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High pretreatment static and dynamic alpha-fetoprotein values predict reduced overall survival in hepatocellular carcinoma

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Abstract

Background: Hepatocellular carcinoma is one of the most lethal cancers worldwide. Novel prognostic and/or predictive biomarkers are urgently needed to improve patient management. Alpha-fetoprotein (AFP) is a well-established and widely used biomarker for hepatocellular carcinoma. However, diagnostic accuracy of static AFP values is limited and the clinical potential is a matter of ongoing scientific discussion.

Objective: We here evaluated the prognostic impact of pretreatment static and dynamic AFP variables on overall survival of hepatocellular carcinoma patients in a Western cohort.

Methods: Patients with confirmed hepatocellular carcinoma ($n = 809$) treated at the Johannes Gutenberg University Mainz between 1998 and 2014 and two available pretreatment AFP-values (AFP-slope) were retrospectively analysed. Clinicopathological baseline parameters, pretreatment static values and AFP-slope were assessed. Prognostic impact was determined by Kaplan–Meier analyses and Cox regression models.

Results: High static and dynamic AFP variables prior to therapy were associated with reduced survival rates of hepatocellular carcinoma patients. Several known clinical parameters such as Child–Pugh B ($p < 0.01$) and C stage ($p < 0.001$), portal vein thrombosis ($p < 0.001$) and extrahepatic spread ($p < 0.001$) were confirmed as independent predictors for overall survival. Addition of static and/or dynamic AFP variable resulted in higher time-dependent area under the curves. Notably, in patients with more favourable prognosis, AFP-slope prior to therapy was a slightly stronger predictor for overall survival compared with static AFP values.

Conclusion: Static and dynamic AFP variables prior to therapy are predictive for overall survival of hepatocellular carcinoma patients. Addition of AFP-slope to established prognostic parameters might improve prognostic classification for a

subgroup of hepatocellular carcinoma patients with preserved liver function and without portal vein tumour thrombosis.

KEYWORDS
alpha-fetoprotein, biomarker, hepatocellular carcinoma, prognosis, survival

Key Summary

- Alpha-fetoprotein (AFP) is the most commonly used biomarker for hepatocellular carcinomas (HCCs), but accuracy of static and dynamic AFP values is limited and the prognostic significance is under debate.
- High static and dynamic AFP variables prior to therapy are associated with reduced survival rates of HCC patients across different tumour stages and treatment modalities.
- In patients with more favourable prognosis, AFP-slope prior to therapy was a better predictor for overall survival in comparison with static AFP values.
- Addition of AFP-slope to established prognostic parameters might improve prognostic classification for a subgroup of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and leading cause of death in cirrhotic patients.[1] HCC is the sixth most common cancer worldwide and shows a rising incidence in the Western world. Between 2005 and 2015 HCCs were the second leading cause of cancer related years of life lost.[2] The majority of HCCs develop on the background of chronic liver injury and, most commonly, liver cirrhosis. Predisposing risk factors include chronic viral hepatitis, alcohol abuse and metabolic disorders.[3] Due to an impaired liver function and late diagnosis, only a minority of patients is amenable to curative treatment such as resection, orthotopic liver transplantation (OLT) or local ablation. More than two-thirds of HCC patients are diagnosed in intermediate and advanced stages of disease, when therapeutic options are limited to locoregional and systemic therapies.[4–6] Despite new approaches in interventional and systemic treatment modalities, prognosis of HCC remains decidedly poor and novel biomarkers for accurate prediction of prognosis as well as selection of optimal treatment strategies are urgently needed to improve patient management.[4] Alpha-fetoprotein (AFP) is the most widely used and validated biomarker for HCCs since the 1970s. Routine clinical use of AFP in HCC diagnosis and surveillance has been under extensive debate over recent years due to a low sensitivity and specificity, especially in detecting small HCCs.[7,8] However, due to improved imaging modalities for HCC diagnosis, routine AFP measurements in HCC surveillance are no longer endorsed by HCC guidelines of the American Association for the Study of Liver Diseases (AASLD)[5] and the European Association for the Study of the Liver (EASL).[4]

