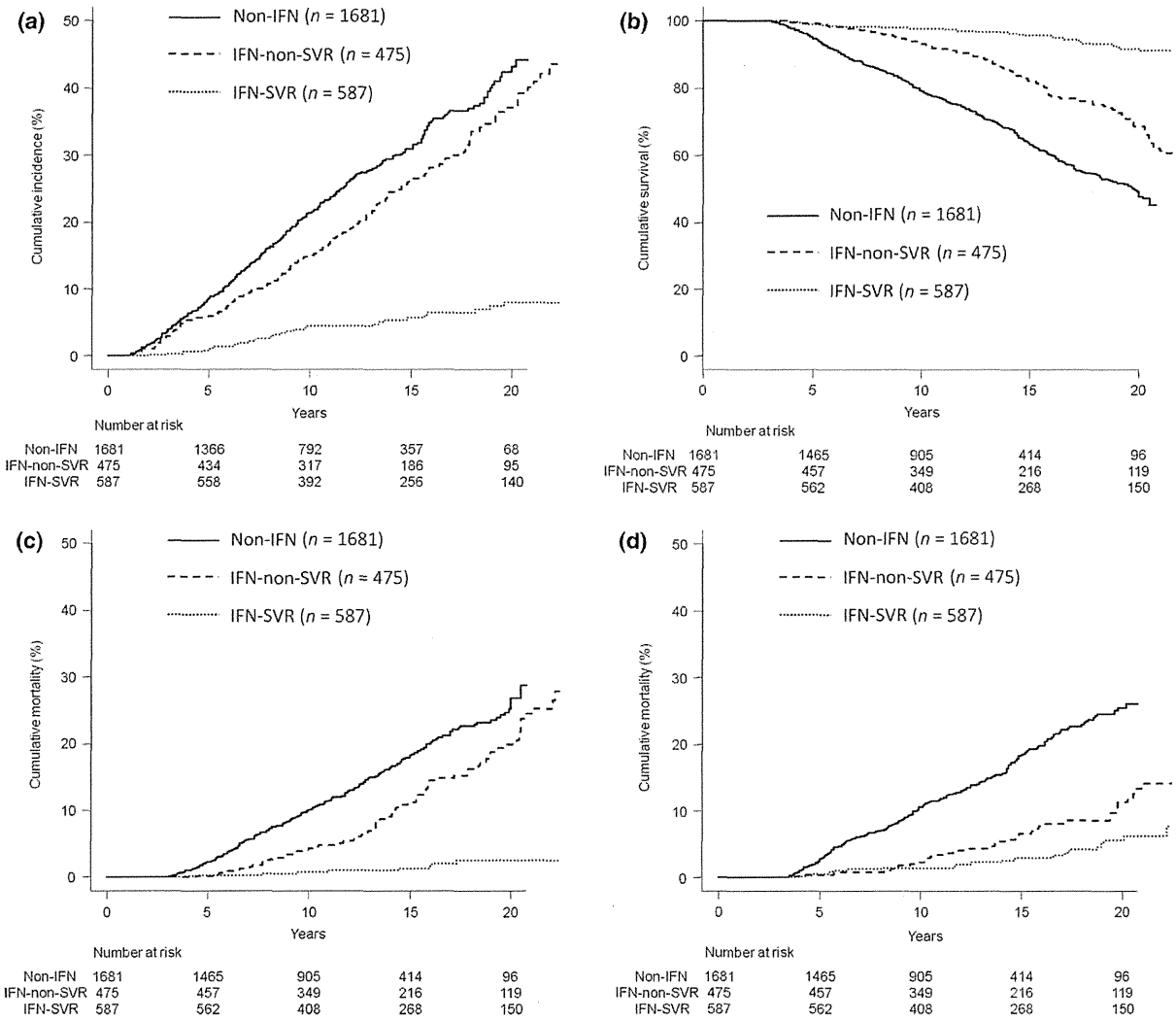

Fig. 2. (a) Curves for cumulative incidence of HCC in Cohort 1 patients. The incidence of HCC differed significantly between IFN-SVR group and IFN-non-SVR group ($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$), and IFN-non-SVR group and non-IFN group ($P = 0.015$), after Holm correction (log-rank test). (b) Curves for cumulative survival from all-cause mortality in Cohort 1 patients. The survival from all-cause mortality differed significantly between IFN-SVR group and IFN-non-SVR group ($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$) and IFN-non-SVR group and non-IFN group ($P < 0.001$), after Holm correction (log-rank test). (c) Curves for cumulative mortality from liver-related diseases in Cohort 1 patients. The mortality from liver-related diseases differed significantly between IFN-SVR group and IFN-non-SVR group ($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$), and IFN-non-SVR group and non-IFN group ($P = 0.002$), after Holm correction (Gray test). (d) Curves for cumulative mortality from non–liver-related diseases in Cohort 1 patients. The mortality from non–liver-related diseases differed significantly between IFN-SVR group and IFN-non-SVR group ($P = 0.016$), IFN-SVR group and non-IFN group ($P < 0.001$) and IFN-non-SVR group and non-IFN group ($P < 0.001$), after Holm correction (Gray test). IFN, interferon; SVR, sustained virological response.

($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$), and IFN-non-SVR group and non-IFN group ($P < 0.001$), respectively, after Holm correction.

Figure 2c shows the curves for mortality from liver-related diseases in Cohort 1 patients stratified by the IFN-based therapy and SVR status. The respective 5-, 10-, 15- and 20-year cumulative mortality rates were 0.3%, 0.8%, 1.4% and 2.5% in the IFN-SVR group, 0.2%, 4.3%, 11.3% and 20.0% in the IFN-non-SVR group, and 2.3%, 10.1%, 18.3% and 26.8% in the non-IFN group. The mortality from liver-related diseases differed significantly between IFN-SVR group and IFN-non-SVR group ($P < 0.001$), IFN-SVR group and non-IFN group ($P < 0.001$), and IFN-non-SVR group and non-IFN group ($P = 0.002$), respectively, after Holm correction.

