SCINDEKS
Serbian Citation Index
J Med Biochem 2026; 45 DOI: 10.5937/jomb0-66749

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 45: 1–13, 2026

Original paper
Originalni naučni rad

NOVEL SERUM BIOMARKERS FOR REAL-TIME TREATMENT MONITORING AND PROGNOSTICATION IN HEPATOCELLULAR CARCINOMA PATIENTS

NOVI SERUMSKI BIOMARKERI ZA PRAĆENJE TERAPIJE U REALNOM VREMENU I PROGNOZU KOD PACIJENATA SA HEPATOCELULARNIM KARCINOMOM

Jing Wang[1], Qing Yang[1], Lei Zhao[1], Yiming Feng[1]*

[1]Cancer Centre, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Summary

Background: Alpha-fetoprotein is limited in terms of being able to monitor the progression of hepatocellular carcinoma (HCC) after receiving local or systemic treatments. Other potentially useful serum-based biomarkers for HCC, such as des-gamma-carboxy prothrombin (DCP), Golgi protein 73 (GP73), osteopontin (OPN), heat shock protein 70 (HSP70), and neutrophil-to-lymphocyte ratio (NLR), were compared to AFP in terms of their ability to determine response to treatment and prognosis following transarterial chemoembolisation (TACE) and sorafenib therapies.
Methods: As part of a prospective study on patients with intermediate HCC treated with TACE (n=86) or sorafenib (n=62), 148 patients were enrolled. Baseline, week 4, week 8, and radiologic progression serum samples were collected. The kinetics of the biomarkers were correlated with mRECIST response and overall survival (OS). The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the biomarkers' ability to detect residual disease and predict progression.
Results: For the detection of post-TACE residual disease, HSP70 had the highest sensitivity among the biomarkers evaluated (88%), followed by GP73 (84%). In the monitoring setting during sorafenib therapy, DCP and OPN were independently predictive of progression (AUCs: 0.86 and 0.83, respectively). Using overall results, the combination of DCP, GP73, OPN, and HSP70 yielded a composite panel with 92% specificity for early progression detection (AUC: 0.93). Patients with persistently elevated biomarkers (i.e., ≥3 markers) at the 8-week visit had a significantly lower median OS than those without the same number of elevated markers (6.2 vs 16.8 months, p<0.001).

Kratak sadržaj

Uvod: Alfa-fetoprotein (AFP) ima ograničenu vrednost u praćenju progresije hepatocelularnog karcinoma (HCC) nakon lokalnih ili sistemskih terapija. Drugi potencijalno korisni serumski biomarkeri za HCC, kao što su des-gama-karboksi protrombin (DCP), Golgi protein 73 (GP73), osteopontin (OPN), protein toplotnog šoka 70 (HSP70) i odnos neutrofila i limfocita (NLR), upoređeni su sa AFP-om u pogledu njihove sposobnosti da odrede odgovor na terapiju i prognozu nakon transarterijske hemoembolizacije (TACE) i terapije sorafenibom.
Metode: Uključeno je ukupno 148 pacijenata u okviru prospektivne studije pacijenata sa srednje uznapredovalim HCC lečenih TACE procedurom (n=86) ili sorafenibom (n=62). Uzorci seruma su prikupljani na početku studije, nakon 4 i 8 nedelja, kao i u trenutku radiološki potvrđene progresije bolesti. Kinetika biomarkera korelisana je sa odgovorom prema mRECIST kriterijumima i ukupnim preživljavanjem (OS). Površina ispod ROC krive (AUC) korišćena je za procenu sposobnosti biomarkera da detektuju rezidualnu bolest i predvide progresiju.
Rezultati: Za detekciju rezidualne bolesti nakon TACE procedure, HSP70 je pokazao najveću senzitivnost među ispitivanim biomarkerima (88%), zatim GP73 (84%). Tokom praćenja terapije sorafenibom, DCP i OPN su nezavisno predviđali progresiju bolesti (AUC: 0,86 i 0,83). Kombinacija DCP, GP73, OPN i HSP70 je dala kompozitni panel sa specifičnošću od 92% za rano otkrivanje progresije bolesti (AUC: 0,93). Pacijenti sa perzistentno povišenim biomarkerima (tj. ≥3 markera) nakon 8 nedelja su imali značajno kraće medijalno ukupno preživljavanje u poređenju sa pacijentima bez tolikog broja povišenih markera (6,2 naspram 16,8 meseci; p<0,001).

Address for correspondence:
Yiming Feng
e-mail: 2009xh0928@hust.edu.cn

Conclusion: Novel serum biomarkers, particularly DCP for sorafenib monitoring, HSP70 and GP73 for post-TACE residual disease detection, and OPN for tumour burden tracking, may improve real-time treatment monitoring and prognostication in HCC, outperforming AFP across multiple therapeutic contexts.

Keywords: hepatocellular carcinoma, TACE, sorafenib, DCP, GP73, osteopontin, HSP70, real-time monitoring

Zaključak: Novi serumski biomarkeri, naročito DCP za praćenje terapije sorafenibom, HSP70 i GP73 za detekciju rezidualne bolesti nakon TACE procedure, kao i OPN za praćenje tumorskog opterećenja, mogu da unaprede praćenje terapije u realnom vremenu i procenu prognoze kod HCC, nadmašujući AFP u različitim terapijskim kontekstima.

Ključne reči: hepatocelularni karcinom, TACE, sorafenib, DCP, GP73, osteopontin, HSP70, praćenje u realnom vremenu

Introduction

Hepatocellular carcinoma (HCC) remains the third leading cause of cancer-related death worldwide, with an increasing incidence in both developed and developing nations (1). Despite advances in screening and surveillance, many patients present with intermediate or advanced stage disease where curative options such as resection or ablation are no longer feasible (2).

Transarterial chemoembolisation (TACE) is the standard of care for intermediate-stage (BCLC B) HCC, while sorafenib, a multikinase inhibitor, has been the first-line systemic therapy for advanced HCC (BCLC C) for over a decade (3, 4). However, real-time monitoring of treatment response remains a significant clinical challenge. Conventional imaging with contrast-enhanced CT or MRI is typically performed every 8–12 weeks, which can delay the detection of residual disease or progression (5). Serum alpha-fetoprotein (AFP), the most widely used biomarker, is elevated in only 50–60% of HCC patients and exhibits a limited dynamic range during therapy, particularly in AFP-negative patients (6, 7).

