Original research

Validation of Serological Models for Staging and Prognostication of HCC in Patients from Japanese Nationwide Survey

Hidenori Toyoda[1], hmtoyoda@spice.ocn.ne.jp

Takashi Kumada[1], hosp3@omh.ogaki.gifu.jp

Toshifumi Tada[1], tadat0627@gmail.com

Philip J. Johnson[2], Philip.Johnson@liverpool.ac.uk

Namiki Izumi[3], izumi012@musashino.jrc.or.jp

Masumi Kadoya[4], kadoyam@shinshu-u.ac.jp

Norihiro Kokudo[6], KOKUDO-2SU@h.u-tokyo.ac.jp

Shoji Kubo[7], m7696493@msic.med.osaka-cu.ac.jp

Yutaka Matsuyama[8], matuyama@epistat.m.u-tokyo.ac.jp

Osamu Nakashima[9], osamu31@med.kurume-u.ac.jp

Michiie Sakamoto[10], msakamot@z5.keio.jp

Tadatoshi Takayama[11], takayama.tadatoshi@nihon-u.ac.jp

Masatoshi Kudo[12], m-kudo@med.kindai.ac.jp

Liver Cancer Study Group of Japan, kangan@nihon-kangan.jp

[1]Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan, [2]Institute of Translational Medicine, University of Liverpool, Liverpool, U.K., [3]Department of Gastroenterology, Musashino Red Cross Hospital, Tokyo, Japan, [4]Department of Radiology, Shinshu University School of Medicine, Matsumoto, Japan, [5]Department of Gastroenterology, Kanazawa University Hospital, Kanazawa, Japan, [6]Hepato-Biliary-Pancreatic Surgery Division. Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, [7]Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, Kobe University Graduate School of Medicine, Kobe, Japan, [8]Department of Biostatics, School of Public Health University of Tokyo, Tokyo, Japan, [9]Department of Clinical Laboratory Medicine, Kurume University Hospital, Kurume, Japan, [10]Department of Pathology, Keio University School of Medicine, Tokyo, Japan, [11]Department of Digestive Surgery, Nihon University School of Medicine, Tokyo, Japan, [12]Department

of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan.

Keywords: hepatocellular carcinoma, staging, serological markers, tumor progression, liver function, prognosis

Contact information

Hidenori Toyoda, MD, PhD

Department of Gastroenterology, Ogaki Municipal Hospital

4-86 Minaminokawa, Ogaki, Gifu, 503-8502 JAPAN

telephone: +81-584-81-3341, facsimile: +81-584-75-5715

email: hmtoyoda@spice.ocn.ne.jp

List of Abbreviations

HCC, hepatocellular carcinoma; T-Bil, total bilirubin; ALB, albumin; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; LCSGJ, Liver Cancer Study Group of Japan; CT, computed tomography; MRI, magnetic resonance imaging; AIC, Akaike information criteria; HCV, hepatitis C virus; HBV, hepatitis B virus.

Financial Support

The authors declare that there is no grants or other financial support on this manuscript.

Abstract

Two serology-based staging models of patients with hepatocellular carcinoma (HCC), the BALAD and BALAD-2 models, were applied to a Japanese cohort of a nationwide follow-up survey of HCC. The ability of these models to predict the progression of HCC and the deterioration of liver function and to assess prognosis was evaluated. BALAD and BALAD-2 scores were calculated in 6816 patients from a cohort of Japanese nationwide survey based on the serum levels of five markers (bilirubin, albumin, *lens culinaris* agglutinin-reactive alpha-fetoprotein, alpha-fetoprotein, and des-gamma-carboxy prothrombin) measured at the time of HCC diagnosis. The associations of these scores with the progression of HCC and liver function and with survival rates were analyzed. There were good correlations between BALAD and BALAD-2 scores and the progression of HCC and Child-Pugh class. Both staging scores accurately categorized patients into risk groups with different survival rates. BALAD-2 showed superior discrimination of patient survival compared with the original BALAD. *Conclusions*: Serology-based staging models, especially the BALAD-2 model, were useful for staging and prognostication of survival in a cohort of

Japanese patients with HCC from a nationwide survey.

Introduction

We previously proposed a serology-based staging system for hepatocellular carcinoma (HCC), the BALAD scoring model, for assessing the prognosis of patients with HCC (1). This scoring system is based solely on the serum levels of five parameters, namely, total bilirubin (T-Bil), albumin (ALB), *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3), alpha-fetoprotein (AFP), and des-gamma-carboxy prothrombin (DCP). The model predicted the outcome of patients with HCC with high discrimination (1). Recently, Fox et al. proposed an improved serologic staging model, BALAD-2, that was developed using a more sophisticated statistical method that treated variables in a continuous manner (2). Both BALAD and BALAD-2 were shown to have excellent prognostic discrimination in international settings (2, 3), despite large differences across regions in both HCC progression and survival after diagnosis.