Figure 2d shows the curves for mortality from non–liver-related diseases in Cohort 1 patients stratified by

the IFN-based therapy and SVR status. The respective 5-, 10-, 15- and 20-year cumulative mortality rates were 0.5%, 1.5%, 3.0% and 5.6% in the IFN-SVR group, 0.4%, 2.4%, 6.5% and 11.4% in the IFN-non-SVR group, and 2.8%, 10.7%, 18.5% and 25.5% in the non-IFN group. The mortality from non–liver-related diseases differed significantly between IFN-SVR group and IFN-non-SVR group ($P = 0.016$), IFN-SVR group and non-IFN group ($P < 0.001$), and IFN-non-SVR group and non-IFN group ($P < 0.001$), respectively, after Holm correction.

Patient characteristics stratified by the presence or absence of HCV after propensity score matching (Cohort 2)

The baseline characteristics of the 618 study patients after propensity score matching are summarized in Table 2. There were no significant differences in between the IFN-SVR and non-IFN (control) groups.

In the IFN-SVR group, mortality rates from liver-related and non–liver-related diseases were 27.8% (5/18) and 72.2% (13/18) respectively. Conversely, in non-IFN patients, mortality rates from liver-related and non–liver-related diseases were 53.2% (33/62) and 46.8% (29/62) respectively ($P = 0.066$).

Cumulative incidence of HCC, survival from all-cause mortality, and mortality from liver-related and non–liver-related diseases in Cohort 2 patients

Figure 3a shows the curves for the incidence of HCC in Cohort 2 patients stratified by the presence or absence of HCV. The respective 5-, 10-, 15- and 20-year cumulative incidences of HCC were 1.3%, 4.8%, 5.4% and

Table 2. Characteristics of the Cohort 2 patients stratified by the presence or absence of HCV after propensity score matching ($n = 618$)