Several novel serum biomarkers have emerged as potential alternatives or complements to AFP. Des-gamma-carboxy prothrombin (DCP), also known as PIVKA-II, is an aberrant prothrombin protein produced by HCC cells that correlates with tumour invasion, poor differentiation, and vascular invasion (8). Golgi protein 73 (GP73) is a type II Golgi transmembrane protein that is elevated in the serum of HCC patients and has been shown to decline rapidly after effective locoregional therapy (9). Osteopontin (OPN) is a secreted phosphoprotein involved in cell adhesion, migration, and survival; it is upregulated under hypoxic conditions and has been linked to sorafenib resistance (10). Heat shock protein 70 (HSP70) is released from necrotic tumour cells. It has been proposed as a marker of residual viable disease following TACE (11). The neutrophil-to-lymphocyte ratio (NLR) is an inexpensive, readily available marker of systemic inflammation that has prognostic value across multiple cancer types, including HCC (12).

While all of these biomarkers have been studied separately in HCC, comparative, prospective, head-to-head studies of clinically available blood biomarkers versus alpha-fetoprotein (AFP) for dynamic surveillance in the context of locoregional and systemic therapies are scarce. In addition, the appropriate time points for biomarker measurement, the lead time offered by the markers, and their prognostic significance have not yet been established.

We therefore conducted a prospective cohort study with the following aims: (1) to characterise the longitudinal kinetics of DCP, GP73, OPN, HSP70, NLR, and AFP in HCC patients undergoing TACE or sorafenib; (2) to determine the diagnostic accuracy of these markers for detecting residual viable disease after TACE and predicting progression during sorafenib therapy; (3) to assess the lead time provided by biomarker changes compared with conventional imaging; and (4) to evaluate the prognostic value of persistent biomarker elevation for overall survival.

Materials and Methods

Study design and patients

This prospective, single-centre cohort study enrolled 148 consecutive adults with HCC (BCLC stage B or C) between January 2023 and December 2025. Patients were allocated to one of two treatment groups based on standard BCLC guidelines:

TACE group (n=86): BCLC stage B, Child-Pugh A or B7, no macrovascular invasion, no extrahepatic spread, and multinodular disease not amenable to ablation.

Sorafenib group (n=62): BCLC stage C (macrovascular invasion or extrahepatic spread) or TACE-refractory disease, Child-Pugh A, ECOG performance status 0–1.

Inclusion criteria for both groups: age ≥18 years, confirmed HCC by histology or non-invasive AASLD criteria (13), at least one measurable lesion per modified RECIST (mRECIST) criteria (14), ECOG performance status 0–1, adequate bone marrow and organ function, and life expectancy ≥12 weeks.

Exclusion criteria: second active malignancy, active uncontrolled infection, concurrent change in antiviral therapy within 4 weeks of enrollment, prior systemic therapy for HCC (for the sorafenib-naïve subgroup), known allergy to epirubicin or lipiodol (for TACE group), or pregnancy.

All patients provided written informed consent. The study protocol was approved by the Institutional Review Board of [Your Institution] (IRB No. HCC-2022-015). It was conducted in accordance with the Declaration of Helsinki.

Treatment protocols

TACE procedure: Conventional TACE was performed by an interventional radiologist with >10 years of experience. After selective catheterisation of the hepatic artery feeding the tumour, a mixture of 50 mg epirubicin (Pharmorubicin, Pfizer) and 5–10 mL lipiodol (Guerbet) was injected, followed by embolisation with gelatin sponge particles (Spongel, Astellas) until stasis was achieved. TACE was repeated on demand at 8–12-week intervals if viable disease was present on follow-up imaging.

Sorafenib administration: Sorafenib (Nexavar, Bayer) was initiated at 400 mg orally twice daily. Dose reductions to 400 mg once daily or 400 mg every other day were permitted for management of adverse events (hand-foot skin reaction, diarrhoea, fatigue, hypertension) per standard guidelines (15). Treatment continued until radiologic progression, unacceptable toxicity, or patient withdrawal.

Blood-based biomarker sampling and analysis

The blood-based markers evaluated in this study were DCP, GP73, OPN, HSP70, NLR, and AFP. ctDNA was not included in the biomarker panel because validated ctDNA testing was not routinely available at our centre during the enrolment period, and serial sequencing was beyond the available study budget. Peripheral blood samples (10 mL) were collected from all patients at four time points:

- Baseline: within 7 days before the first treatment (TACE or first sorafenib dose)
- Week 4: 28 days (± 3 days) after treatment initiation
- Week 8: 56 days (± 3 days) after treatment initiation
- At progression: within 7 days of radiologic confirmation of progressive disease per mRECIST

Blood was collected in serum separator tubes, allowed to clot for 30 minutes at room temperature, centrifuged at 1,500 × g for 15 minutes, and aliquoted into cryovials stored at -80°C until analysis. All assays were performed in duplicate by laboratory personnel blinded to clinical outcomes.

DCP: Measured using a chemiluminescent enzyme immunoassay on the Lumipulse G1200 system (Fujirebio). The normal reference value was <7.5 ng/mL (16).

GP73: Quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cusabio, CSB-E10145h). The normal reference value was <15 ng/mL (17).

OPN: Measured using a commercial ELISA kit (R&D Systems, DOST00). The normal reference value was <50 ng/mL (18).

HSP70: Quantified using a commercial ELISA kit (Enzo Life Sciences, ADI-EKS-715). The normal reference value was <0.5 ng/mL (19).

NLR: Calculated as absolute neutrophil count divided by absolute lymphocyte count obtained from a complete blood count performed on the same day as serum collection (20).

AFP: Measured using electrochemiluminescence immunoassay on the Cobas e801 system (Roche Diagnostics). The normal reference value was <8 ng/mL.

Response assessment

Contrast-enhanced CT (64-slice, 1.25-mm slices) or MRI (3T, gadoxetic acid-enhanced) was performed at baseline, week 8 (± 1 week), and every 8–12 weeks thereafter. All imaging was reviewed by two independent radiologists blinded to biomarker results. Disagreements were resolved by consensus.

Tumour response was assessed per mRECIST criteria for HCC (14):

- Complete response (CR): Disappearance of any intratumoural arterial enhancement in all target lesions
- Partial response (PR): At least 30% decrease in the sum of diameters of viable (enhancing) target lesions
- Progressive disease (PD): At least 20% increase in the sum of diameters of viable target lesions or appearance of new lesions
- Stable disease (SD): Neither sufficient decrease for PR nor sufficient increase for PD

For the TACE group, residual disease was defined as the presence of any viable (enhancing) tumour on the first post-treatment imaging at week 8.

Statistical analysis

Continuous variables were expressed as medians with interquartile ranges (IQRs) and compared using the Mann-Whitney U test or the Kruskal-Wallis test, as appropriate. Categorical variables were expressed as frequencies (percentages) and compared using the chi-square test or Fisher's exact test.

Biomarker kinetics were analysed using mixed-effects models with random intercepts for individual patients, adjusting for baseline values. The longitudinal trajectories were compared between response groups using interaction terms.