However, despite the low accuracy in a diagnostic setting, the value of AFP in prediction of overall survival (OS) has been repeatedly demonstrated and measurement is still implemented in most established prognostic staging algorithms.[5,9] Especially with respect to patient allocation for liver transplantation, AFP seems to possess considerable predictive value.[10]

Interestingly, several recent studies further delineate a strong impact on both recurrence rates and OS when AFP *dynamics* rather than static values are assessed prior to liver transplantation.[11–15] Unlike single AFP values, AFP trends and changes might better reflect the biological traits of tumours. However, clinical potential of these dynamic AFP-slopes as prognostic and/or predictive markers needs to be more precisely defined. We here evaluated the prognostic role of pretreatment serum static as well as dynamic AFP variables on OS of 809 patients with HCC in a German cohort and investigated their predictive significance across different treatment modalities including OLT, HCC resection, transarterial chemoembolization (TACE) and systemic therapy.

MATERIALS AND METHODS

Demographics

Patients diagnosed with HCC at the University Medical Centre of the Johannes Gutenberg University Mainz between 1998 and 2014 were included in this retrospective analysis (Mainz cohort). HCC diagnosis was based on histological or radiologic findings according to AASLD/EASL criteria.[4,5,16] End of follow-up was 30 June 2017. A total of 1706 patients were identified from our HCC registry. Exclusion criteria were: (i) less than two AFP values or (ii) an interval between two AFP measurements of less than 7 days or more than 365 days, (iii) patients with mixed hepato/-cholangio cellular carcinoma as (Figure 1). Fifty patients were censored due to loss to follow-up. Baseline parameters concerning patient status (sex, age, performance status), tumour characteristics (tumour size, tumour number, extrahepatic spread, Barcelona Clinic Liver Cancer [BCLC] stage), Child–Pugh score, aetiology of underlying liver disease, presence of portal vein thrombosis, as well as static pretreatment AFP values and AFP dynamics within one year of the remaining 809 patients were

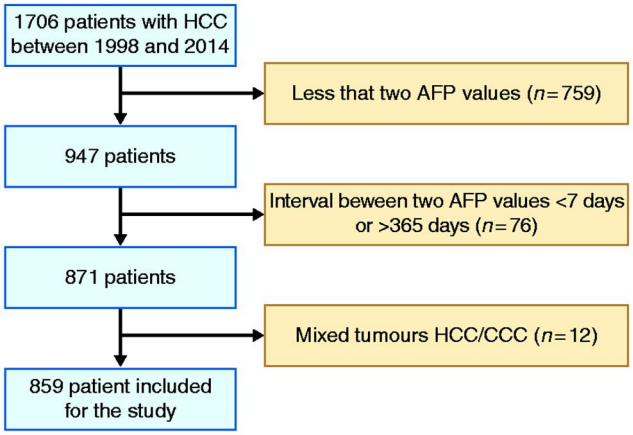

FIGURE 1 Flow chart of the study. AFP, alpha-fetoprotein; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma

collected from a prospectively maintained database of our clinical registry unit.[17] The study was approved by the responsible ethics committee for the retrospective analysis of clinical data.

Tumour characteristics

All patients were classified according to the BCLC classification.[18] Eastern Co-operative Oncology Group performance status and treatment was retrieved from medical records or records from the clinical registry. Tumour size, extrahepatic spread and presence of portal vein thrombosis was documented based on resected specimen or radiological assessment as applicable.