	IFN-SVR ($n = 309$)	Non-IFN ($n = 309$)	*P*-value
Age (years)*	54.0 (46.0–60.0)	55.0 (47.0–60.0)	0.277**
Sex (female/male)	152/157	152/157	1.000***
AST (IU/L)*	40 (27–63)	42 (27–67)	0.646**
ALT (IU/L)*	50 (30–92)	50 (31–91)	0.691**
Albumin (g/dl)*	4.2 (4.0–4.4)	4.2 (4.0–4.4)	0.773**
Total bilirubin (mg/dl)*	0.6 (0.5–0.8)	0.5 (0.4–0.7)	0.052**
Prothrombin time (%)*	100.0 (91.0–106.0)	100.0 (92.3–109.0)	0.507**
Platelet count ($\times 10^4/mm^3$)*	17.6 (14.7–22.2)	17.7 (14.6–22.0)	0.616**
AFP (ng/ml)*	3.0 (1.7–5.4)	3.0 (1.9–5.5)	0.464**
APRI*	0.57 (0.35–1.09)	0.64 (0.32–1.22)	0.574**
HCV genotype (1/2/unknown)	164/124/21	176/118/15	0.456***
HCV RNA ($\log_{10}$ IU/ml)*	5.5 (4.2–6.1)	5.6 (4.5–6.0)	0.858**
Follow-up duration (years)*	13.2 (8.4–19.6)	13.5 (8.8–17.3)	0.083**
Development of HCC	17	56	
Deaths	18	62	
Causes			
Liver-related diseases	5/18 (27.8%)	33/62 (53.2%)	0.066***
HCC	5	26	
Liver failure	0	7	
Non–liver-related diseases	13/18 (72.2%)	29/62 (46.8%)	
Malignancies	8/13 (61.5%)	12/29 (41.4%)	
Digestive malignancies	5	7	
Respiratory malignancies	0	2	
Haematological malignancies	1	2	
Other	2	1	
Non-malignant diseases	5/13 (38.5%)	17/29 (58.6%)	
Digestive diseases	0	1	
Respiratory diseases	0	2	
Cardiovascular diseases	0	5	
Renal diseases	0	5	
Cerebrovascular diseases	4	4	
Injury	1	0	
Other	0	0	
Propensity score	0.46524 (0.25324–0.62539)	0.46505 (0.25346–0.62589)	1.000**

*Values are expressed as medians (interquartile range).

**Mann–Whitney *U* test.

***Chi-square test.

IFN, interferon; SVR, sustained virological response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, α-foetoprotein; APRI, aspartate aminotransferase-to-platelet ratio index; HCV, hepatitis C virus; HCC, hepatocellular carcinoma.