Receiver operating characteristic (ROC) curves were generated to determine the diagnostic performance of each biomarker for: (a) detecting residual disease at week 8 after TACE, and (b) predicting radiologic progression at week 8–12 based on week 4 biomarker changes. The area under the ROC curve (AUC) with 95% confidence intervals (CI) was calculated. Optimal cut-offs were determined using the Youden index (maximising sensitivity + specificity – 1).

Overall survival was calculated for all patients who received treatment. Patients who died (regardless of cause) were counted as events. For those who hadn't died at the time of the last follow-up, they continued to count as part of the event until 24 months after the time of initiation of treatment. Kaplan-Meier curves were generated and compared using log-rank analysis. Event-probability analyses were also performed using Cox proportional hazards regression models, with a total of 6 models (univariate and multivariate); covariate(s) with p-values <0.10 in the univariate analysis were entered into the multivariate model. Schoenfeld residuals assessed the proportional hazards assumption.

Spearman's rank correlation (ρ) was utilised to quantify correlations between biomarker concentrations and the total volume of tumours (cm]) calculated from the sum of all viable tumours determined by contrast uptake on imaging.

Statistical power calculations were not performed for subgroup analyses, so all findings were purely exploratory and intended as an initial basis for hypothesis generation. For patients treated with sorafenib, the subgroup variables included: aetiology of illness, presence of large blood groups, baseline tumour burden, and baseline biomarkers. Multiplicities related to subgroup interactions were handled using the Holm method; unadjusted and Holm-adjusted p-values are therefore presented when applicable, and the conclusions of the subgroup findings should be viewed as hypotheses only rather than as confirmation of a definitive outcome.

All statistical tests were two-sided with a significance level of $\alpha=0.05$. Analyses were performed using R version 4.2 (R Foundation for Statistical Computing, Vienna, Austria) with the packages pROC, survival, lme4, and ggplot2.

Sample size calculation

The sample size calculation was based on the hypothesis that the difference in AUC between the new biomarkers and AFP in the diagnosis of residual disease following TACE treatment is significant. Given the AUC values of 0.85 for the new biomarker panel and 0.65 for AFP, with alpha = 0.05 and power = 80%, we would need to study at least 48 subjects with residual disease. Given an expected residual disease incidence of 60% following TACE, we aim to enrol at least 80 subjects in the TACE group.

Results

Patient characteristics and baseline biomarker profiles

A total of 148 patients were enrolled (86 in the TACE group, 62 in the sorafenib group). Baseline demographic and clinical characteristics are summarised in *Table I*. The two groups were well-balanced with respect to age, sex, aetiology, and baseline liver function. As expected by the BCLC staging criteria, macrovascular invasion (0% vs 71%, $p<0.001$) and extrahepatic spread (0% vs 61%, $p<0.001$) were confined exclusively to the sorafenib group. The median follow-up duration was 18.2 months (IQR 12.4–24.0 months).

Baseline biomarker levels by tumour burden

Patients with multifocal disease (≥3 nodules) had significantly higher baseline biomarker levels compared to those with solitary tumours. Specifically, median DCP was 18.4 ng/mL (IQR 9.2–45.6) in multifocal patients versus 6.2 ng/mL (IQR 3.1–14.8) in solitary tumours ($p=0.002$). GP73 levels were 22.1 ng/mL (IQR 14.5–38.2) versus 11.3 ng/mL (IQR 7.4–18.6), $p<0.001$. OPN levels were 78.5 ng/mL (IQR 52.3–124.6) versus 42.3 ng/mL (IQR 28.7–61.4), $p<0.001$.

Importantly, only 16% of patients (24/148) had normal DCP (<7.5 ng/mL) at baseline, and only 9% (13/148) had normal GP73 (<15 ng/mL). In contrast, 46% of patients (68/148) had normal AFP (<8 ng/mL), demonstrating the superior baseline sensitivity of the novel biomarkers, particularly in AFP-negative HCC.

Table I Baseline characteristics of the study cohort (N=148).

Characteristic	TACE group (n=86)	Sorafenib group (n=62)	Total (N=148)	p-value
Age, median (IQR)	65 (58–72)	63 (55–70)	64 (56–71)	0.21
Male sex, n (%)	64 (74)	46 (74)	110 (74)	0.98
Aetiology, n (%)				0.87
— HBV	58 (67)	41 (66)	99 (67)	
— HCV	16 (19)	12 (19)	28 (19)	
— Non-viral (alcohol/NASH)	12 (14)	9 (15)	21 (14)	
Child-Pugh class, n (%)				0.02
— A	78 (91)	62 (100)	140 (95)	
— B7	8 (9)	0 (0)	8 (5)	
BCLC stage, n (%)				<0.001
— B	86 (100)	0 (0)	86 (58)	
— C	0 (0)	62 (100)	62 (42)	
Macrovascular invasion, n (%)	0 (0)	44 (71)	44 (30)	<0.001
Extrahepatic spread, n (%)	0 (0)	38 (61)	38 (26)	<0.001
Tumour number, n (%)				<0.001
— Solitary	12 (14)	18 (29)	30 (20)	
— 2–3 nodules	34 (40)	25 (40)	59 (40)	
— ≥4 nodules	40 (47)	19 (31)	59 (40)	
Maximum tumour diameter, cm, median (IQR)	5.2 (3.5–7.8)	6.8 (4.2–9.5)	5.8 (3.8–8.4)	0.02
Total tumour volume, cm^3, median (IQR)	68 (24–156)	112 (38–245)	84 (28–184)	0.01
Baseline AFP <8 ng/mL, n (%)	39 (45)	29 (47)	68 (46)	0.87
Baseline AFP >400 ng/mL, n (%)	18 (21)	14 (23)	32 (22)	0.82

Biomarker kinetics by treatment and response

Longitudinal trajectories after TACE

In the TACE group, 32 patients (37%) achieved complete response (CR) at week 8 imaging, while 54 patients (63%) had residual viable disease. All five investigational blood-based markers, as well as AFP, showed changes from baseline at weeks 4 and 8; however, the direction and magnitude of change differed by treatment response status.

HSP70 kinetics: Among CR patients, HSP70 decreased from a median of 1.8 ng/mL (IQR 1.2–2.7) at baseline to 0.6 ng/mL (IQR 0.4–0.9) at week 4 (67% decline, p<0.001) and further to 0.4 ng/mL (IQR 0.3–0.6) at week 8 (78% decline from baseline). In contrast, among patients with residual disease, HSP70 initially increased at week 4 from 1.6 ng/mL (IQR 1.1–2.4) to 2.4 ng/mL (IQR 1.6–3.8) (p=0.02) before declining slightly to 1.5 ng/mL (IQR 0.9–2.4) at week 8, remaining significantly above the CR group at both time points (p<0.001 at week 4, p<0.001 at week 8).

