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CLINICAL INVESTIGATION INTERVENTIONAL ONCOLOGY

# Prognosticating Survival in Hepatocellular Carcinoma with Elevated Baseline Alpha-fetoprotein Treated with Radioembolization Using a Novel Laboratory Scoring System: Initial Development and Validation

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## Abstract

*Aims* To investigate laboratory parameters as predictors of overall survival (OS) for hepatocellular carcinoma (HCC) treated with radioembolization and develop/validate a scoring system.

*Methods* With IRB approval, we included all patients with baseline alpha-fetoprotein (AFP) > 100 ng/dL from our prospectively acquired HCC radioembolization database. Neutrophil–lymphocyte ratio, albumin–bilirubin (ALBI), and AFP were measured at baseline and at 1-, 3-, and 6-month post-radioembolization Landmarks. OS was assessed from these Landmarks. Univariate/multivariate analyses were performed to evaluate OS predictability of these parameters. Baseline Imaging, Laboratory, and Combination scoring systems were developed. Developing/validating groups were created to investigate/validate the score's OS predictability. Time-dependent receiver operating characteristics (ROC) were evaluated. Patients were stratified into groups I, II, and III by using 25th and 75th percentile cutoffs according to change in Laboratory Score from baseline.

*Results* 345/401 (86%), 238/401 (59%), and 167/401 (42%) patients had laboratory parameters available at the 1-, 3-, and 6-month Landmarks, respectively. ALBI and AFP were significant OS prognosticators at all Landmarks. The Laboratory Score [ALBI + (0.3 × LnAFP)] was developed/internally validated to predict OS from these Landmarks. Areas under the curve of time-dependent ROCs of the Baseline Imaging vs. Laboratory scores in predicting patient OS post 3 and 6 months Landmarks were 0.56 versus 0.82 and 0.57 versus 0.77, respectively. OS differences in groups I, II, and III according to change in Laboratory Score from baseline were significant ($p < 0.001$).

*Conclusions* Post-radioembolization AFP and ALBI scores were significant OS prognosticators. A decrease in post-therapeutic Laboratory Score, which combines AFP and ALBI, correlates with an improved OS.

**Keywords** Hepatocellular carcinoma (HCC) · Alpha-fetoprotein (AFP) · TARE · Radioembolization · HCC survival · Laboratory prognosticators for HCC survival

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## Introduction

Hepatocellular carcinoma (HCC) usually occurs in a background of cirrhosis which compromises and complicates survival prognostication [1]. HCC survival is multifactorial, and various imaging and laboratory survival predictors have been investigated [2–4]. In this analysis, we studied routine laboratory parameters which are representative of the various parameters that are thought to affect outcomes (inflammation, liver function, and tumor activity). These may predict survival in HCC following yttrium-90 transarterial radioembolization (TARE). These factors included neutrophil-to-lymphocyte ratio (N:L), albumin–bilirubin grade (ALBI grade) and alpha-fetoprotein (AFP).

N:L provides information on neutrophil (inflammation) and lymphocyte (regulatory/immune pathway) counts [5, 6]. N:L in cancer patients integrates the detrimental effects of neutrophila and lymphopenia (reflecting decreased immune response) and has emerged as a useful survival prognosticator [7–10]. N:L also has a prognostic role in patients with unresectable HCC [11].

Liver health is reflected by the synthetic and excretory hepatic functions, with the magnitude of injury/disease represented by the degree of hypoalbuminemia and hyperbilirubinemia. The ALBI scoring/grading system has been developed as an objective index of liver dysfunction, based solely on albumin and bilirubin level [12]. It has also been suggested to more accurately predict patients' mortality than Child–Pugh Class and has been validated as a prognostic index across all Barcelona Clinic for Liver Cancer (BCLC) stages in HCC [13].

Serum AFP level also plays an important role in representing the pathobiological features of HCC identified as prognostic factors [14–16]. Elevated AFP has been correlated with (a) poorly differentiated HCC; (b) large tumor size; (c) multifocal tumor; and (d) tumors with macrovascular invasion [16–20]. A decrease in post-therapeutic AFP prognosticates survival [21]. A combination of AFP, AFP-L3 (an AFP ligand), and protein induced by vitamin K absence-II (PIVKA-II) such as the GALAD score represents the optimum tumor marker assessment tool in HCC [22]. We do not perform all three tumor marker tests routinely at our institution.

Many scoring systems are being studied/developed into determine survival predictability. A recent study by Spearifico et al. suggests the role of a score based on extent of portal vein thrombosis, bilirubin level, and tumor burden in patients with HCC and PVT [23]. In this study, we evaluated the survival predictability of laboratory prognosticators (reproducible and routinely performed) including ALBI grade, AFP response, and N:L. A scoring system combining significant laboratory predictors of survival was developed and internally validated.

## Methods

This study was compliant with the Health Insurance Portability and Accountability Act (HIPAA) and approved by the Northwestern University Institutional Review Board. Diagnostic criteria for HCC were defined by established institutional guidelines (arterial phase hyperenhancement and venous/delayed phase washout) [24]. Patients were selected for TARE in our institutional multidisciplinary HCC conference.

### Patient Cohort

We selected patients with elevated baseline (pre-TARE) AFP $>$ 100 ng/dL [25]. This cutoff was chosen as Tateishi et al. have shown that when baseline AFP $\leq$ 100 ng/dL, no significant change in AFP levels is observed following therapy [25]. Additionally, there are trials being designed for patients with baseline AFP $>$ 100 ng/dL such as "A Study of Ramucirumab Versus Placebo in Participants with HCC and Elevated Baseline AFP (REACH-2)." We excluded patients with laboratory values unavailable at baseline or at the post-TARE (1, 3, or 6 months) Landmarks.

### Evaluation/Staging

All patients underwent pretreatment assessment consisting of history, laboratory, and imaging workup. Baseline staging was performed by CP (Child–Pugh; liver function), UNOS TNM (United Network for Organ Sharing; tumor, node, and metastases), and BCLC classification systems [24]. Tumor characteristics included distribution, maximum dimension of the index lesion, focality (solitary or multifocal), extrahepatic metastases, portal vein thrombosis, and imaging cirrhosis. Baseline laboratory values included bilirubin, albumin, neutrophil and lymphocyte counts, and AFP.