In the present study, we applied these two serologic staging models to 24029 patients with HCC in Japan, where surveillance of HCC is established and patients are diagnosed with HCC earlier and have longer survival than in Western and other Asian

countries. Since 1965, the Liver Cancer Study Group of Japan (LCSGJ) has been conducting biannual nationwide follow-up surveys and prospectively collecting data on patients with HCC in Japan. We conducted this retrospective study based on these prospectively collected data which included variables for BALAD and BALAD-2 scores.

Patients and Methods

Patients and Treatments

A total of 66554 patients with primary liver cancer were prospectively registered biannually by LCSGJ from more than 750 participating institutions from January 2000 to December 2007 using a registration/questionnaire sheet with more than 180 questions. Data regarding three tumor markers for HCC, specifically AFP, AFP-L3, and DCP, were beginning with the 16th survey. Therefore, the current study used the data from 2000 (16th survey) to 2007 (18th survey, the latest). The data from 24029 patients contained all the laboratory variables necessary for calculating the BALAD and BALAD-2 scores, i.e., serum ALB, T-Bil, AFP, AFP-L3, and DCP at diagnosis of HCC, as well as the final

prognosis (Figure 1). HCC was diagnosed on the basis of imaging studies, clinical data, or histopathologic studies at each institution. Treatment types were determined by the treatment algorithm for HCC proposed by Japanese guidelines (4). The patients were prospectively followed up at each institution. Most patients underwent ultrasonography and measurements of tumor markers every 3 or 4 months, and enhanced computed tomography (CT) or magnetic resonance imaging (MRI) every 6 or 12 months, according to the protocol of the Japanese guidelines (4). The prognosis of these registered patients was followed until confirmation of death in every survey. Although this study protocol was not submitted to the institutional review board of each institution that participated in the nationwide survey, the data collection and registration of patients with HCC were conducted with the approval of each institution.

BALAD and BALAD-2 scores were assessed in terms of their association with liver dysfunction, based on Child-Pugh class, and the progression of HCC on imaging examinations; progression was assessed based on tumor size, tumor multiplicity, portal vein invasion, and tumor stage. In addition, we used univariate and multivariate analyses to investigate whether BALAD and BALAD-2 scores discriminated the patient

survival. Tumor staging was according to TNM criteria of LCSGJ (Supplementary table) (5).

Calculating BALAD and BALAD-2 Scores

BALAD and BALAD-2 scores were calculated based on AFP, AFP-L3, DCP, ALB, and T-Bil levels measured in the serum sample obtained from each patient at the time of HCC diagnosis. The original BALAD score was calculated by simply summing the serum levels of factors indicating both tumor progression (AFP, AFP-L3, and DCP) and liver function (ALB and T-Bil) (1). The cut-offs for the elevations of AFP, AFP-L3, and DCP were 400 ng/dL, 15%, and 100 mAU/mL, respectively (1). Liver function was categorized based on serum ALB and T-Bil levels according to the method of Tateishi et al. (6). ALB level was categorized as above 3.5 g/dL, 2.8–3.5 g/dL, or below 2.8 g/dL, and scored as 0, 1, or 2, respectively. T-Bil level was categorized as below 1.0 mg/dL, 1.0–2.0 mg/dL, or above 2.0 mg/dL, and scored as 0, 1, or 2, respectively. Liver function was then categorized based on the sum of these 2 scores as A (0 or 1), B (2 or 3), or C (4). The BALAD score is based on the total number of elevated tumor markers

and liver function scores.

The BALAD-2 score is calculated using the equation:

Linear predictor (xb) = 0.02*(AFP – 2.57) + 0.012*(AFP-L3 – 14.19) + 0.19*(ln(DCP) – 1.93) + 0.17*((T-Bil [μmol/L]$^{1/2}$) – 4.50) – 0.09*(ALB [g/L] – 35.11) , where T-Bil 1 mg/dL = 17.1μmol/L, and AFP was capped at 50000 units. Both AFP and DCP are modeled as per 1000 units. Patients are stratified into four prognostic groups according to previously described cut-offs, resulting in four grades: score 1 (low risk, ≤ -1.74), score 2 (–0.91 to > –1.74), score 3 (0.24 to > –0.91), and score 4 (high risk, > 0.24) (2).

Because the actual values of AFP less than 15 ng/dL and DCP less than 40 mAU/mL were not documented but described simple as “normal” in the data of nationwide follow-up surveys by LCSGJ, we randomly assigned the number 1 to 14 ng/dL for AFP and 1 to 39 for DCP mAU/mL in cases with normal levels of these markers. We assigned 0% for AFP-L3 in cases with undetectable AFP-L3.