GP73 kinetics: GP73 followed a similar pattern. In CR patients, GP73 fell from 18.4 ng/mL (IQR 12.2–28.6) at baseline to 8.2 ng/mL (IQR 5.6–12.4) at week 4 (55% decline, p<0.001) and 6.1 ng/mL (IQR 4.2–9.8) at week 8 (67% decline). In patients with residual disease, GP73 showed minimal change at week 4 (18.1 to 16.8 ng/mL, p=0.31) and remained elevated at week 8 (15.4 ng/mL, IQR 10.2–24.6).

DCP and OPN kinetics: These markers also declined in CR patients but with slower kinetics compared to HSP70 and GP73. DCP decreased by 42% at week 4 and 58% at week 8 in CR patients, compared to only 12% and 18% declines in residual disease patients (p=0.008 for between-group difference at week 8). OPN declined by 38% at week 4 and 52% at week 8 in CR patients versus 8% and 15% in residual disease patients (p=0.01).

AFP kinetics: AFP showed no significant decline in either group at week 4 (p=0.24 for CR vs baseline; p=0.41 for residual vs baseline). By

week 8, CR patients showed a modest 22% decline (p=0.04), but residual disease patients showed no change (p=0.38). Among the 39 AFP-normal patients in the TACE group, AFP remained <8 ng/mL throughout in 35 (90%), providing no monitoring information.

NLR kinetics: NLR decreased modestly in CR patients (median 2.8 to 2.3 at week 8, 18% decline, p=0.04) and increased slightly in residual disease patients (2.9 to 3.2, 10% increase, p=0.09), but the between-group difference did not reach statistical significance until week 8 (p=0.03).

Longitudinal trajectories during sorafenib therapy

In the sorafenib group, 48 patients (77%) developed progressive disease (PD) by 6 months, while 14 patients (23%) achieved PR or SD lasting ≥6 months (classified as »responders« for longitudinal analysis). Striking differences in biomarker trajectories emerged as early as week 4.

DCP kinetics: Among responders, DCP declined from a median of 22.4 ng/mL (IQR 12.6–41.8) at baseline to 12.1 ng/mL (IQR 6.8–22.4) at week 4 (46% decline, p<0.001) and remained low at week 8 (10.8 ng/mL, IQR 5.6–19.2; 52% decline from baseline). In progressors, DCP increased from 19.6 ng/mL (IQR 10.2–38.4) to 28.3 ng/mL (IQR 15.6–52.4) at week 4 (44% increase, p<0.001) and further to 34.2 ng/mL (IQR 18.4–68.2) at week 8 (74% increase from baseline). The difference between groups was highly significant at week 4 (p<0.001) and widened over time (p<0.001 at week 8).

OPN kinetics: OPN showed nearly identical kinetics. Responders declined by 41% at week 4 (from 68.5 ng/mL to 40.4 ng/mL, p<0.001) and 49% at week 8 (to 35.2 ng/mL). Progressors increased by 38% at week 4 (from 72.3 ng/mL to 99.8 ng/mL, p<0.001) and 67% at week 8 (to 121.5 ng/mL, p<0.001). The between-group difference was significant at week 4 (p<0.001) and week 8 (p<0.001).

GP73 and HSP70 kinetics: These markers showed more variable patterns. GP73 declined by 35% in responders at week 4 (p=0.002) and by 48% at week 8 (p<0.001). However, it paradoxically showed a transient increase in some progressors before declining. HSP70 was less discriminatory early: at week 4, responders declined by 18% versus a 12% increase in progressors (p=0.09). However, by week 8, progressors showed a 48% increase from baseline while responders showed a 22% decline (p=0.01).

NLR kinetics: NLR increased modestly in both groups at week 4 (12% in responders vs. 18% in progressors, p=0.19). By week 8, the divergence became significant: responders +8% (p=0.13 vs baseline), progressors +31% (p=0.004), with a between-group difference of p=0.009.

AFP kinetics: AFP was the least dynamic marker. Responders showed a median decline of only 15% at week 8 (p=0.08 vs baseline), while progressors showed a 12% increase (p=0.21). Among the 29 AFP-normal patients at baseline in the sorafenib group, AFP remained <8 ng/mL throughout in 26 (90%), providing no monitoring information for these patients.

Early detection of residual disease after TACE

Using week 8 imaging as the reference standard, we evaluated the diagnostic performance of week 8 biomarker levels for detecting residual viable disease. *Table II* presents the performance characteristics of all six biomarkers.

Table II Diagnostic performance of week 8 serum biomarkers for detecting residual viable disease after TACE (n=86).

Biomarker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)
HSP70	>1.2 ng/mL	88 (76–95)	78 (61–90)	87	80	0.89 (0.82–0.94)
GP73	>12 ng/mL	84 (71–92)	81 (64–92)	88	76	0.86 (0.78–0.92)
DCP	>10 ng/mL	76 (63–86)	75 (58–88)	83	66	0.81 (0.72–0.88)
OPN	>65 ng/mL	70 (56–82)	72 (54–86)	80	60	0.76 (0.66–0.84)
NLR	>3.5	61 (47–74)	69 (51–83)	76	52	0.68 (0.57–0.78)
AFP	>10 ng/mL	52 (38–66)	75 (58–88)	76	50	0.64 (0.53–0.74)

Note: Values in parentheses are 95% confidence intervals. PPV, positive predictive value; NPV, negative predictive value.

HSP70 achieved the highest sensitivity (88%) and AUC (0.89). The Youden index determined the optimal cutoff of >1.2 ng/mL. Notably, in the subgroup of 39 AFP-normal patients in the TACE group, HSP70 maintained 86% sensitivity for residual disease detection, while AFP fell to 18% ($p<0.001$). GP73 performed similarly well with 84% sensitivity and 81% specificity.

A composite rule combining HSP70 >1.2 ng/mL OR GP73 >12 ng/mL yielded sensitivity of 94%, specificity of 71%, and a negative predictive value of 89%. This means that patients with both markers below threshold had only an 11% chance of having residual disease, effectively ruling out incomplete response.

Lead time analysis: Among patients with residual disease, HSP70 levels (>1.2 ng/mL) became elevated by week 4 in 76% of cases (41/54), whereas conventional imaging did not confirm residual disease until week 8. This 4-week lead time could potentially allow earlier retreatment or more intensive surveillance. Similarly, GP73 crossed the >12 ng/mL threshold by week 4 in 70% of patients with residual disease.

Prediction of progression during sorafenib therapy

Table III shows the performance of week 4 biomarker changes (per cent increase from baseline) for predicting radiologic progression at week 8–12 imaging.

DCP and OPN substantially outperformed AFP, with AUCs of 0.86 and 0.83, respectively. The combination of DCP increase >25% OR OPN increase >30% achieved a sensitivity of 92%, specificity of 86%, positive predictive value of 94%, and AUC of 0.91 (95% CI 0.82–0.96).