### Radioembolization

Radioembolization was performed with yttrium-90 glass microspheres using previously described methodology [26, 27].

### Follow-Up

The following three parameters were reviewed at 1-, 3- and 6-month Landmarks following TARE for the following laboratory variables.

*N:L*

Absolute values of neutrophils and lymphocytes counts were reviewed and N:L was calculated at baseline, 1-, 3-, and 6-month Landmarks. N:L $> 5$ and N:L $\leq 5$ were compared for survival analyses. Historically, different N:L cutoffs have been used and proposed ranging from 2 to 5 [28]. We chose 5 as a cutoff value to increase specificity of a high N:L.

*ALBI Grade*

ALBI grade at baseline, 1-, 3-, and 6-month Landmarks was calculated using the following formula [12]:

$$\text{ALBI} = \log_{10}(\text{bilirubin} \times 17.1) \times 0.66 - (\text{albumin} \times 10) \times 0.085$$

ALBI score was converted to ALBI grade as follows [12]:

| ALBI score | ALBI grade |
|---|---|
| $\leq -2.60$ | I |
| $> -2.60$ to $\leq -1.39$ | II |
| $> -1.39$ | III |

*AFP*

AFP at 1-, 3-, and 6-months Landmarks was compared to baseline. AFP response was determined at all Landmarks and defined as $\geq 50\%$ decrease from baseline [21].

### Statistical Methodology

All analyses were performed in SPSS 24.0 and R 3.3.2. R package "survival ROC" was applied. $p < 0.05$ was considered significant for the analyses. The details of the statistical methods used are presented in the eMethods. These are summarized below.

Landmark method is recommended in oncology guidelines for time-dependent analyses. For this analysis, 1-, 3-, and 6-month post-TARE Landmarks were used. The 401 patients were randomized to the following groups: (a) score development and (b) score validation. In order to determine independent risk factors for overall survival (OS), Cox regression was applied. For each Landmark, we included baseline characteristics and the laboratory parameters (at the specified Landmark) as independent variables. The outcome was OS at the post-TARE Landmark. The following scores were developed: (a) Laboratory Score, (b) Baseline Imaging Score, and (c) Combination Score. The validation of the Laboratory, Baseline Imaging, and Combination scores was performed in the validating group. Time-dependent receiver operating characteristics (ROCs) of these three scoring systems at 1, 3, and 6 months were plotted. Areas under the curve (AUCs) of time-dependent ROCs were calculated.

Change in the Laboratory Score was also used to determine treatment response.

$$\text{Change in lab score} = \text{Lab score (baseline)} - \text{Lab score (Landmark)}$$

The change in Laboratory Score was calculated as above. A positive change in the score represents a decrease in the score at the specific post-treatment Landmark and vice versa. All cutoffs were chosen at the 25th and 75th percentile as represented in eTable 3, and patients were grouped into three groups based on these cutoffs.

## Results

### Baseline Characteristics

Four hundred one patients were included for these analyses (eFigure 1). One hundred forty (35%) tumors were $< 5$ cm (range of sizes 1.2–22 cm). One hundred ninety-six (36%) tumors were solitary. At baseline, 215 (54%) had PVT and 57 (14%) had metastases (eTable 1). 40/401 (10%) patients underwent liver transplantation.

### Baseline Laboratory Findings

The median values of baseline bilirubin and albumin were 1.1 mg/dL (range 0.2–13.1) and 3.0 mg/dL (range 1.0–4.3), respectively. At baseline, 18 patients had ALBI grade I, 254 had grade II, and 129 had grade III. The median value for neutrophil and lymphocyte counts was 3.2 * $10^3$/mcL (range 0.6 * $10^3$/mcL–20.7 * $10^3$/mcL) and 1.0 * $10^3$/mcL (range 0.1 * $10^3$/mcL–4.5 * $10^3$/mcL), respectively. The median value for AFP was 1529 ng/dL (range 100.1–465,859).

### One-Month Landmark

Three hundred forty-five patients were analyzed. The median OS from the 1-month Landmark was 10.5 months (95% CI 8.7–13.0) ($p < 0.0001$) (eTable 2).

*N:L*

Median OS (95% CI) for N:L $\leq 5$ was 14.1 months (10.5–24.4) and for N:L $> 5$ was 8.3 months (7.0–11.0) ($p = 0.0002$).

*ALBI*

Median OS for ALBI I was not reached (NR). Median OS (95% CI) for ALBI grade II was 12.8 months (10.0–15.6) and for grade III was 5.03 months (3.8–6.8) ($p < 0.0001$).

*AFP*

Median OS (95% CI) for AFP responders and non-responders was 14.1 (13.0–18.2) and 7.03 months (5.7–7.9), respectively ($p < 0.0001$).

N:L, ALBI, and AFP were significant on multivariate analysis at the 1-month Landmark (Table 1).

### Three-Months Landmark

Two hundred and thirty-eight patients were analyzed. The median OS from the 3-month Landmark was 11.0 months (95% CI 8.9–12.1) ($p < 0.0001$) (eTable 2).

*N:L*

Median OS (95% CI) for N:L $\leq 5$ was 14.7 months (11.8–26) and for N:L $> 5$ was 5.9 months (95% CI 4.7–8.9) ($p < 0.0001$).

*ALBI*

Median OS (95% CI) for ALBI grades I, II, and III was 44.3 months, 11.3 months (9.9–14.7), and 5.5 months (4.3–8.5), respectively ($p < 0.0001$).

*AFP*

Median OS (95% CI) for AFP responders and non-responders was 14.7 (11.8–22.4) and 5.2 months (3.9–6.0), respectively ($p < 0.0001$).

ALBI and AFP were significant on multivariate analysis at the 3-month Landmark (Table 1).

### Six-Month Landmark

One hundred sixty-seven patients were analyzed. The median OS from the 6-month Landmark was 11.7 months (95% CI 9.0–18) ($p < 0.0001$) (eTable 2).

*N:L*

Median OS (95% CI) for N:L $\leq 5$ was 19.4 months (11.7–32) and for N:L $> 5$ was 8.3 months (5.03–10.6) ($p = 0.0008$).