Statistical Analyses

Differences in percentages between groups were analyzed using the chi-square

test. Differences in means of quantitative values were analyzed using the Mann-Whitney U test. Changes in percentages and quantitative values of increases in BALAD and BALAD-2 scores were analyzed with the Cochran-Armitage test and Jonckheere-Terpstra test, respectively. The date of HCC diagnosis was defined as time zero for calculations of survival rates. Survival was defined as the time from diagnosis to death, or last follow-up if death had not occurred. Patients who died were not censored, while surviving patients were censored. The Kaplan-Meier method was used to calculate survival rates, and the log-rank test was used to analyze differences in survival.

The Cox proportional hazard regression model with backward elimination method was used for multivariate analysis. The factors analyzed were age, sex, Child-Pugh class, tumor size, tumor number, portal vein invasion, tumor stage, treatment, and BALAD and BALAD-2 scores. Statistical analysis was performed using JMP statistical software, version 11.0.0 (Macintosh version; SAS Institute, Cary, NC). All P values were derived from two-tailed tests, with $P<0.05$ accepted as statistically significant.

Results

Baseline Patient Characteristics

The median follow-up period after diagnosis was 19.2 months, and the 25th and 75th percentiles were 8.4 and 39.6 months, respectively. Table 1 shows the characteristics of study patients. Males comprised 70.1% of patients, and the mean age was 66.9 years. In the majority of patients, hepatitis C virus (HCV) antibody was positive and was as the cause of chronic liver disease. More than 70% of patients had Child-Pugh (7) class A liver function and HCC was stage I or II in more than 60% of the patients. Serum AFP and DCP levels were below the normal cut-offs (15 ng/mL and 40 mAU/mL) in 33.7% and 42.9% of patients, respectively. Serum AFP-L3 was undetectable in 40.3% of patients.

On calculation of BALAD and BALAD-2 scores, patients were rated as having a BALAD score of 0, 1, 2, 3, 4, and 5 in 9658 (40.2%), 6756 (28.1%), 4135 (17.2%), 2751 (11.4%), 499 (2.1%), and 230 (1.0%) of cases, respectively. BALAD-2 score was 1, 2, 3, and 4 in 7827 (32.6%%), 6772 (28.2%%), 5510 (22.9%%), and 3920 (16.3%) of

patients, respectively.

Association of BALAD and BALAD-2 scores with Progression of HCC and Liver Function

Table 2 shows patient backgrounds as well as data on liver dysfunction and tumor progression based on BALAD and BALAD-2 scores. Increases in these scores were significantly associated with increased tumor size as well as higher percentages of patients with worse liver function (Child-Pugh A to C), multiple tumors, portal vein invasion, and increased TNM stage. The associations of BALAD-2 scores with liver dysfunction and tumor progression were more marked and consistent than those of BALAD scores.

Prognostic Significance of BALAD and BALAD-2 scores

Patients' median survival times and overall 3- and 5-year survival rates are shown in Table 3. Increases in both BALAD and BALAD-2 scores were associated with shortened median survival times and decreased 3- and 5-year survival rates. There were

no overlaps in the 95% confidence intervals of median survival times between BALAD-2 scores, whereas there were some overlaps with BALAD. Multivariate analysis showed that BALAD and BALAD-2 scores were associated with patient survival independent of Child-Pugh class, tumor stage, and treatment (Table 4).

Discrimination of Patient Survivals by BALAD and BALAD-2 scores

Figure 2 shows the post-diagnosis survival curves of patients based on BALAD and BALAD-2 scores. Both scores show good discriminatory ability for patient survival rates. In particular, there was no overlap in the 95% confidence intervals of survival curves when categorized by BALAD-2 scores. Figure 3 shows the post-diagnosis survival curves by BALAD-2 scores according to the etiology of background liver disease. BALAD-2 scores maintained a good discriminatory ability in all three patient subgroups without overlap of survival curves between scores. When patients were grouped based on the treatment of HCC (Figure 4), BALAD-2 scores proved equally discriminatory in all treatment classes without overlap of survival curves. In contrast, BALAD scores showed several overlaps between scores when patients were grouped by

etiology or treatment (Supplementary figures 1 and 2).

Discussion

The staging systems of HCC for assessment of patient outcomes are based on features that influence prognosis, which are broadly classified into two categories, namely, progression of tumors and severity of underlying liver dysfunction. Several staging systems / prognostic scores that combine these factors have been developed (8-13). In terms of staging, the progression of HCC is primarily evaluated by morphology, i.e., the size and number of tumors and the presence of portal vein invasion (5,14,15). Such evaluations are based mainly on imaging studies and postoperative pathologic examinations in patients who have undergone hepatic resection or transplantation. However, estimating tumor progression using imaging studies has several shortcomings. The detectability of liver tumors, which influences the determination of tumor multiplicity, depends on the resolution of the imaging modality (ultrasonography, CT, or MRI) and their quality, as well as the skill of the sonographer in case of ultrasonography. Recent advancements in imaging technology, such as

multidetector-row CT (16,17), and MRI (18), have improved the detection of hepatic nodules, resulting in upstaging of HCC progression. In addition, discrepancies between imaging findings and pathologic results are often found in patients who undergo hepatic resection. With imaging studies, it is often difficult to detect microvascular invasion of HCC or minute satellite nodules, both of which are found in pathologic analysis after resection and result in upstaging of HCC progression.