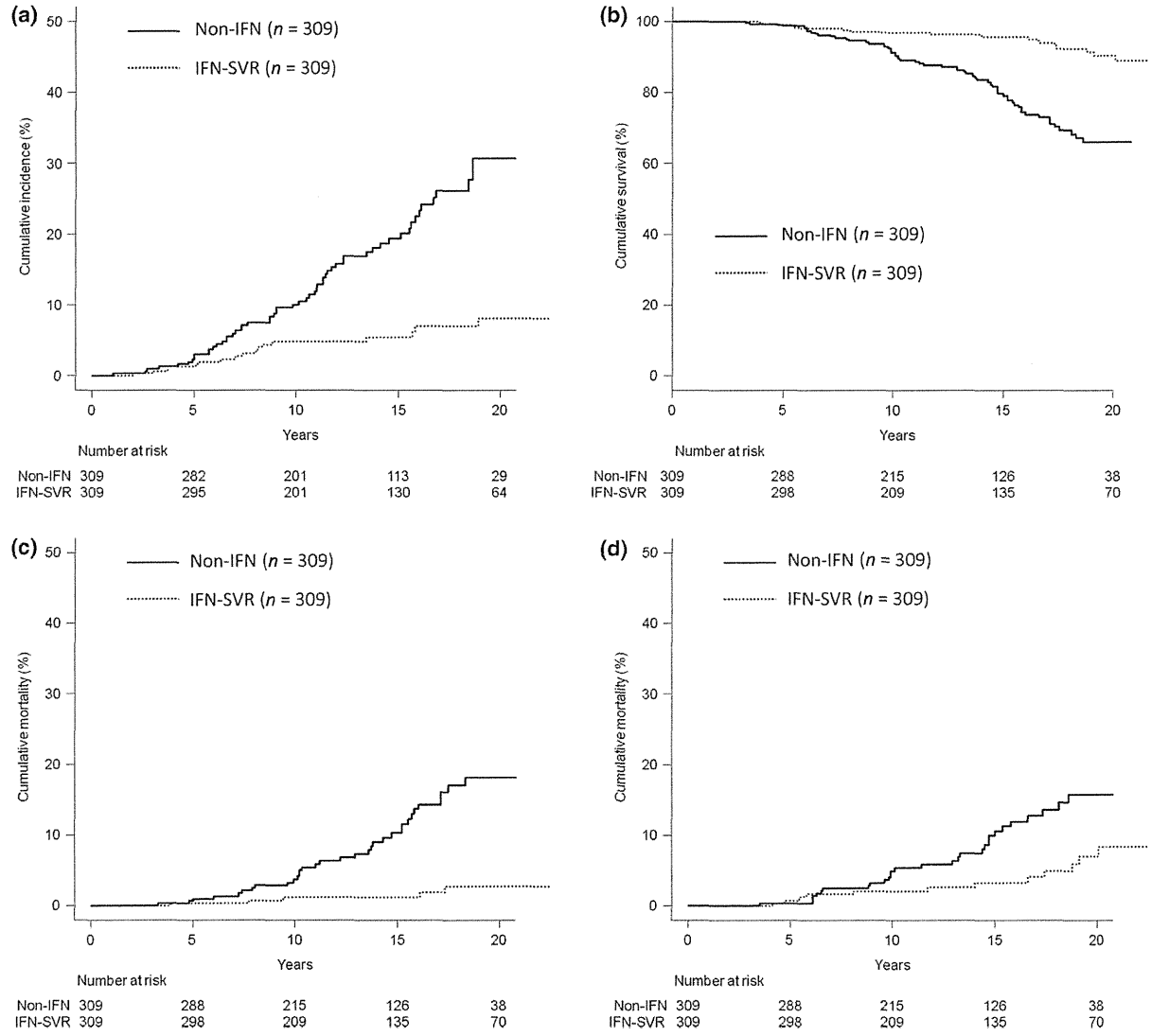

Fig. 3. (a) Curves for cumulative incidence of HCC in Cohort 2 patients. There is a significant difference between IFN-SVR patients and non-IFN patients ($P < 0.001$; log-rank test). (b) Curves for cumulative survival from all-cause mortality in Cohort 2 patients. There is a significant difference between IFN-SVR patients and non-IFN patients ($P < 0.001$; log-rank test). (c) Curves for cumulative mortality from liver-related diseases in Cohort 2 patients. There is a significant difference between IFN-SVR patients and non-IFN patients ($P < 0.001$; Gray test). (d) Curves for cumulative mortality from non–liver-related diseases in Cohort 2 patients. There is a significant difference between IFN-SVR patients and non-IFN patients ($P = 0.011$; Gray test). IFN, interferon; SVR, sustained virological respons.

8.1% in the IFN-SVR group, and 3.0%, 10.0%, 19.3% and 30.7% in the non-IFN group ($P < 0.001$) (HR, 0.275; 95% CI, 0.159–0.473; $P < 0.001$).

Figure 3b shows the curves for survival from all-cause mortality in Cohort 2 patients stratified by the presence or absence of HCV. The respective 5-, 10-, 15- and 20-year cumulative survival rates were 99.0%, 96.7%, 95.6% and 90.2% in the IFN-SVR group, and 98.7%, 91.3%, 79.1% and 66.0% in the non-IFN group ($P < 0.001$) (HR, 0.265; 95% CI, 0.156–0.448; $P < 0.001$).

Figure 3c shows the curves for mortality from liver-related diseases in Cohort 2 patients stratified by the presence or absence of HCV. The respective 5-, 10-, 15- and 20-year cumulative mortality rates were 0.3%, 1.2%, 1.2% and 2.8% in the IFN-SVR group, and 1.0%, 3.7%, 10.3% and 18.1% in the non-IFN group ($P < 0.001$) (HR, 0.149; 95% CI, 0.058–0.380; $P < 0.001$).

Figure 3d shows the curves for mortality from non–liver-related diseases in Cohort 2 patients stratified by the presence or absence of HCV. The respective 5-, 10-, 15- and 20-year cumulative mortality rates were 0.7%, 2.1%, 3.3% and 7.0% in the IFN-SVR group, and 0.3%, 5.0%, 10.6% and 15.8% in the non-IFN group ($P = 0.011$) (HR, 0.439; 95% CI, 0.231–0.834; $P = 0.012$).