*ALBI*

Median OS (95% CI) for ALBI grades I, II, and III was 31.9 months (8.5–95), 15.7 months (11.3–29.9) and 5.8 months (3.0–10.1) ($p = 0.0005$).

*AFP*

Median OS (95% CI) for AFP responders and non-responders was 25.4 months (13.9–41.3) and 6.6 months (3.0–8.3), respectively ($p < 0.0001$).

ALBI and AFP were significant on multivariate analysis at the 6-month Landmark (Table 1).

### Scoring System

*Laboratory Score*

In the predicting group, the final multivariable Cox regression models with laboratory parameters as independent variables at baseline and 1, 3, and 6 months Landmarks are listed in Table 1. From the multivariable Cox regressions in the developing group, we detected that ALBI score and AFP measured at each Landmark were independent risk factors of mortality at all Landmarks. The linear components of these regression models are listed below:

| Baseline | Baseline ALBI score + 0.290 * Ln(Baseline AFP) |
|---|---|
| 1 month | ALBI score 1 m + 0.573 * Ln(AFP 1 m) |
| 3 months | ALBI score 3 m + 0.209 * Ln(AFP 3 m) |
| 6 months | ALBI score 6 m + 0.337 * Ln(AFP 6 m) |

The coefficient of LnAFP and ALBI scores remained robust at all Landmarks, and we estimated the Laboratory Score to be:

**Table 1** Multivariable cox regression at each landmark

| Time | Laboratory parameters | | | Baseline imaging parameters | | | Baseline imaging and laboratory parameters | | |
|---|---|---|---|---|---|---|---|---|---|
| | Variable | Exp (B) (95% CI) | *p* value | Variable | Exp (B) (95% CI) | *p* value | Variable | Exp (B) (95% CI) | *p* value |
| Baseline | Female sex | 0.62 (0.43–0.92) | 0.016 | Ascites | 1.88 (1.3–2.8) | 0.001 | Ascites | 1.76 (1.20–2.6) | 0.004 |
| | Baseline ALBI score | 1.93 (1.46–2.56) | < 0.001 | Multiple lesions | 2.49 (1.6–3.7) | < 0.001 | Multiple lesions | 2.11 (1.41–3.15) | < 0.001 |
| | Ln (baseline AFP) | 1.21 (1.11–1.32) | < 0.001 | Extrahepatic metastasis | 1.91 (1.12–3.24) | 0.017 | Extrahepatic metastasis | 1.95 (1.14–3.32) | 0.015 |
| | Ln (baseline N:L) | 1.34 (1.05–1.71) | 0.017 | PVT | 2.62 (1.82–3.78) | <0.001 | PVT | 2.25 (1.54–3.28) | < 0.001 |
| | | | | | | | Laboratory score (baseline) | 1.54 (1.25–1.89) | < 0.001 |
| One month | Ln (baseline AFP) | 0.81 (0.66–0.99) | 0.043 | Ascites | 2.04 (1.34–3.11) | 0.001 | Ascites | 1.95 (1.28–2.99) | 0.002 |
| | Ln (AFP 1 m) | 1.47 (1.22–1.78) | < 0.001 | Multiple lesions | 2.29 (1.52–3.44) | < 0.001 | Multiple lesions | 1.75 (1.16–2.65) | 0.008 |
| | Ln (N:L 1 m) | 1.46 (1.08–1.97) | 0.014 | PVT | 2.71 (1.84–3.99) | < 0.001 | PVT | 2.25 (1.51–3.36) | < 0.001 |
| | ALBI score 1 m | 1.97 (1.49–2.6) | < 0.001 | | | | Laboratory score (1 month) | 1.71 (1.40–2.09) | < 0.001 |
| Three months | Baseline ALBI score | 0.39 (0.22–0.73) | 0.003 | Ascites | 1.83 (1.06–3.17) | 0.03 | PVT | 2.06 (1.31–3.26) | 0.002 |
| | ALBI score 3 m | 3.64 (2.03–6.55) | < 0.001 | Multiple lesions | 2.10 (1.30–3.41) | 0.003 | Laboratory score (3 months) | 1.99 (1.60–2.50) | < 0.001 |
| | Ln (AFP 3 m) | 1.31 (1.18–1.45) | < 0.001 | PVT | 1.88 (1.19–2.98) | 0.007 | | | |
| Six months | ALBI score 6 m | 2.30 (1.41–3.77) | 0.001 | Ascites | 3.25 (1.68–6.29) | <0.001 | Ascites | 2.24 (1.11–4.52) | 0.024 |
| | Ln (AFP 6 m) | 1.32 (1.18–1.49) | < 0.001 | Multiple lesions | 2.04 (1.12–3.7) | 0.02 | Laboratory score (6 months) | 2. 4 (1.74–3.15) | < 0.001 |

Laboratory Parameters: *For overall Cox regression, patient sex, age, baseline ALBI score, AFP [(transformed to Ln(Baseline AFP) and N:L (transformed to Ln(Baseline N:L)] were included in the model. **For 1-month Cox regression, patient sex, age, baseline ALBI score, AFP [transformed to Ln(Baseline AFP)] and N:L [transformed to Ln(Baseline N:L)], and 1-month measured ALBI score (ALBI 1 m), AFP [transformed to Ln(AFP 1 m)] and N:L [transformed to Ln(N:L 1 m)] were included in the model. ***For 3-month COX regression, patient sex, age, baseline ALBI score, AFP [transformed to Ln(Baseline AFP)] and N:L [transformed to Ln(Baseline N:L)], and 3-month measured ALBI score (ALBI 3 m), AFP [transformed to Ln(AFP 3 m)] and N:L [transformed to Ln(N:L 3 m)] were included in the model. ****For 6-month COX regression, patient sex, age, baseline ALBI score, AFP [transformed to Ln(Baseline AFP)] and N:L [transformed to Ln(Baseline N:L)], and 6-month measured ALBI score (ALBI 6 m), AFP [transformed to Ln(AFP 6 m)] and N:L [transformed to Ln(N:L 6 m)] were included in the model

Baseline Imaging Parameters: presence of ascites, presence of PVT, multiple lesions (compared to single lesion), presence of extrahepatic metastasis, and maximum diameter of the lesion [transformed to Ln(maximum diameter)] were included in the regression

Baseline Imaging Parameters/Laboratory Score: presence of ascites, presence of PVT, multiple lesions (compared to single lesion), presence of extrahepatic metastasis, and maximum diameter of the lesion [transformed to Ln(maximum diameter)], as well as the most recent lab score were included in the regression

ALBI score + 0.3 × LnAFP

*Baseline Imaging Score*

The final multivariable Cox regression models with baseline imaging parameters as independent variables at baseline and 1-, 3-, and 6-month Landmarks are listed in Table 1. The linear components of these regression models were used for the Baseline Imaging Score. The values in this score system are assigned as follows: if extrahepatic metastasis, PVT, multiple lesions and/or ascites are present, they are assigned 1, otherwise 0.