Liver dysfunction in patients with HCC is usually estimated based on the Child-Pugh classification (7). This classification takes into account the presence and controllability of ascites and hepatic encephalopathy, in addition to prothrombin time and levels of serum ALB and T-Bil. However, the presence and controllability of ascites and hepatic encephalopathy are largely subjective. Therefore, HCC staging that is based on the morphological evaluation of tumor progression and on liver dysfunction as determined by Child-Pugh classification cannot be fully objective, and therefore cannot be standardized across regions.

The results of the present study, based on the data of a nationwide follow-up survey showed that staging systems based solely on serology were well associated with

the progression of HCC and liver dysfunction, and had excellent discriminatory ability for survival in Japanese patients with HCC. The scores of the BALAD and BALAD-2 models were associated with the survival of patients with HCC independent of Child-Pugh class and the morphological features of HCC (Table 4). Previous findings on the usefulness of the BALAD and BALAD-2 models for the prognostication of patients with HCC (1-3) were replicated in this large HCC cohort in Japan, where the survival of patients is long in comparison to Western and other Asian countries. Japanese patients are diagnosed at a much earlier stage, because individuals of the Japanese population who are at risk of HCC (those with chronic liver disease) are more rigorously screened than in Western and other Asian countries, and hence are much more likely to receive potentially curative therapy (19).

The three markers that are incorporated in the BALAD and BALAD-2 scores have the advantage of being commercially available, with regulatory approval in Japan, the United States, and Europe. All three markers are well documented to have prognostic significance when used individually (20-22) and in combination (23). In addition, previous studies revealed the prognostic significance of serum ALB and T-Bil

levels as liver function measures in patients with HCC (6,24). The combination of these serological indicators of tumor progression and liver function accurately reflected the state of patients with HCC at diagnosis and categorized the risk of death thereafter, at least in Japan where the main etiology of HCC is HCV, many cases are diagnosed in the early stage, and the majority of patients have Child-Pugh class A liver function. In addition, these serological models, especially BALAD-2 model, maintained discriminatory ability in patient subgroups with hepatitis B virus (HBV) or non-HBV/HCV, or in those with intermediate or advanced stages, in addition to HCV-related HCC or early-stage HCC.

When comparing the original BALAD and BALAD-2 models, the latter had a marginally better discrimination. The overlap between risk groups was less evident for BALAD-2 (Table 4 and Figure 2). The superior discrimination of BALAD-2 model was enhanced when patients were grouped by disease stages. Also, regarding the association between BALAD and BALAD-2 scores and tumor progression and liver function, increases in BALAD-2 scores showed more consistent association with the progression of HCC and the deterioration of liver function (Table 3).

However, the serology-based staging models have several limitations. First, staging on the basis of serum markers is not applicable to diagnosis, although another serology-based model for the diagnosis of HCC has been reported (3,25). Although the selected treatments had close associations with the scores, especially of BALAD-2, these staging models cannot be used for treatment planning. In addition, these models are not applicable in patients who are taking drugs such as warfarin or vitamin K that can influence the levels of tumor markers, and it should be noted that the use of such drugs could not be verified in this study cohort.

There are several limitations of this study. The study patients were a part of all patients in nationwide survey (36.1%) in whom five laboratory variables necessary for calculating the BALAD and BALAD-2 scores were available, although the distributions of HCC stage and Child-Pugh class, as well as tumor size, number, and portal vein invasion, were same between 24029 study patients and 42525 patients excluded from the study due to the lack of laboratory variables (data not sown). In addition, actual levels of AFP and DCP were not available in patients with AFP below 15 ng/dL and patients with DCP below 40 mAU/mL and values within these reference ranges were

randomly assigned for the calculation of BALAD-2 scores in these cases. Finally, the prognoses of patients who underwent transplantation based on these scores are not known, because few patients with HCC are treated with transplantation in Japan.

In conclusion, we evaluated the prognostic significance of the serology-based BALAD and BALAD-2 scoring models in Japanese patients with HCC in a cohort of a nationwide follow-up survey, and confirmed that these models are applicable for these patients.