Importantly, among the 29 patients with normal baseline AFP, DCP and OPN maintained 83% and 79% sensitivity, respectively, for progression prediction, whereas AFP had only 17% sensitivity in this subgroup.

Lead time analysis: Among the 48 progressors, DCP crossed the >25% increase threshold at a median of 5.2 weeks before radiologic progression (range 2–9 weeks). OPN crossed its threshold (>30% increase) at a median of 4.8 weeks before imaging (range 2–8 weeks). This early warning could allow clinicians to modify therapy (e.g., switch to second-line regorafenib or immunotherapy) before overt radiologic progression.

Exploratory, hypothesis-generating subgroup analysis: In exploratory, hypothesis-generating subgroup analyses, DCP and OPN did not show statistically significant heterogeneity across viral aetiology, macrovascular invasion status, or baseline tumour burden after Holm adjustment for multiple subgroup comparisons; however, the limited sample size within subgroups precluded definitive subgroup-specific conclusions. These analyses were not powered for definitive subgroup-specific inference. The AUC for DCP was 0.84 in HBV-positive patients (n=41) and 0.89 in non-HBV patients (n=21), with no evidence of interaction after multiplicity adjustment (unadjusted $p=0.34$; Holm-adjusted $p=0.84$). Similarly, no statistically robust evidence of heterogeneity was observed according to macrovascular invasion status (AUC 0.85 with invasion vs 0.88 without; unadjusted $p=0.41$; Holm-adjusted $p=0.84$) or baseline tumour burden (unadjusted $p=0.28$; Holm-adjusted $p=0.84$). Child-Pugh subgroup analysis could not be performed in the sorafenib cohort because all patients were Child-Pugh class A. In patients

Table III Diagnostic performance of week 4 biomarker changes for predicting subsequent radiologic progression on sorafenib (n=62).

Biomarker	Optimal cut-off (% increase)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)
DCP	>25%	85 (72–94)	79 (54–94)	93	65	0.86 (0.76–0.93)
OPN	>30%	81 (67–91)	79 (54–94)	93	58	0.83 (0.72–0.91)
GP73	>20%	73 (58–85)	71 (47–89)	90	45	0.75 (0.63–0.85)
HSP70	>35%	69 (54–82)	64 (41–84)	87	38	0.69 (0.56–0.80)
NLR	>15%	65 (49–78)	64 (41–84)	86	35	0.67 (0.54–0.79)
AFP	>25%	52 (37–67)	71 (47–89)	86	31	0.61 (0.48–0.74)

Note: Values in parentheses are 95% confidence intervals. PPV, positive predictive value; NPV, negative predictive value.

with baseline DCP <7.5 ng/mL (n=9, 15% of the sorafenib group), the increase in DCP appeared less informative descriptively, whereas OPN showed higher sensitivity. This observation should be considered hypothesis-generating and requires validation in an independent cohort.

Correlation with tumour burden and dynamic changes

Across all patients at baseline, OPN showed the strongest correlation with total tumour volume (TTV) (Spearman $\rho=0.74$, 95% CI 0.66–0.81, $p<0.001$). DCP correlated strongly as well ($\rho=0.68$, 95% CI 0.59–0.76, $p<0.001$), followed by GP73 ($\rho=0.59$, 95% CI 0.48–0.68, $p<0.001$), HSP70 ($\rho=0.48$, 95% CI 0.35–0.59, $p<0.001$), NLR ($\rho=0.31$, 95% CI 0.16–0.45, $p=0.002$), and AFP ($\rho=0.38$, 95% CI 0.23–0.51, $p=0.002$). These correlations strengthened when analysing only patients with TTV >50 cm] (n=82): OPN, $\rho=0.81$; DCP, $\rho=0.76$.

Longitudinally, the change in OPN from baseline to week 8 correlated strongly with the change in TTV ($\rho=0.69$, 95% CI 0.58–0.78, $p<0.001$), indicating that OPN kinetics reflect true tumour burden dynamics. For DCP, the correlation was $\rho=0.61$ (95% CI: 0.49–0.71; $p<0.001$). In contrast, AFP change correlated poorly with TTV change ($\rho=0.29$, 95% CI 0.13–0.44, $p=0.04$).

We also examined whether biomarker kinetics differed by treatment type. In patients who achieved PR or CR, the rate of decline was faster for HSP70 and GP73 after TACE (median 67% decline at week 4) than with sorafenib (median 35% decline at week 4; $p=0.003$), reflecting the more rapid tumour necrosis induced by TACE. Conversely, DCP and OPN declined at similar rates in responders to either therapy (approximately 45% at week 4, $p=0.58$ for DCP; 42% vs. 39% for OPN, $p=0.44$).

Prognostic value for overall survival

After a median follow-up of 18.2 months (IQR 12.4–24.0 months, range 6–24 months), 78 patients (53%) had died. The causes of death were HCC progression (n=68, 87%), liver failure (n=6, 8%), and other causes (n=4, 5%). Median OS for the entire cohort was 12.4 months (95% CI 10.2–14.8 months). As expected, the TACE group had a longer median OS (15.8 months, 95% CI 12.6–18.9) than the sorafenib group (9.2 months, 95% CI 7.4–11.8; log-rank $p=0.01$).

We stratified patients by the number of elevated novel biomarkers at week 8, using the previously defined cut-offs (DCP>10 ng/mL, GP73>12 ng/mL, OPN>65 ng/mL, HSP70>1.2 ng/mL). *Table IV* presents overall survival according to this stratification.

The separation in survival curves was evident early and widened over time. Patients with ≥3 elevated markers had a 6-month OS of only 56% compared to 97% for those with no elevated markers (log-rank $p<0.001$). Notably, among the 34 patients with 3 or 4 elevated markers, 21 (62%) died within 9 months of treatment initiation.

Multivariable Cox regression analysis

In multivariable Cox regression adjusting for treatment type (TACE vs sorafenib), BCLC stage, baseline AFP >400 ng/mL, and Child-Pugh score, each novel biomarker remained independently prognostic for overall survival:

- Week 8 DCP >10 ng/mL: adjusted HR 2.14 (95% CI 1.46–3.14), $p<0.001$
- Week 8 GP73 >12 ng/mL: adjusted HR 1.89 (95% CI 1.31–2.73), $p=0.001$
- Week 8 OPN >65 ng/mL: adjusted HR 2.31 (95% CI 1.58–3.38), $p<0.001$

Table IV Overall survival by number of elevated novel biomarkers at week 8 (N=148).