*Combination Score*

For the Combination Score, the final multivariable Cox regression model with baseline imaging parameters as independent variables at baseline and 1, 3, and 6 months Landmarks are listed in Table 1. The linear components of these regression models were used for the Combination Score. The values in this scoring system are assigned in the same way as in the Baseline Imaging Score.

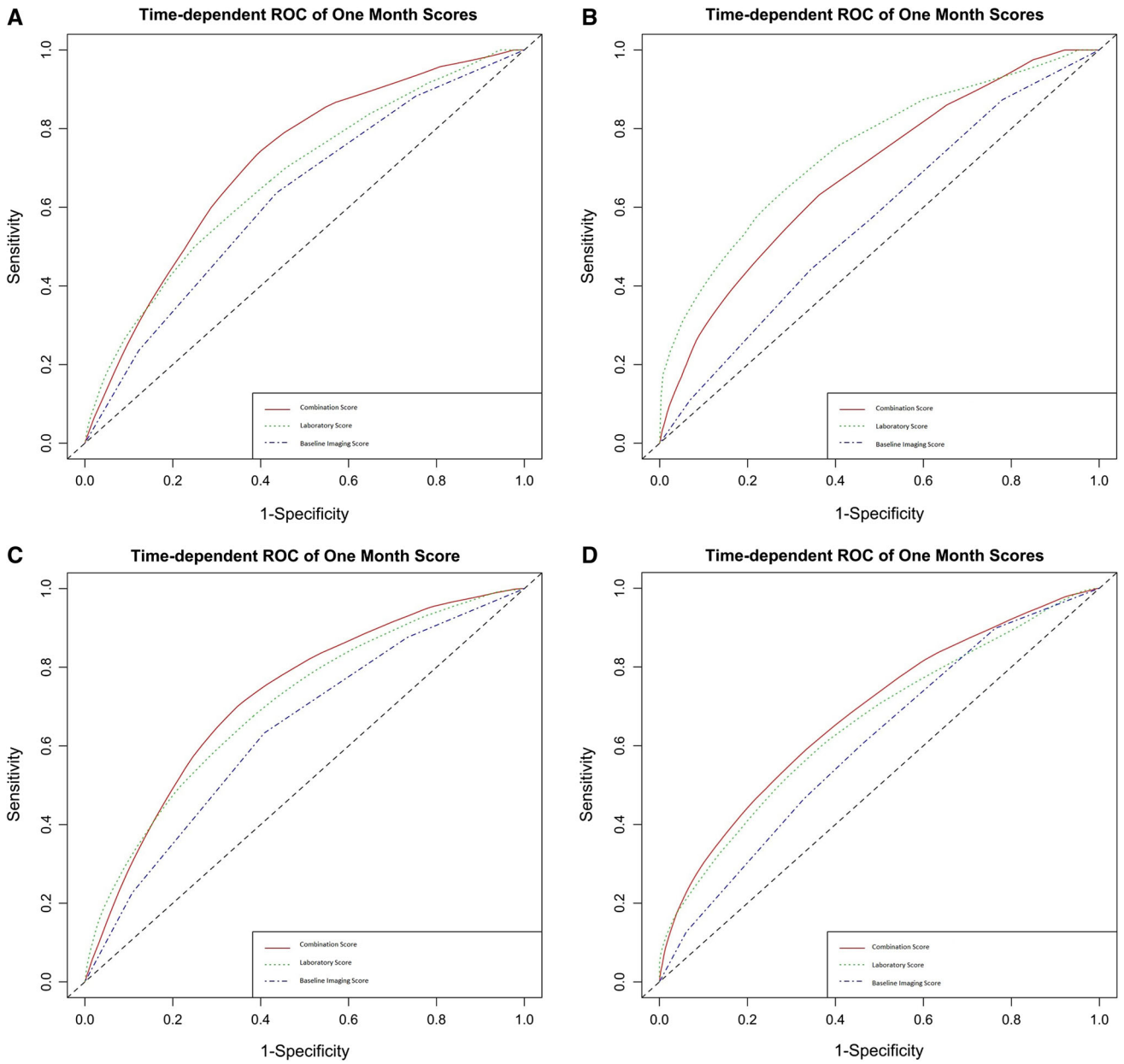


**Fig. 1** **A** One-month scores predict 1-month survival in developing group. **B** One-month scores predict 1-month survival in validating group. **C** One-month score predict 3-months survival in developing group. **D** One-month scores predict 3-months survival in validating group

### Time-Dependent ROC Curves of the Scoring Systems

Areas under the curve (AUC) of time-dependent ROCs of the Baseline Imaging Score vs. Laboratory Score in predicting patient OS 1 month post 1, 3, and 6 months Landmarks were 0.62 versus 0.68, 0.56 versus 0.82, and 0.57 versus 0.77, respectively (Figs. 1A–D, 2A–D, 3A–D). AUCs in predicting patient OS 3 months post 1, 3, and 6 months Landmarks were 0.64 versus 0.70, 0.58 versus 0.72, and 0.55 versus 0.79, respectively (Table 2).

*Change in Laboratory Score*

Table 3 presents OS of the three groups from each Landmark in patients stratified as groups I, II, and III. The differences in OS between the three groups were significant ($p < 0.001$) in the developing and validating groups.