References

1. Toyoda H, Kumada T, Osaki Y, Oka H, Urano F, Kudo M, et al. Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. Clin Gastroenterol Hepatol 2006; 4: 1528-1536.
2. Fox R, Berhane S, Teng M, Cox T, Tada T, Toyoda H, et al. Biomarker-based prognosis in hepatocellular carcinoma: validation and extension of the BALAD model. Br J Cancer 2014; 110: 2090-2098.
3. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, et al. Role of Galad and Balad-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol (in press).
4. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence–based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008; 38: 37-51.
5. The Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 5th ed. (English Ed.) Kaneraha & Co. Ltd: Tokyo, 2009.

6. Tateishi R, Yoshida H, Shiina S, Imamura H, Hasegawa K, Teratani T, et al. Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. Gut 2005; 54: 419-425.
7. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-649.
8. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985; 56: 918-928.
9. The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. Hepatology 1998; 28: 751-755.
10. Chevret S, Trinchet J-C, Mathieu D, Rachad AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. J Hepatol 1999; 31: 133-141.
11. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999; 19: 329-338.

12. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. Cancer 2002; 94: 1760-1769.

13. Kudo M, Chung H, Haji S, Osaki Y, Oka H, Seki T, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. Hepatology 2004; 40: 1396-1405.

14. International Union Against Cancer (UICC): Liver. In: Sobin LH, Wittekind CH, eds. TNM Classification of Malignant Tumours. 6th ed. New York, NY: Wiley, 2002: 81-83.

15. Vauthey JN, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, et al. Simplified staging for hepatocellular carcinoma. J Clin Oncol 2002; 127: 603-608.

16. Kawata S, Murakami T, Kim T, Hori M, Federle MP, Kumano S, et al. Multidetector CT: diagnostic impact of slice thickness on detection of hypervascular hepatocellular carcinoma. Am J Roentgenol 2002; 179: 61-66.

17. Ichikawa T, Erturk SM, Araki T. Multiphasic contrast-enhanced multidetector-row CT of liver: contrast-enhancement theory and practical scan protocol with a combination of fixed injection duration and patients' body-weight-tailored dose of contrast material. Eur J Radiol 2006; 58: 165-176.

18. Kim HD, Lim YS, Han S, An J, Kim GA, Kim SY, et al. Evaluation of early-stage hepatocellular carcinoma by magnetic resonance imaging with gadoxetic acid detects additional lesions and increases overall survival. Gastroenterology 2015; 148: 1371-1382.

19. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Impact of surveillance on survival of patients with initial hepatocellular carcinoma: a study from Japan. Clin Gastroenterol Hepatol 2006; 4: 1170-1176.

20. Toyoda H, Kumada T, Osaki Y, Oka H, Kudo M. Role of tumor markers in assessment of tumor progression and prediction of outcomes in patients with hepatocellular carcinoma. Hepatol Res 2007; 37: S166-S171.

21. Nagaoka S, Yatsuhashi H, Hamada H, Yano K, Matsumoto T, Daikoku M, et al. The des-γ-carboxy prothrombin index is a new prognostic indicator for hepatocellular

carcinoma. Cancer 2003; 98: 2671-2677.

22. Nouso K, Kobayashi Y, Nakamura S, Kobayashi S, Takayama H, Toshimori J, et al. Prognostic importance of fucosylated alpha-fetoprotein in hepatocellular carcinoma patients with low alpha-fetoprotein. J Gastroenterol Hepatol 2011; 26: 1195-1200.

23. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol 2006; 4: 111-117.

24. Johnson PJ, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. J Clin Oncol 2015; 33: 550-558.

25. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. Cancer Epidemiol Biomarkers Prev 2014; 23: 144-153.

Figure Legends

Figure 1. Scelection flowchart of the study patients in a cohort of a Japanese nationwide survey of patients with HCC.

Figure 2. Survival rates of patients with hepatocellular carcinoma (HCC) after diagnosis based on (A) BALAD and (B) BALAD-2 scores evaluated by serum levels of the following measured at the diagnosis of HCC: ALB, T-Bil, AFP, AFP-L3, and DCP. Dotted lines, 95% confidence intervals (CIs). BALAD-2 had an excellent discriminatory ability for patient survival with little overlap of 95% CIs between groups.

Figure 3. Survival rates of patients with hepatocellular carcinoma (HCC) after diagnosis by BALAD-2 scores evaluated by serum levels of the following measured at the diagnosis of HCC: ALB, T-Bil, AFP, AFP-L3, and DCP. A) Patients with hepatitis B virus (HBV) infection; B) Patients with hepatitis C virus (HCV) infection; C) Patients without hepatitis virus infection (non-HBV/HCV).

Figure 4. Survival rates of patients with hepatocellular carcinoma (HCC) who undergone A) curative, B) intermediate, and C) palliative or no treatment after diagnosis by BALAD-2 scores evaluated by serum levels of the following measured at the diagnosis of HCC: ALB, T-Bil, AFP, AFP-L3, and DCP.