Discussion

This study, an analysis of a large number of chronic HCV patients with long-term follow-up, showed that the eradication of HCV reduced the incidence of HCC and increased the survival from all-cause mortality, including that caused by non–liver-related diseases. Approximately 70% of IFN-SVR patients died as a result of non–liver-related diseases, while half of non-IFN patients died from non–liver-related diseases. Of the IFN-SVR patients who died because of non–liver-related diseases, approximately 60% died from malignancies other than HCC, compared to more than 30% in the non-IFN patients. Additionally, in the IFN-treated group, SVR reduced significantly the incidence of HCC and increased the survival from all-cause mortality, including that caused by non–liver-related diseases compared with non-SVR (Cohort 1). Similarly, propensity score matching performed to reduce biases associated with the selection of study patients showed that the eradication of HCV reduced the incidence of HCC and increased survival from all-cause mortality, including non–liver-related diseases (Cohort 2). Further, more than 70% of IFN-SVR patients and approximately 50% of non-IFN patients in this cohort died due to non–liver-related diseases.

Retrospective studies with a duration of HCV infection between 20 and 30 years reported the following incidence rates: cirrhosis, 17–55%; HCC, 1–23%; and liver-related deaths, 1–23% (21, 22). Numerous extrahepatic manifestations of HCV infection have also been reported. Cacoub *et al.* (23) found that almost 40% of patients with HCV developed at least one extrahepatic manifestation during the course of disease. Most of the available data are regarding HCV-related autoimmune and lymphoproliferative disorders, from benign mixed cryoglobulinaemia to frank lymphoma, which is consistent with HCV lymphotropism.

van der Meer *et al.* (9) reported that IFN-SVR was associated with reduced risk of all-cause mortality (HR, 0.26) and reduced risk of liver-related mortality or transplantation (HR, 0.06) in the 530 patients with chronic HCV. However, the impact of IFN-SVR on non–liver-related mortality including the causes of death was not sufficiently investigated in their report.

Additionally, in a prospective study by Lee *et al.* (24) that adjusted for multivariate factors such as age and sex, HCV-antibody–positive patients with detectable HCV RNA levels had significantly higher rates of mortality from both liver-related and non–liver-related diseases than either HCV-antibody–positive patients with undetectable levels of HCV RNA or HCV-antibody–negative patients. These authors also reported that while all-cause mortality was increased in HCV-antibody–positive patients with detectable HCV RNA levels, it was similar between HCV-antibody–positive patients with undetectable levels of HCV RNA and HCV-antibody–negative patients. In their study, of the 975 HCV-antibody–positive patients who had samples available for testing, HCV RNA was undetectable in 298 (30.6%) patients and was detectable in 677 (69.4%).

Although this study used a retrospective design, it included a large number of HCV-antibody–positive patients with detectable HCV RNA levels who were treated at a single medical centre located in a region of 400 000 inhabitants. Further, patient outcomes, including non–liver-related mortality and causes of death, were compared in detail between patients with HCV eradication and patients with chronic HCV infection. Additionally, we analysed propensity scores to match patients based on age, sex, AST level, ALT level, albumin level, total bilirubin level, prothrombin time, platelet count, AFP level and HCV genotype, all covariates associated with the prognosis of patients with HCV. We demonstrated that antiviral therapy aimed at HCV eradication in patients with chronic HCV reduced all-cause mortality, including that caused by non–liver-related diseases. Conversely, the study by Lee *et al.* did not provide details regarding the antiviral therapy administered to HCV-antibody–positive patients, or delineate patient characteristics, including the number of patients after multivariate factor adjustment among the HCV-antibody–positive patients with detectable HCV RNA levels, HCV-antibody–positive patients with undetectable levels of HCV RNA, and HCV-antibody–negative patients.

Recently, direct-acting antiviral (DAA) therapies without IFN have raised the possibility of a new era of chronic HCV treatment marked by increased SVR rates, shorter and simpler regimens, and minimal treatment-related side effects. It is assumed that in the future, these therapies will increase the rate of HCV eradication in patients with chronic HCV infection, and therefore reduce not only liver-related mortality but non–liver-related mortality as well. In this study, therefore, we compared the HCV eradicated patients by the treatment and the patients with persistent infection of untreated HCV using the propensity score matching. Since a recent study (25) suggested that maintenance peg-IFN therapy reduced the incidence of HCC, it is possible that IFN-based therapy suppresses hepatocarcinogenesis via a mechanism separate from HCV eradication. Future prospective studies of DAA therapies for patients with chronic HCV are warranted, in particular to evaluate the incidence of HCC and all-cause mortality, including that caused by non–liver-related diseases.