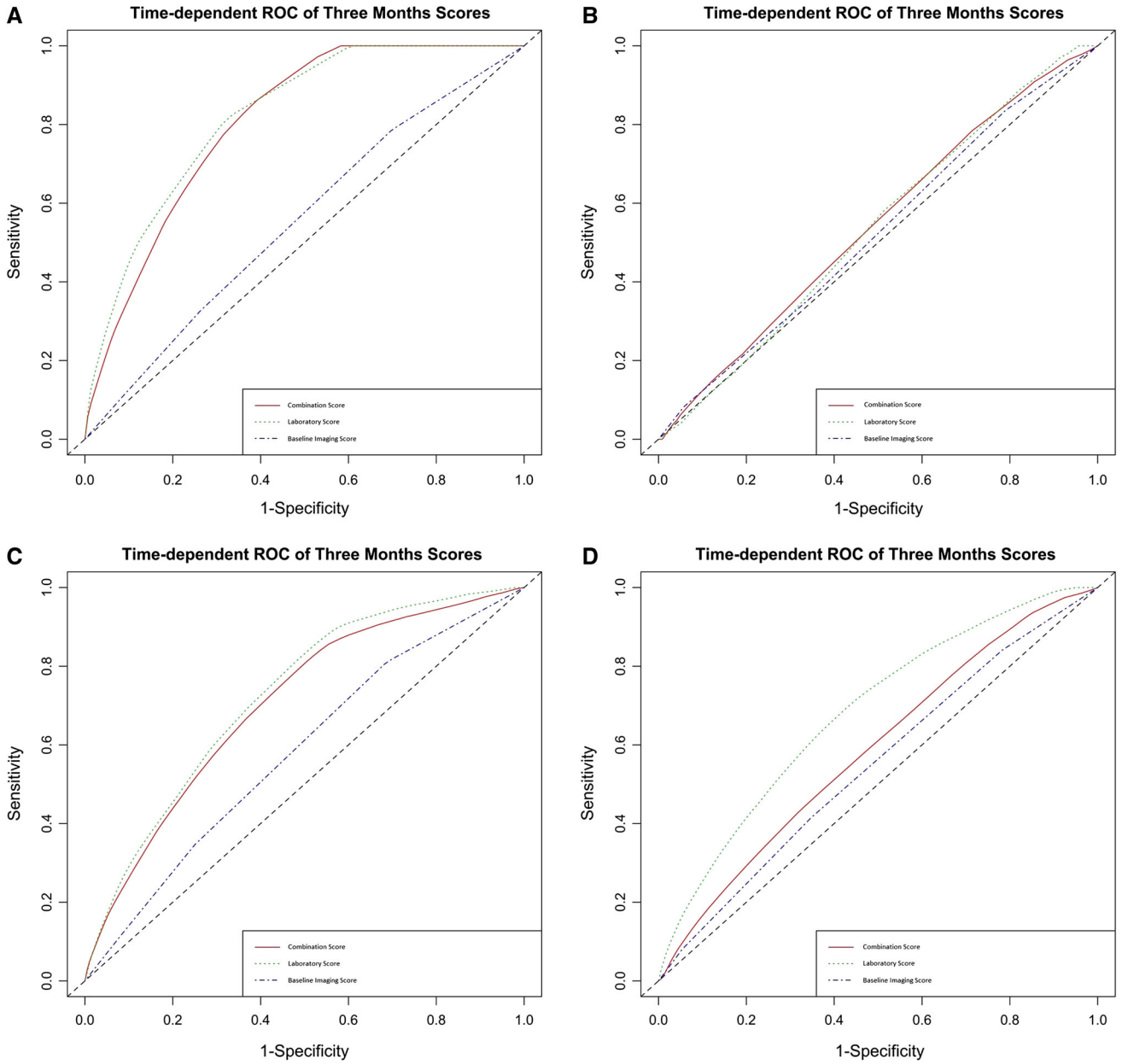


**Fig. 2** **A** Three-month scores predict 1-month survival in developing group. **B** Three-month scores predict 1-month survival in validating group. **C** Three-month scores predict 3-months survival in developing group. **D** Three-month scores predict 3-months survival in validating group