Supplementary figure 1. Survival rates of patients with hepatocellular carcinoma (HCC) after diagnosis by original BALAD scores evaluated by serum levels of the following measured at the diagnosis of HCC: ALB, T-Bil, AFP, AFP-L3, and DCP. A) Patients with hepatitis B virus (HBV) infection; B) Patients with hepatitis C virus (HCV) infection; C) Patients without hepatitis virus infection (non-HBV/HCV).

Supplementary figure 2. Survival rates of patients with hepatocellular carcinoma (HCC) who undergone A) curative, B) intermediate, and C) palliative or no treatment after diagnosis by original BALAD scores evaluated by serum levels of the following measured at the diagnosis of HCC: ALB, T-Bil, AFP, AFP-L3, and DCP.

Table 1. Patient characteristics (n = 24029)

Age (mean ± SD, years) (median, IQR)	66.9 ± 9.6 (68, 61-74)
Sex ratio (male / female)	16850 (70.1) / 7179 (29.9)
HBsAg (positive / negative)	3724 (16.0) / 19618 (84.0)
HCV-Ab (positive / negative)	16352 (69.5) / 7186 (30.5)
Child-Pugh class (A / B / C)**	17533 (74.3) / 5230 (22.1) / 846 (3.6)
Albumin (mean ± SD, g/dL)	3.63 ± 0.56
Total bilirubin (mean ± SD, mg/dL)	1.13 ± 1.44
Prothrombin (mean ± SD, %)	81.2 ± 16.6
Platelet count (mean ± SD, x1000/mL)	127 ± 71
Tumor size (mean ± SD, cm) (median, IQR)	3.92 ± 3.70 (2.8, 2.0–4.5)
≤2 cm / >2 cm	7966 (34.2) / 15318(65.8)
Number of tumors (single/multiple)	13381 (57.0) / 10107 (43.0)
Portal vein invasion (absent/present)*	19876 (88.1) / 2680 (11.9)
AFP (median, IQR)*, <15 ng/mL (%)	175.0 (46.0–974.5), 8086 (33.7)
<400 ng/mL / ≥ 400 ng/mL	21560 (89.7) / 2469 (10.3)
AFP-L3 (median, IQR)*, undetected (%)	21.0 (5.6–49.7), 9682 (40.3)
<15% / ≥ 15%	20152 (83.7) / 3877 (16.3)
DCP (median, IQR)*, <40 mAU/mL	283.0 (92.0–1240.0), 10297 (42.9)
<100 mAU/mL / ≥ 100 mAU/mL	19037 (79.2) / 4992 (20.8)
TNM stage (I / II / III / IV)	4791 (22.3) / 8943 (41.6) / 5684 (26.4) / 2081 (9.7)
Treatment (resection / LAT / TACE / others / none)	6859 (28.6) / 8600 (35.8) / 6221 (25.9) / 1378 (5.8) / 934 (3.9)

Percentages are given in parentheses.

HBsAg, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin-reactive AFP; DCP, des-gamma-carboxy prothrombin; LAT, locoregional ablative therapies; TACE, transarterial chemoembolization.

Data were missing in 687 cases for HBsAg, 491 for HCV-Ab, 420 for Child-Pugh class,

706 for prothrombin time, 256 for platelet counts, 745 for tumor size, 541 for tumor number, 1473 for portal vein invasion, 2530 for TNM stage, and 37 for treatment.
*Based on imaging studies.

Table 2. Association of BALAD and BALAD-2 scores with hepatitis viral infection, liver function, tumor progression, and treatment in patients with hepatocellular carcinoma (n = 24029)