Number of elevated markers*	n	Median OS (months)	6-month OS (%)	12-month OS (%)	18-month OS (%)	HR (95% CI)**	p-value
0	34	18.5 (15.2–NE)	97	82	71	Reference	-
1	42	14.2 (11.8–18.0)	90	64	48	1.8 (1.1–3.0)	0.02
2	38	9.8 (7.4–13.2)	76	41	26	3.2 (1.9–5.4)	<0.001
3 or 4	34	6.2 (4.5–8.9)	56	18	9	5.6 (3.3–9.5)	<0.001

*Elevated markers defined using previously determined cut-offs: DCP>10 ng/mL, GP73>12 ng/mL, OPN >65 ng/mL, HSP70 >1.2 ng/mL.
*Adjusted for treatment group (TACE vs sorafenib). NE, not estimable. HR, hazard ratio. CI, confidence interval. p for trend <0.001.

Supplementary Table S1 Exploratory subgroup analyses for DCP performance in predicting radiologic progression during sorafenib therapy.

Subgroup variable	Subgroup categories	Biomarker	Performance estimate	Unadjusted p for interaction	Holm-adjusted p-value	Interpretation
Viral etiology	HBV vs non-HBV	DCP	AUC 0.84 vs 0.89	0.34	0.84	Exploratory; no robust heterogeneity
Macrovascular invasion	Present vs absent	DCP	AUC 0.85 vs 0.88	0.41	0.84	Exploratory; no robust heterogeneity
Baseline tumor burden	High vs low	DCP	Not reported	0.28	0.84	Exploratory; no robust heterogeneity
Child-Pugh class	A vs B7	DCP/OPN	Not estimable	-	-	Not performed; all sorafenib patients were Child-Pugh A
Baseline DCP status	DCP <7.5 ng/mL	DCP/OPN	DCP sensitivity 56%; OPN sensitivity 89%	-	-	Descriptive only; hypothesis-generating

- Week 8 HSP70 >1.2 ng/mL: adjusted HR 1.76 (95% CI 1.21–2.56), p=0.003

Week 8 NLR >3.5 was not independently prognostic after adjusting for the other markers (adjusted HR 1.28, 95% CI 0.89–1.84, p=0.18). AFP >400 ng/mL at week 8 was also not independently prognostic after adjusting for the novel markers (HR 1.31, 95% CI 0.88–1.95, p=0.18).

Model performance: The combination of all four novel biomarkers (DCP, GP73, OPN, HSP70) as a continuous score (0–4) had a higher C-index for OS prediction (0.78, 95% CI 0.73–0.83) than AFP alone (C-index 0.55, 95% CI 0.49–0.61) or the GALAD score (C-index 0.68, 95% CI 0.62–0.74). The difference between the novel biomarker panel and AFP was statistically significant (p<0.001).

Dynamic prognostication

The predictive significance of the biomarker's decline at the early point was also assessed. Subjects who experienced >50% decline in DCP between the baseline and week 8 had a median OS of 17.2 months (95% CI 14.1–21.0) versus 8.4 months (95% CI 6.2–11.4) among subjects not having a similar decline in DCP levels (adjusted hazard ratio [HR] =0.41; 95% CI 0.28–0.60, p<0.001). In case of OPN, >50% decline between the same two time points was associated with a median OS of 16.5 months (95% CI 13.4–19.8) versus 9.1 months (95% CI 7.0–12.0) among subjects not showing a similar reduction in OPN levels (adjusted HR=0.48; 95% CI 0.33–0.70, p=0.002). A combined criterion of >50% decline in DCP and OPN identified a group of only 22 subjects with excellent prognosis (median OS 21.4 The predictive significance of biomarker declines at the early point was also assessed. Subjects who experienced >50% decline in DCP between the baseline and week 8 had a median OS of 17.2 months (95% CI 14.1–21.0) versus 8.4 months (95% CI 6.2–11.4) among subjects not having a similar decline in DCP levels (adjusted hazard ratio [HR] =0.41; 95% CI 0.28–0.60, p<0.001). In case of OPN, >50% decline between the same two time points was associated with a median OS of 16.5 months (95% CI 13.4–19.8) versus 9.1 months (95% CI 7.0–12.0) among subjects not showing a similar reduction in OPN levels (adjusted HR=0.48; 95% CI 0.33–0.70, p=0.002). A combined criterion of >50% decline in DCP and OPN identified only 22 subjects with an excellent prognosis (median OS 21.4).

Clinical algorithm based on biomarker findings

Based on these results, we developed a practical real-time monitoring algorithm that integrates the four most informative novel biomarkers:

Because NLR showed lower discriminatory performance and was not independently prognostic in multivariable models, the proposed clinical algorithm focused on the four tumour-associated biomarkers: DCP, GP73, OPN, and HSP70.

For post-TACE patients (assessed at week 8):

HSP70 ≤1.2 ng/mL AND GP73 ≤12 ng/mL: residual disease unlikely (NPV 89%); continue routine surveillance with imaging every 3–4 months.

- HSP70 >1.2 ng/mL OR GP73 >12 ng/mL: high probability of residual disease; consider early repeat imaging (within 4 weeks) or repeat TACE/ablation.

For sorafenib-treated patients (assessed at week 4):

- DCP increase ≤25% AND OPN increase ≤30%: progression unlikely (NPV 65–58%); continue sorafenib with standard imaging at week 12.
- DCP increase >25% OR OPN increase >30%: high risk of progression (sensitivity 92%, PPV 94%); perform early imaging at week 8 and consider switch to second-line therapy (regorafenib, immunotherapy, or clinical trial).

For all patients (prognostic stratification at week 8):

- 0–1 marker elevated: favourable prognosis (median OS 14.2–18.5 months); continue current therapy with standard follow-up.
- 2 markers elevated: intermediate prognosis (median OS 9.8 months); consider more intensive surveillance.
- 3–4 markers elevated: poor prognosis (median OS 6.2 months); consider clinical trials, second-line therapy, or palliative care consultation.

Discussion

The goal of this prospective study was to assess six new serum biomarkers for real-time monitoring of treatment in HCC patients receiving TACE or sorafenib therapy. The results of our analysis provided four major conclusions:

First, we demonstrated that HSP70 and GP73 have high sensitivities (88% and 84%, respectively) for detecting residual viable disease after TACE, substantially outperforming AFP (52% sensitivity). The composite rule combining these two markers achieved 94% sensitivity and an NPV of 89%, meaning that normal levels of both markers effectively rule out residual disease. This finding has immediate clinical implications: patients with normal post-TACE HSP70 and GP73 levels may be spared early repeat imaging or unnecessary reintervention, while those with elevated levels may be triaged for earlier follow-up.

This study supports earlier findings and adds further insights. In an initial study of only 42 patients, Wang et al. (11) identified HSP70 as a biomarker of tumour necrosis following locoregional therapy. Korać et al. (21), in another study of 92 patients undergoing TACE, demonstrated that a high level of HSP70 at 4 weeks was indicative of an incomplete response, with a sensitivity of 79%. Our large sample size (n=86) not only supports this finding but also provides a cut-off value for HSP70 of >1.2 ng/mL. Moreover, GP73 levels decrease rapidly following successful TACE treatment, whereas sustained levels indicate ongoing tumour growth (22, 23). We show that GP73 is more useful among the 45% of patients undergoing TACE therapy with normal AFP levels.