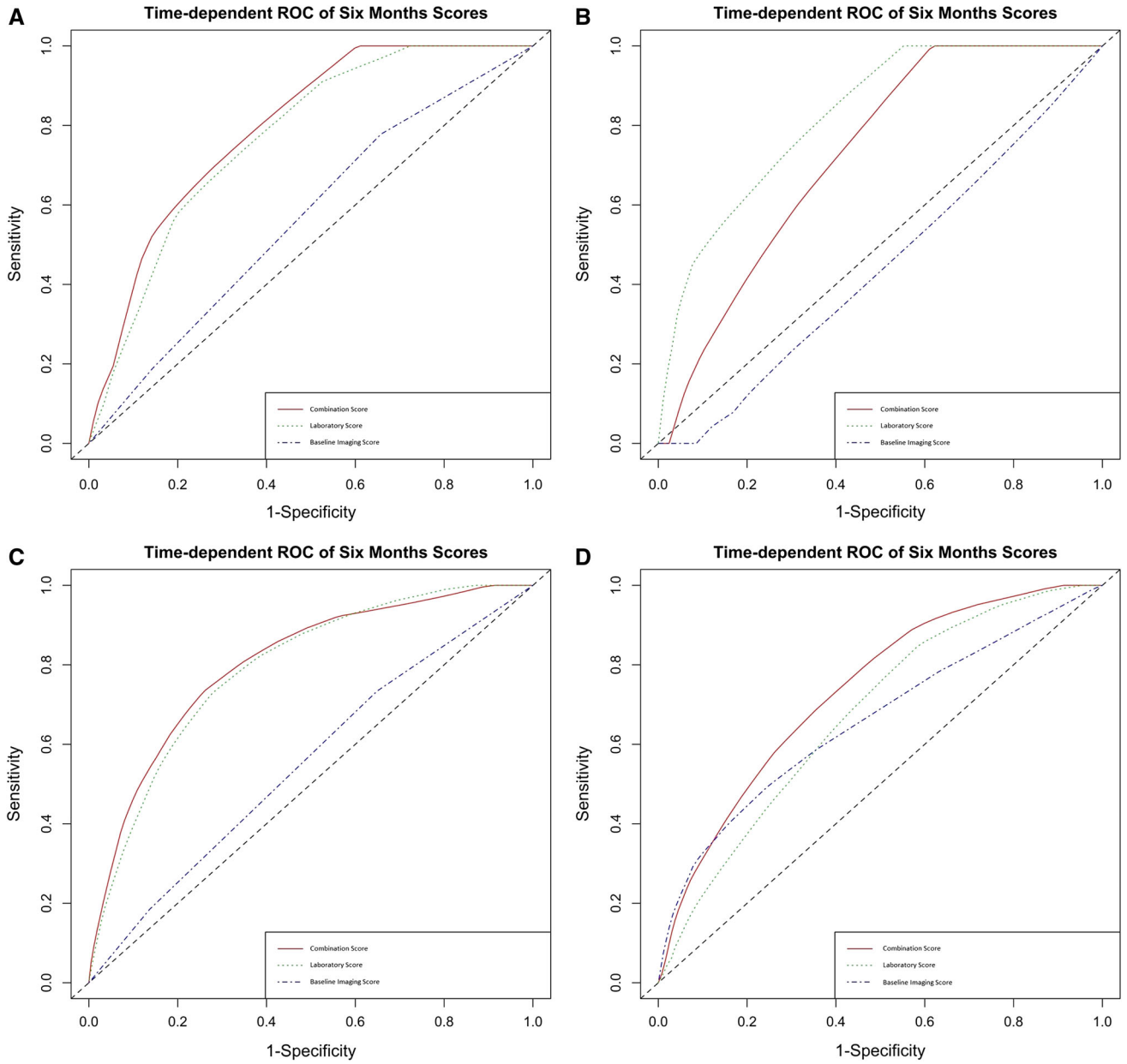


**Fig. 3** **A** Six-month scores predict 1-month survival in developing group. **B** Six-month scores predict 1-month survival in validating group. **C** Six-month scores predict 3-months survival in developing group. **D** Six-month scores predict 3-months survival in validating group

## Discussion

Increasing HCC incidence demands accurate post-therapeutic prediction of survival. Imaging response using the recommended Response Evaluation Criteria in Solid Tumor (RECIST) 1.1 takes time to occur (median of 6 months from treatment) [29]. Laboratory analyses are routinely performed as part of standard-of-care workup in HCC patients, including liver functions (ALBI), complete blood count which can be used to calculate the N:L (inflammatory/immune), and tumor marker (AFP).

This analysis evaluated the three aforementioned parameters in a cohort of 401 patients with HCC treated with TARE who had AFP $>$ 100 ng/dL at baseline. We used the Landmark method to mitigate guarantee-time bias and study the relationship of time-dependent laboratory parameters and survival [30]. The Landmark method determines patient laboratory parameter status at a specified post-therapeutic time point. OS is then estimated from that time point onward. Patients who die before the Landmark are excluded, minimizing confounding effects and permitting survival analysis in patients with favorable biology (liver function and tumor). The baseline, 1-, 3-, and

**Table 2** AUCs of time-dependent ROCs in the validating group

| | Prediction of survival at 1 month post-measurement | Prediction of survival at 3 months post-measurement |
|---|---|---|
| Baseline scores | | |
| Baseline imaging | 0.726 | 0.729 |
| Laboratory | 0.668 | 0.628 |
| Combination | 0.781 | 0.744 |
| One-month scores | | |
| Baseline imaging | 0.624 | 0.637 |
| Laboratory | 0.676 | 0.704 |
| Combination | 0.714 | 0.723 |
| Three-month scores | | |
| Baseline imaging | 0.555 | 0.580 |
| Laboratory | 0.822 | 0.725 |
| Combination | 0.806 | 0.704 |
| Six-month scores | | |
| Baseline imaging | 0.566 | 0.552 |
| Laboratory | 0.770 | 0.791 |
| Combination | 0.795 | 0.803 |

6-month (± 1 month) Landmarks were deemed clinically relevant [31].

Increased inflammation is represented by an increased N:L and may be used as a noninvasive diagnostic marker [32]. N:L represents local pathophysiological reaction of the body/tissue to the tumor which can in turn predict survival [33, 34]. In our analysis, N:L was a significant OS prognosticator on univariate analyses of survival at all Landmarks and was significant on multivariate analysis at the 1-month Landmark.

The extent of disease (cirrhosis) in non-tumoral hepatic parenchyma is important in HCC survival [1, 35]. Assessment of liver function and its inclusion in follow-up is important as degree of hepatic parenchymal injury is a competing cause of death [36, 37]. ALBI, a new evidence-based system, was introduced and validated externally and internally in large cohorts [12, 38, 39]. In a systemic review of prognostic variables in cirrhosis, serum bilirubin and albumin were the two most important and prominent individual prognosticators [40]. Hence, we used ALBI in our study as the marker of liver function. ALBI was a significant OS prognosticator on univariate and multivariate analyses at all three Landmarks.

AFP has been used as diagnostic, screening and prognostic tool for HCC [41–43]. Chan et al. investigated AFP response following chemotherapy and concluded that AFP response is an independent prognostic factor for survival in HCC following chemotherapy [44]. AFP response occurs earlier than RECIST imaging response [45]. AFP was a significant OS prognosticator on univariate and multivariate analyses at all three Landmarks.

Bruix et al. have recently shown that survival benefit of sorafenib is greater in patients with low N:L, low AFP, and macrovascular invasion [28]. Many other investigators are evaluating the role of laboratory values and imaging characteristics as survival prognosticators in HCC [23]. It is recommended to have collaboration in future analyses which may lead to prospective validation and standardization of these parameters.

As seen in Table 2, Laboratory Scores showed better OS predictability than Baseline Imaging Scores at 1, 3, and 6 months. The Combination Score also demonstrated improved survival predictability when compared to Baseline Imaging Score at all Landmarks. We propose using the Laboratory Score (AFP response and ALBI) for post-therapeutic follow-up. External validation of the proposed combinations in retrospective and prospective analyses is recommended.