BALAD score	0 (n = 9568)	1 (n = 6756)	2 (n = 4135)	3 (n = 2751)	4 (n = 499)
Age	67.4 ± 8.8	67.5 ± 9.6	66.3 ± 10.2	65.4 ± 10.6	63.9 ± 9.6
Sex-male	6561 (67.9)	4879 (72.2)	2875 (69.5)	1995 (72.5)	363 (72.8)
HBsAg positive	1169 (12.5)	1002 (15.2)	781 (19.5)	605 (22.6)	106 (22.0)
HCV-Ab positive	7283 (76.9)	4540 (68.6)	2594 (64.2)	1544 (57.2)	278 (57.1)
Child-Pugh class-A	7992 (84.3)	5195 (78.4)	2665 (65.6)	1659 (61.2)	22 (4.5)
Child-Pugh class-B	1480 (15.6)	1377 (20.8)	1170 (28.8)	818 (30.2)	300 (60.7)
Child-Pugh class-C	214 (0.1)	57 (0.8)	229 (5.6)	233 (8.6)	172 (34.8)
Platelet count	117 ± 61	131 ± 69	132 ± 78	147 ± 89	125 ± 86
Tumor size	2.65 ± 2.62	4.05 ± 3.04	4.81 ± 4.31	6.42 ± 5.02	6.13 ± 5.05
Tumor number-solitary	6217 (65.1)	3813 (57.5)	2038 (50.7)	1130 (43.1)	128 (27.7)
Tumor number-multiple	3328 (34.9)	2822 (42.5)	1979 (49.3)	1492 (56.9)	334 (72.3)
Portal vein invasion –*	8957 (98.1)	5795 (91.3)	3143 (81.1)	1649 (64.8)	254 (56.0)
Portal vein invasion +*	171 (1.9)	552 (8.7)	732 (18.9)	897 (35.2)	200 (44.0)
TNM stage-1	3120 (35.5)	1048 (17.3)	459 (12.5)	135 (5.6)	23 (5.8)
TNM stage-2	3935 (44.8)	2765 (45.6)	1429 (38.9)	714 (29.7)	76 (19.0)
TNM stage-3	1603 (18.3)	1838 (30.3)	1223 (33.2)	841 (35.0)	137 (34.3)
TNM stage-4	119 (1.4)	413 (6.8)	567 (15.4)	713 (29.7)	163 (40.9)
Treatment-resection	2422 (25.1)	2273 (33.7)	1283 (31.1)	826 (30.1)	45 (9.0)
Treatment-LAT	5190 (53.9)	2054 (30.4)	923 (22.4)	356 (12.9)	63 (12.6)
Treatment-TACE	1792 (18.6)	1974 (29.3)	1342 (32.5)	901 (32.8)	167 (33.5)
Treatment-others	118 (1.2)	285 (4.2)	377 (9.1)	424 (15.4)	109 (21.8)
Treatment-none	117 (1.2)	162 (2.4)	203 (4.9)	241 (8.8)	115 (23.1)

BALAD-2 score	1 (n=7827)	2 (n=6772)	3 (n=5510)	4 (n=3920)	*P* value
Age	67.5 ± 9.3	67.6 ± 9.4	66.8 ± 9.7	64.6 ± 9.9	<0.0001

Sex-male	5560 (71.0)	4699 (69.4)	3828 (69.5)	2763 (70.5)	0.3006
HBsAg positive	1170 (15.4)	983 (14.9)	842 (15.7)	729 (19.2)	<0.0001
HCV-Ab positive	5453 (71.2)	4744 (71.2)	3749 (69.4)	2406 (62.9)	<0.0001
Child-Pugh class-A	7542 (96.7)	5691 (85.7)	3491 (64.6)	899 (23.3)	<0.0001
Child-Pugh class-B	257 (3.3)	943 (14.2)	1863 (34.5)	2167 (56.2)	<0.0001
Child-Pugh class-C	2 (0.0)	5 (0.1)	48 (0.9)	791 (20.5)	<0.0001
Platelet count	139 ± 63	126 ± 66	120 ± 76	117 ± 86	<0.0001
Tumor size	3.00 ± 2.88	3.70 ± 3.48	4.54 ± 4.13	5.53 ± 5.10	<0.0001
Tumor number-solitary	5232 (67.7)	3909 (58.7)	2737 (51.0)	1503 (40.3)	<0.0001
Tumor number-multiple	2499 (32.3)	2747 (41.3)	2632 (49.0)	2229 (59.7)	<0.0001
Portal vein invasion –	7209 (97.1)	5843 (92.0)	4357 (84.5)	2467 (68.0)	<0.0001
Portal vein invasion +	217 (2.9)	505 (8.0)	797 (15.5)	1161 (32.0)	<0.0001
TNM stage-1	2209 (30.9)	1324 (21.6)	817 (16.7)	441 (13.2)	<0.0001
TNM stage-2	3403 (47.6)	2727 (44.6)	1861 (38.0)	952 (28.5)	<0.0001
TNM stage-3	1383 (19.4)	1704 (27.9)	1559 (31.9)	1038 (31.1)	<0.0001
TNM stage-4	153 (2.1)	360 (5.9)	657 (13.4)	911 (27.2)	<0.0001
Treatment-resection	2776 (35.5)	2217 (32.8)	1348 (24.5)	518 (13.2)	<0.0001
Treatment-LAT	3530 (45.2)	2509 (37.1)	1701 (30.9)	860 (22.0)	<0.0001
Treatment-TACE	1302 (16.7)	1716 (25.4)	1831 (33.3)	1372 (35.0)	<0.0001
Treatment-others	117 (1.5)	211 (3.1)	428 (7.8)	622 (15.9)	<0.0001
Treatment-none	88 (1.1)	107 (1.6)	194 (3.5)	934 (13.9)	<0.0001

Percentages are given in parentheses.