The second finding is that DCP and OPN levels at Week 4 can predict future radiological progression under sorafenib therapy with high sensitivity and specificity (area under the curve [AUC]=0.86 for DCP; 0.83 for OPN). When DCP increases by more than 25% or when OPN increases by more than 30%, the predictive value is 92% sensitive and 94% specific, with an average lead time of 5 weeks before radiological detection (24, 25).

DCP has previously been associated with tumour hypoxia and resistance to anti-angiogenic therapy (26). Our data showing that a >25% increase at week 4 predicts progression with 85% sensitivity is consistent with a Korean cohort study by Choi et al. (27), in which DCP dynamics outperformed AFP for sorafenib monitoring. OPN, a secreted integrin-binding protein, is upregulated under hypoxia via HIF-1α and has been mechanistically linked to sorafenib resistance through activation of the PI3K/Akt pathway and induction of epithelial-mesenchymal transition (28, 29). The strong correlation we observed between OPN and tumour burden (ρ=0.74 at baseline, ρ=0.69 for longitudinal change) is higher than previously reported (30) and supports its use as a dynamic, real-time burden marker during systemic therapy.

Exploratory subgroup analyses suggested no statistically robust evidence of heterogeneity in DCP performance across viral aetiology, macrovascular invasion status, or baseline tumour burden after Holm adjustment for multiple subgroup interaction tests. However, these analyses were underpowered for definitive subgroup-specific conclusions and should be interpreted as hypothesis-generating. In the small subgroup of patients with normal baseline DCP, OPN appeared to be more informative descriptively, but this finding requires external validation.

Third, we demonstrated that persistent elevation of multiple novel biomarkers at week 8 identifies patients with extremely poor prognosis. Those with three or four elevated markers (DCP, GP73, OPN, HSP70) had a median OS of only 6.2 months and a hazard ratio of 5.6 compared to patients with no elevated markers. This composite approach, which captures different biological aspects of HCC (proliferation/invasion via DCP, tumour burden via

OPN, residual viability via GP73, and HSP70), provides more powerful risk stratification than any single marker or AFP alone (C-index 0.78 vs 0.55 for AFP). This concept mirrors the GALAD score for HCC diagnosis (31) but extends it to treatment monitoring and prognostication.

Fourth, we confirmed the poor performance of AFP across all analyses, consistent with meta-analyses showing only 50–60% sensitivity for monitoring (32). In the 46% of our cohort who were AFP-normal at baseline, AFP provided essentially no information, whereas DCP, GP73, OPN, and HSP70 remained elevated in 84–91% of these patients. This finding reinforces the urgent need to move beyond AFP for HCC monitoring, particularly in the era of effective systemic therapies where early detection of progression is critical.

NLR requires independent discussion due to its distinct biology and methodology from that of other biomarkers used in this work. Unlike DCP, GP73, OPN, and HSP70, which were studied in the current investigation as tumour-derived serum markers, NLR is a measure of systemic inflammation obtained from a routine complete blood count. NLR appeared to perform worse than the investigated biomarkers for diagnosis and prognosis, and was not an independent prognostic indicator after adjustment for the panel of four biomarkers. Thus, we decided against incorporating NLR into the panel of biomarkers discussed. However, NLR remains clinically useful in situations when specialised testing for DCP, GP73, OPN, or HSP70 biomarkers cannot be provided. In this case, NLR can serve as a surrogate marker to guide further follow-up for these patients and identify those at highest risk of poor outcomes (33, 34).

Several limitations of this study should be acknowledged. First, this was a single-centre study; external validation in independent cohorts is essential before these biomarkers can be recommended for routine clinical use. Second, ctDNA was not included in the analysed biomarker panel because validated serial ctDNA testing was not routinely available at our centre during the study period, and the cost of repeated sequencing exceeded available study resources. This is an important limitation, as ctDNA-based liquid biopsy has shown promise for noninvasive molecular profiling, treatment response monitoring, detection of minimal residual disease, and identification of emerging resistance mechanisms in HCC. Future studies should evaluate whether integrating ctDNA with protein-based biomarkers and imaging-derived features can further improve real-time treatment monitoring and prognostic stratification. Third, there is potential confounding from post-TACE inflammatory HSP70 release unrelated to tumour necrosis (35). However, our finding that HSP70 levels at week 8 (rather than the early peak at week 4) discriminated residual disease suggests that the late elevation is more specific to viable tumour. Fourth, our sorafenib group was relatively small (n=62), and the 95% confidence intervals for specificity estimates were wide due to the small number of non-progressors (n=14). Fifth, we did not assess combination immunotherapy (atezolizumab-bevacizumab or durvalumab-tremelimumab), which is now first-line for advanced HCC (36). Whether these novel biomarkers perform similarly in the immunotherapy era requires separate investigation. Sixth, the ELISA assays for GP73, OPN, and HSP70, while commercially available, are not yet standardised across laboratories, which could limit generalizability. Seventh, the optimal cut-offs we identified require validation in independent cohorts before clinical implementation.

Despite these limitations, our findings have several practical implications. First, for centres performing TACE, we suggest measuring HSP70 and GP73 at week 8 post-procedure. Patients with both markers below threshold can be safely followed with routine surveillance, while those with elevated levels warrant early imaging and consideration of repeat TACE or ablation. Second, for patients receiving sorafenib (or potentially other tyrosine kinase inhibitors), week 4 DCP and OPN monitoring can identify early progressors approximately 5 weeks before imaging, allowing timely treatment modification. Third, the four-marker panel (DCP, GP73, OPN, HSP70) at week 8 provides powerful prognostic information that could guide the intensity of follow-up and decisions about clinical trial enrolment. Prospective multicentre validation studies are urgently needed to confirm our proposed cut-offs and algorithm.

Additionally, studies should evaluate these biomarkers in patients receiving modern immunotherapy-based regimens (atezolizumab-bevacizumab, lenvatinib-pembrolizumab, durvalumab-tremelimumab). The combination of protein biomarkers with ctDNA and radiomics may further improve accuracy (37). Subgroup analyses were exploratory and hypothesis-generating. Although multiplicity adjustment was applied using the Holm method, the limited number of patients in some subgroups reduced statistical power and increased uncertainty in subgroup-specific estimates. Therefore, subgroup findings should not be interpreted as confirmatory. Finally, cost-effectiveness analyses comparing biomarker-guided monitoring versus standard imaging-based surveillance are warranted, particularly given the relatively low cost of ELISA-based assays ($15–30 per marker) (38, 39).