## Clinical Utility

To bring a clinical perspective to this proposed Laboratory Score, we grouped patients based on a change in the Laboratory Score at each Landmark. The differences in OS between the three groups were significant ($p < 0.001$) in the developing and validating groups (Table 3 and eFigure 2). For example, at the 6-month Landmark in the developing group, OS in groups 1, 2, and 3 were 6, 13, and 52 months, respectively. Group 3 in all Landmarks is the group with a decreasing post-treatment AFP (tumor necrosis) and/or ALBI score (improved liver function). If externally validated, this score may be used for post-therapeutic prognosis and at the 1-month Landmark, it may help make early changes in management before any imaging evidence of response or progression.

## Strengths and Limitations

Strengths to this analysis include: (a) Landmark methods decreased guarantee-time bias and minimizing unknown confounders; (b) multiple statistical methods with similar findings strengthen the conclusions; (c) the study consists of a cohort with representation of all HCC stages; and (d) internal validation of the score.

Limitations of this study include: (a) retrospective model; (b) single treatment modality; (c) single-center

**Table 3** Change in laboratory score correlated to overall survival

| | Cutoffs | Developing | | | | | Validating | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Count | Median OS | 1-year OS | 3-year OS | 5-year OS | Count | Median OS | 1-year OS | 3-year OS | 5-year OS |
| 1 month | | | | | | | | | | | |
| I | < 0.06 | 45 | 5.1 (3.8, 7.8) | 20% | 9% | 9% | 53 | 4.6 (3.3, 7.4) | 21% | 9% | n/a |
| II | 0.06 to 0.64 | 85 | 11.6 (6.7, 6.8) | 49% | 20% | 18% | 65 | 9.1 (7.5, 18.2) | 47% | 16% | 13% |
| III | > 0.64 | 44 | 34.9 (12.8, NR) | 69% | 48% | 40% | 52 | 16.3 (14.1, NR) | 71% | 41% | 27% |
| | | $p < 0.001$ | | | | | $p < 0.001$ | | | | |
| 3 months | | | | | | | | | | | |
| I | < − 0.13 | 31 | 3.9 (2.3, 8.5) | 10% | n/a | n/a | 28 | 4.6 (2.6, 10.2) | 18% | 9% | n/a |
| II | − 0.13 to 1.10 | 58 | 11.8 (7.6, 2.4) | 46% | 23% | 19% | 67 | 11.3 (6.7, 18.6) | 48% | 18% | 18% |
| III | > 1.10 | 29 | 26.6 (16.2, NR) | 73% | 41% | 31% | 25 | 26.3 (12.0, NR) | 67% | 30% | 20% |
| | | | $p < 0.001$ | | | | | $p < 0.001$ | | | |
| 6 months | | | | | | | | | | | |
| I | < − 0.13 | 22 | 5.5 (1.7, 8.4) | 20% | n/a | n/a | 23 | 3.7 (2.4, 9.1) | 12% | n/a | n/a |
| II | − 0.13 to 1.32 | 40 | 13.2 (8.8, 3.6) | 55% | 35% | 35% | 43 | 11.7 (9.0, NR) | 48% | 33% | 33% |
| III | > 1.32 | 21 | 51.6 (23.6, NR) | 78% | 69% | 46% | 18 | 26.9 (14.1, NR) | 82% | 29% | 19% |
| | | | $p < 0.001$ | | | | | $p < 0.001$ | | | |

I (< 25th percentile); II (25th to 75th percentile); III (> 75th percentile)

based; (d) only patients with AFP > 100 ng/dL (although this allows for studying percent change in AFP values, the role of findings presented in this manuscript is limited in HCC patients with AFP < 100 ng/dL); (e) given low numbers at each Landmark, sub-stratification by HCC staging systems (such as BCLC) was not possible; (f) no imaging response was included (this is a focus of an ongoing research project at our institution); (g) the AUCs were not compared to each other as no validated comparison method was available for time-dependent ROCs.

## Conclusion

AFP, N:L, and ALBI grades are objective and reproducible values from routinely performed blood tests. As seen in this analysis using Landmark methodology, AFP response and ALBI are significant prognostic indicators of overall survival at all three Landmarks. This study provides an internally validated score combining these parameters. External validation of the proposed Laboratory Score in other cohorts of HCC is recommended.

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**Compliance with Ethical Standards**

**Conflict of interest** RS, RJL and LK are advisors to BTG. None of the other co-authors report any conflict of interest.

## References

1. Schutte K, Bornschein J, Malfertheiner P. Hepatocellular carcinoma–epidemiological trends and risk factors. Dig Dis. 2009;27(2):80–92.