HBsAg, hepatitis B virus surface antigen; HCV-Ab, hepatitis C virus antibody; LAT, locoregional ablative therapies including radiofrequency ablation and percutaneous ethanol injection; TACE, transarterial chemoembolization.

Data were missing in 687 cases for HBsAg, 491 for HCV-Ab, 420 for Child-Pugh class, 706 for prothrombin time, 256 for platelet counts, 745 for tumor size, 541 for tumor number, 1473 for portal vein invasion, 2530 for TNM stage, and 37 for treatment.

*Based on imaging studies.

Table 3. Median survival times and 3- and 5-years survival rates for BALAD and BALAD-2 scores in patients with hepatocellular carcinoma (n = 24029)

		N	Median survival (years)	95% CI	3-year survival (%)	95% CI	5-year survival (%)	95% CI
BALAD score	0	9658	7.7	7.3–9.4	85.2	84.3–86.1	68.0	66.3–69.5
	1	6576	5.4	5.1–5.8	70.0	68.5–71.4	52.4	50.4–54.4
	2	4135	3.7	3.3–4.0	55.2	53.1–57.1	41.7	39.3–44.1
	3	2751	2.0	1.8–2.2	41.0	38.6–43.4	29.4	26.6–32.1
	4	499	0.8	0.8–0.9	24.4	19.4–29.7	15.9	11.0–21.5
	5	230	0.3	0.2–0.4	10.8	6.2–16.9	7.0	3.1–13.0
BALAD-2 score	1	7827	9.7	8.3–	86.9	85.9–87.8	72.5	70.8–74.1
	2	6772	5.8	5.5–6.2	74.8	73.4–76.2	55.8	53.8–57.8
	3	5510	3.8	3.6–4.0	57.1	55.4–58.9	40.4	38.2–42.6
	4	3920	1.8	1.7–1.9	37.7	35.7–39.5	25.4	23.2–27.7

CI, confidence interval.

Table 4. Multivariate analysis with backward elimination method for factors associated with survival after diagnosis in patients with hepatocellular carcinoma (n = 24029)

Factor		Multivariate analysis	
		P value	Relative risk (95% CI)
Age	per 1.0	<0.0001	1.011 (1.008–1.014)
Sex	female		
	male	<0.0001	1.128 (1.062–1.198)
Child-Pugh class	A		1
	B	<0.0001	1.631 (1.534–1.736)
	C	<0.0001	1.779 (1.565–2.022)
Tumor size	per 1.0	<0.0001	1.019 (1.014–1.024)
Portal vein invasion*	Absent		1
	Present	<0.0001	1.320 (1.193–1.460)
TNM stage	1		1
	2	<0.0001	1.279 (1.172–1.397)
	3	<0.0001	1.757 (1.601–1.929)
	4	<0.0001	2.890 (2.528–3.304)
Treatment	None		1
	Resection	<0.0001	0.278 (0.245–0.315)
	LAT	<0.0001	0.362 (0.319–0.410)
	TACE	<0.0001	0.520 (0.462–0.585)
	Others	<0.0001	0.730 (0.641–0.832)
BALAD score	0		1
	1	<0.0001	1.443 (1.339–1.556)
	2	<0.0001	1.892 (1.743–2.053)
	3	<0.0001	2.579 (2.358–2.821)
	4	0.0005	2.634 (2.247–3.087)
	5	0.0005	3.846 (3.104–4.766)

Factor		Multivariate analysis	
		P value	Relative risk (95% CI)

Age	per 1.0	<0.0001	1.011 (1.008–1.014)
Sex	female		
	male	<0.0001	1.129 (1.063–1.199)
Child-Pugh class	A		1
	B	<0.0001	1.293 (1.207–1.386)
	C	<0.0001	1.598 (1.406–1.816)
Tumor size	per 1.0	<0.0001	1.021 (1.016–1.026)
Number of tumors	single		
	multiple	0.0065	1.117 (1.032–1.211)
Portal vein invasion*	Absent		1
	Present	<0.0001	1.263 (1.137–1.404)
TNM stage	1		1
	2	<0.0001	1.384 (1.263–1.516)
	3	<0.0001	2.073 (1.839–2.336)
	4	<0.0001	3.607 (3.044–4.274)
Treatment	None		1
	Resection	<0.0001	0.301 (0.265–0.342)
	LAT	<0.0001	0.356 (0.314–0.404)
	TACE	<0.0001	0.525 (0.467–0.591)
	Others	<0.0001	0.770 (0.676–0.878)
BALAD-2 score	1		1
	2	<0.0001	1.505 (1.387–1.632)
	3	<0.0001	2.128 (1.957–2.314)
	4	<0.0001	2.816 (2.545–3.116)

CI, confidence interval; LAT, locoregional ablative therapies; TACE, transarterial chemoembolization.

*Based on imaging studies.