In this study, there were significant differences between the IFN-non-SVR group and non-IFN group in the incidence of HCC and survival from all-cause mortality including non–liver-related diseases. Both groups had persistent HCV infection, therefore, the difference of the incidence of HCC or all-cause mortality might be because of another confounder (e.g. healthier patients received IFN-based therapy).

This study has several limitations. First, findings from propensity score analyses might be limited by biases

related to unmeasured and hidden covariates (e.g. such as mental illness and risky drug behaviours). One-to-one matching based on propensity scores resulted in the inclusion of fewer patients in the analysis. Two other limitations of this study were its hospital-based subject population and its retrospective nature. In addition, this study findings was tempered by limiting to patients engaged in long-term follow-up in hospital. Although our hospital is the only general hospital serving a large number of nearby patients with chronic HCV, further prospective studies with community-based subjects are warranted.

In conclusion, non–liver-related diseases during follow-up were the cause of death in approximately 70% of patients in whom HCV was eradicated and in approximately 50% of patients with chronic HCV infection. This study also suggests that the eradication of HCV reduces both liver-related and non–liver-related mortality in patients with chronic HCV infection. Further studies are warranted to confirm these findings in other populations.

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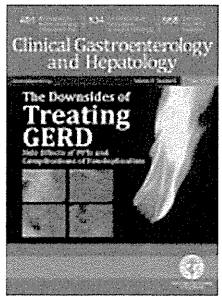

Role of the GALAD and BALAD-2 Serologic Models in Diagnosis of Hepatocellular Carcinoma and Prediction of Survival in Patients

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ROLE OF THE GALAD AND BALAD-2 SEROLOGIC MODELS IN DIAGNOSIS OF HEPATOCELLULAR CARCINOMA AND PREDICTION OF SURVIVAL IN PATIENTS

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Running title: International validation of GALAD and BALAD-2 score

Abbreviations: AFP, alpha-fetoprotein; AUROC, area under ROC curve; DCP, Des-gamma carboxyprothrombin; CLD, chronic liver disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ROC, receiver operating characteristic; USS, ultrasound scan

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Some of the these data were reported in abstract form at The Liver Meeting, The 64th Annual Meeting of the American Association for the Study of Liver Diseases 2013[1] and EASL 2015.

Background & Aims: GALAD and BALAD-2 are statistical models for estimating the likelihood of the presence of hepatocellular carcinoma (HCC) in individual patients with chronic liver disease and survival of patients with HCC, respectively. Both models use objective measures, particularly the serum markers □-fetoprotein (AFP), AFP-L3, and des-□-carboxyprothrombin (DCP). We aimed to

validate these models in an international cohort of patients with HCC and assess their clinical performance.

Methods: We collected data on cancer diagnosis and outcomes of 6834 patients (2430 with HCC and 4404 with chronic liver disease) recruited from Germany, Japan, and Hong Kong. We also collected data from 229 patients with other hepatobiliary tract cancers (cholangiocarcinoma or pancreatic adenocarcinoma) and 92 healthy individuals (controls). For reference, the original UK cohort (on which the GALAD model was initially built and BALAD-2 validated) was included in the analysis. We assessed the effects of tumor size and etiology on GALAD model performance, and its ability to correctly discriminate HCC from other hepatobiliary cancers. We assessed the performance of BALAD-2 in patients with different stages of HCC.

Results: In all cohorts, the area under the receiver operating characteristic curve (AUROC), quantifying the ability of GALAD to discriminate patients with HCC from patients with chronic liver disease, was >0.90—similar to the series on which the model was originally built (AUROC, 0.97). GALAD discriminated patients with HCC from those with other hepatobiliary cancers with an AUROC value of 0.95; values were slightly lower for patients with small, unifocal HCCs, ranging from 0.85 to 0.95. Etiology and treatment of chronic viral hepatitis had no effect on the performance of this model. BALAD-2 analysis assigned patients with HCC to 4 distinct prognostic groups—overall and when patients were stratified according to disease stage.

Conclusions: We validated the performance of the GALAD and BALAD-2 models for diagnosis of HCC and predicting patient survival, respectively (based on levels of the serum markers AFP, AFP-

L3, and DCP) in an international cohort of almost 7000 patients. These systems might be used in HCC surveillance and determination of patient prognosis.

KEY WORDS: liver cancer, prognostic marker, diagnostic, quantification