2. Mgaieth S, Kemp W, Gow P, Fink M, Lubel J, Nicoll A, et al. Impact of viral hepatitis aetiology on survival outcomes in hepatocellular carcinoma: a large multicentre cohort study. J Viral Hepat. 2017;24(11):982–9.
3. Varghese J, Kedarisetty C, Venkataraman J, Srinivasan V, Deepashree T, Uthappa M, et al. Combination of TACE and sorafenib improves outcomes in BCLC stages B/C of hepatocellular carcinoma: a single centre experience. Ann Hepatol. 2017;16(2):247–54.
4. Richani M, Kolly P, Knoepfli M, Herrmann E, Zweifel M, von Tengg-Kobligk H, et al. Treatment allocation in hepatocellular carcinoma: assessment of the BCLC algorithm. Ann Hepatol. 2016;15(1):82–90.
5. Avanzas P, Quiles J, Lopez de Sa E, Sanchez A, Rubio R, Garcia E, et al. Neutrophil count and infarct size in patients with acute myocardial infarction. Int J Cardiol. 2004;97(1):155–6.
6. Ommen SR, Hodge DO, Rodeheffer RJ, McGregor CG, Thomson SP, Gibbons RJ. Predictive power of the relative lymphocyte concentration in patients with advanced heart failure. Circulation. 1998;97(1):19–22.
7. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol. 2005;91(3):181–4.
8. Gibson PH, Cuthbertson BH, Croal BL, Rae D, El-Shafei H, Gibson G, et al. Usefulness of neutrophil/lymphocyte ratio as predictor of new-onset atrial fibrillation after coronary artery bypass grafting. Am J Cardiol. 2010;105(2):186–91.
9. Duffy BK, Gurm HS, Rajagopal V, Gupta R, Ellis SG, Bhatt DL. Usefulness of an elevated neutrophil to lymphocyte ratio in predicting long-term mortality after percutaneous coronary intervention. Am J Cardiol. 2006;97(7):993–6.
10. Halazun KJ, Hardy MA, Rana AA, Woodland DCT, Luyten EJ, Mahadev S, et al. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. Ann Surg. 2009;250(1):141–51.
11. Sukato DC, Tohme S, Chalhoub D, Han K, Zajko A, Amesur N, et al. The prognostic role of neutrophil-to-lymphocyte ratio in patients with unresectable hepatocellular carcinoma treated with radioembolization. J Vasc Interv Radiol JVIR. 2015;26(6):816–24.
12. 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 Off J Am Soc Clin Oncol. 2015;33(6):550–8.
13. Pinato DJ, Sharma R, Allara E, Yen C, Arizumi T, Kubota K, et al. The ALBI grade provides objective hepatic reserve estimation across each BCLC stage of hepatocellular carcinoma. J Hepatol. 2017;66(2):338–46.
14. Carr BI, Kanke F, Wise M, Satomura S. Clinical evaluation of *Lens culinaris* agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. Dig Dis Sci. 2007;52(3):776–82.
15. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, et al. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. Cancer. 2000;88(3):544–9.
16. Yamamoto K, Imamura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, et al. Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. Ann Surg Oncol. 2009;16(10):2795–804.
17. Wang CS, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, et al. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. World J Gastroenterol. 2005;11(39):6115–9.
18. Saffroy R, Pham P, Reffas M, Takka M, Lemoine A, Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. Clin Chem Lab Med. 2007;45(9):1169–79.
19. Sakata J, Shirai Y, Wakai T, Kaneko K, Nagahashi M, Hatakeyama K. Preoperative predictors of vascular invasion in hepatocellular carcinoma. Eur J Surg Oncol. 2008;34(8):900–5.
20. Liu C, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, et al. Value of alpha-fetoprotein in association with clinicopathological features of hepatocellular carcinoma. World J Gastroenterol. 2013;19(11):1811–9.
21. Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, et al. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. J Clin Oncol. 2009;27(34):5734–42.
22. Yang JD, Addissie BD, Mara KC, Harmsen WS, Dai J, Zhang N, et al. GALAD score for hepatocellular carcinoma detection in comparison to liver ultrasound and proposal of GALADUS score. Cancer Epidemiol Biomark Prev. 2018. http://cebp.aacrjournals.org/content/cebp/early/2019/02/21/1055-9965.EPI-18-0281.full.pdf.
23. Spreafico C, Sposito C, Vaiani M, Cascella T, Bhoori S, Morosi C, et al. Development of a prognostic score to predict response to yttrium-90 radioembolization for hepatocellular carcinoma with portal vein invasion. J Hepatol. 2018;68(4):724–32.
24. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100(10):698–711.
25. Tateishi R, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology. 2006;44(6):1518–27.
26. Salem R, Thurston KG. Radioembolization with 90Yttrium microspheres: a state-of-the-art brachytherapy treatment for primary and secondary liver malignancies. Part 1: Technical and methodologic considerations. J Vasc Interv Radiol. 2006;17(8):1251–78.
27. Salem R, Lewandowski RJ, Gates VL, Nutting CW, Murthy R, Rose SC, et al. Research reporting standards for radioembolization of hepatic malignancies. J Vasc Interv Radiol JVIR. 2011;22(3):265–78.
28. Bruix J, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase III studies. J Hepatol. 2017;67(5):999–1008.
29. Riaz A, Miller FH, Kulik LM, Nikolaidis P, Yaghmai V, Lewandowski RJ, et al. Imaging response in the primary index lesion and clinical outcomes following transarterial locoregional therapy for hepatocellular carcinoma. JAMA. 2010;303(11):1062–9.
30. Giobbie-Hurder A, Gelber RD, Regan MM. Challenges of guarantee-time bias. J Clin Oncol. 2013;31(23):2963–9.
31. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. J Clin Oncol. 1983;1(11):710–9.
32. Alkhouri N, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA, Yerian L, et al. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. Liver Int. 2012;32(2):297–302.
33. Schmidt H, Bastholt L, Geertsen P, Christensen IJ, Larsen S, Gehl J, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer. 2005;93(3):273–8.

34. Teramukai S, Kitano T, Kishida Y, Kawahara M, Kubota K, Komuta K, et al. Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of Japan Multinational Trial Organisation LC00-03. Eur J Cancer. 2009;45(11):1950–8.
35. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60(5):277–300.
36. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol. 2008;48(Suppl 1):S20–37.
37. Durand F, Valla D. Assessment of prognosis of cirrhosis. Semin Liver Dis. 2008;28(1):110–22.
38. Li MX, Zhao H, Bi XY, Li ZY, Huang Z, Han Y, et al. Prognostic value of the albumin–bilirubin grade in patients with hepatocellular carcinoma: validation in a Chinese cohort. Hepatol Res. 2017;47(8):731–41.
39. Hickey R, Mouli S, Kulik L, Desai K, Thornburg B, Ganger D, et al. Independent analysis of albumin–bilirubin grade in a 765-patient cohort treated with transarterial locoregional therapy for hepatocellular carcinoma. J Vasc Interv Radiol. 2016;27(6):795–802.
40. de Lope CR, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. J Hepatol. 2012;56(Suppl 1):S75–87.
41. Trevisani F, De Notariis S, Rossi C, Bernardi M. Randomized control trials on chemoembolization for hepatocellular carcinoma: Is there room for new studies? J Clin Gastroenterol. 2001;32(5):383–9.
42. Nomura F, Ohnishi K, Tanabe Y. Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Analysis of 606 patients. Cancer. 1989;64(8):1700–7.
43. Lok AS, Lai CL. alpha-Fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. Hepatology. 1989;9(1):110–5.
44. Chan SL, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, et al. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Clin Oncol. 2009;27(3):446–52.
45. Memon K, Kulik L, Lewandowski RJ, Wang E, Ryu RK, Riaz A, et al. Alpha-fetoprotein response correlates with EASL response and survival in solitary hepatocellular carcinoma treated with transarterial therapies: a subgroup analysis. J Hepatol. 2012;56(5):1112–20.