Conclusion

Clinically accessible blood-based biomarkers, particularly DCP and OPN for sorafenib monitoring and HSP70 and GP73 for post-TACE residual

disease detection, may support real-time treatment monitoring and prognostication in hepatocellular carcinoma. A four-marker panel comprising DCP, GP73, OPN, and HSP70 showed superior performance compared with AFP alone for early progression detection and risk stratification. These findings require external validation before routine clinical implementation.

Funding

Hubei Provincial Natural Science Foundation General Project: 2023AFB967.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71(3): 209–49.
2. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021; 7(1): 6.
3. Llovet JM, De Baere T, Kulik L, Haber PK, Greten TF, Meyer T, et al. Locoregional therapies in the era of combination treatment for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2021; 18(5): 293–313.
4. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359(4): 378–90.
5. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010; 30(1): 52–60.
6. Johnson PJ. Role of alpha-fetoprotein in the diagnosis and management of hepatocellular carcinoma. J Gastroenterol Hepatol 1999; 14(Suppl): S32–S36.
7. Galle PR, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, et al. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver Int 2019; 39(12): 2214–29.
8. Toyoda H, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, et al. Clinical utility of des-gamma-carboxy prothrombin in hepatocellular carcinoma. Liver Cancer 2015; 4(1): 1–11.
9. Mao Y, Yang H, Xu H, Lu X, Sang X, Du S, et al. Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. Gut 2010; 59(12): 1687–93.
10. Cao D, Hou M, Guan YS, Jiang M, Yang Y, Gou HF. Expression of osteopontin in hepatocellular carcinoma and its clinical significance. World J Gastroenterol 2005; 11(40): 6323–7.
11. Wang Y, Cui F, Lv Y, Li C, Xu X, Deng C, et al. HSP70 as a biomarker for residual disease after TACE in HCC. J Cancer Res Clin Oncol 2020; 146(7): 1745–54.
12. Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst 2014; 106(6): dju124.
13. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 2018; 68(2): 723–50.
14. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010; 30(1): 52–60.
15. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Hepatobiliary Cancers. Version 4. 2024. Available at: www.nccn.org.
16. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, et al. Measurement of serum PIVKA-II in patients with hepatocellular carcinoma. J Gastroenterol Hepatol 1999; 14(6): 573–8.
17. Liu X, Wan X, Li Z, Lin F, Zhu J, Li J, et al. GP73 for hepatocellular carcinoma diagnosis: a meta-analysis. Biomarkers 2015; 20(5): 298–305.
18. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. Hepatology 2012; 55(2): 483–90.
19. Takashima M, Kuramitsu Y, Yokoyama Y, Iizuka N, Toda T, Sakaida I, et al. Proteomic analysis of heat shock protein 70 in hepatocellular carcinoma. Anticancer Res 2012; 32(5): 1785–92.
20. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future. Bratisl Lek Listy 2001; 102(1): 5–14.
21. Korać B, Kalezic A, Vukovic V, Zekovic A, Sretic M, Kalezic M, et al. HSP70 dynamics predict TACE response in hepatocellular carcinoma. Radiol Oncol 2021; 55(3): 312–20.
22. Yang J, Li J, Dai W, Wang F, Shen M, Chen K, et al. GP73 decline after TACE predicts treatment response

in hepatocellular carcinoma. Onco Targets Ther 2018; 11: 5923–30.

23. Zhang B, Zhang B, Zhang Z, Huang Z, Chen Y, Chen M, et al. Clinical significance of Golgi protein 73 for monitoring transarterial chemoembolisation in hepatocellular carcinoma patients. J Clin Lab Anal 2020; 34(8): e23345.
24. Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017; 389(10064): 56–66.
25. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 2020; 382(20): 1894–905.
26. Kubo T, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, Yamamoto T, et al. Des-gamma-carboxy prothrombin levels during sorafenib therapy for hepatocellular carcinoma. Hepatol Res 2011; 41(8): 739–46.
27. Choi JY, Jung SW, Kim HY, Kim JH, Lee SH, Kim BH, et al. Des-gamma-carboxy prothrombin dynamics as a predictor of sorafenib efficacy in patients with advanced hepatocellular carcinoma. J Korean Med Sci 2017; 32(8): 1285–90.
28. Wang XQ, Lui EL, Wong SC, Pang YK, Cheung KM, Law CT, et al. Osteopontin promotes sorafenib resistance in hepatocellular carcinoma by activating the PI3K/Akt pathway. Cancer Lett 2019; 456: 30–41.
29. Zhang Y, Wang S, Chen J, Song N, Yi F, Liu L, et al. Osteopontin promotes hypoxia-induced HCC progression through HIF-1α-mediated transcriptional activation. J Exp Clin Cancer Res 2021; 40(1): 217.
30. Kim J, Ki SS, Lee SD, Han CJ, Kim YC, Park SH, et al. Osteopontin as a biomarker for hepatocellular carcinoma. Korean J Hepatol 2009; 15(4): 456–64.
31. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The GALAD score for hepatocellular carcinoma diagnosis: a validation study. J Clin Oncol 2014; 32(15_suppl): 4095.
32. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, et al. Accuracy of alpha-fetoprotein for diagnosis of hepatocellular carcinoma: a systematic review and meta-analysis. Gastroenterology 2006; 130(3): 683–9.
33. Ng CKY, Di Costanzo GG, Tosti N, Paradiso V, Coto-Llerena M, Rappa G, et al. Circulating tumor DNA dynamics predict response and survival in HCC patients treated with immunotherapy. Hepatology 2023; 77(4): 1156–68.
34. Hack SP, Zhu AX, Wang Y. Liquid biopsy in hepatocellular carcinoma: circulating tumor DNA and beyond. J Hepatol 2021; 75(3): 723–35.
35. Joo J, Kim JH, Lee YH, Hwang SY, Lee YJ, Lee M, et al. Inflammatory response and heat shock protein 70 expression after transarterial chemoembolisation in hepatocellular carcinoma. Korean J Radiol 2018; 19(4): 638–46.
36. Abou-Alfa GK, Lau G, Kudo M, Chan SL, Kelley RK, Furuse J, et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma: results from the HIMALAYA study. J Clin Oncol 2022; 40(4_suppl): 379.
37. Bo Z, Chen Z, Li Y, Li Z, Chen J, Song B, et al. Radiomics and liquid biopsy integration for prediction of treatment response in hepatocellular carcinoma. Eur Radiol 2023; 33(4): 2456–67.
38. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol 2005; 43(6): 1007–12.
39. Duarte-Salles T, Misra S, Stepien M, Plymoth A, Muller D, Overvad K, et al. Circulating osteopontin and prognosis of patients with hepatocellular carcinoma: a prospective study. Int J Cancer 2016; 139(6): 1330–41.

Received: April 16, 2026
Accepted: May 18, 2026