AFP cut-offs and slope calculation

Laboratory results, including AFP values prior to therapy, were collected from the hospital information system and patient records. Positive AFP values were defined above a cut-off of 8.8 ng/ml. For Cox regression analyses an AFP cut-off over 400 ng/ml was chosen, since this is the most widely used cut-off in existing staging systems.[9,19,20] Absolute AFP-slope was defined as the difference of two consecutive pretreatment measurements divided by the time between measurements, thus obtaining daily increment/decline: AFP-slope (ng/ml/day) = $(AFP_2 - AFP_1)/T$ (time in days between the two AFP measurements).

Statistical analyses

Statistical analyses were conducted with R (The R Project for Statistical Computing, version 3.4.2; www.r-project.org) and SAS 9.4. Univariate and multivariate Cox regression analyses were performed to identify prognostic factors for OS of HCC patients using PROC PHREG from SAS. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test using Prism GraphPad. *p* Values less than 0.01 were considered statistically significant.

RESULTS

Study population and pretreatment AFP levels and dynamics

A total of 809 patients were included in the study after applying the above-mentioned inclusion criteria. Median time of follow-up was 453 (interquartile range: 1269) days. Baseline characteristics of patients are presented in Table 1. Patients of the study cohort were mainly men, with a median age of 65 years (range: 16–90). The majority of patients (88.4%) presented with liver cirrhosis in Child–Pugh stages A (40%), B (34.5%) and C (13.8). Main aetiology of underlying liver disease was alcohol abuse (39.2%) followed by hepatitis C (24.7%) and hepatitis B infection (12.2%). BCLC stages were represented as 34.9% classified as stage BCLC-A, 22.2% BCLC-B, 28.6% BCLC-C and 14.3% BCLC-D. The majority of patients (94.2%) presented in a good performance status (0–1). A portal vein tumour thrombosis has been found in 20.6% of patients and extrahepatic spread in 8.0%. Predictive impact of static and dynamic AFP variables has been studied across several treatment approaches. Curative therapies included OLT (4.2%), ablation (3.2%) and resection (12.9%) of HCCs. Most patients were treated with TACE (58.7%). Patients with advanced stages of disease received systemic therapy (8.0%) or best supportive care (10.2%). Static AFP values were measured at two different time points, that is, AFP_1 and AFP_2 prior to therapy. For 717 patients (88.6%) the two values were measured within 7–90 days, for 61 patients (7.5%) within 91–180 days and for 31 patients (3.8%) within 181–365 days (Table 1; Figure S1a online). While AFP_1 was collected either during HCC-surveillance or during diagnostic clarification for HCC, AFP_2 was collected mainly during diagnostic work-up for HCC (Figure S1b,c). Intervals between each measurement and start of therapy were similar across different treatment modalities (Table 1). Median AFP level at baseline (AFP_1) within the study population was 26 ng/ml spanning a wide range from 1 to 411.417 ng/ml as shown in Table 1. Two hundred and forty-seven patients (30.5%) initially presented with AFP values below the internal laboratory thresholds of 8.8 ng/ml. Values below 8.8 are considered negative according to our internal laboratory standard. From the 562 (69.5%) patients with positive AFP values 224 (27.7%) had high AFP values (>400 ng/ml). At the second time point 223 (28.8%) of registry unit.[17] The study was approved by the responsible ethics committee for the retrospective analysis of clinical data.

Tumour characteristics

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TABLE 1 Patient and tumour characteristics

Characteristic	
Total number	809
Median age in years	65
Gender, *n* (%)	
Male	667 (82.4)
Female	142 (17.6)
Aetiology of liver disease, *n* (%)	
Alcohol abuse	317 (39.2)
HCV	200 (24.7)
HBV	99 (12.2)
NASH	41 (5.1)
Haemochromatosis	29 (3.6)
Others	123 (15.2)
BCLC at initial diagnosis, *n* (%)	
A	282 (34.9)
B	179 (22.2)
C	232 (28.6)
D	116 (14.3)
ECOG PST, *n* (%)	
0–1	762 (94.2)
2	36 (4.4)
3	5 (0.6)
4	5 (0.6)
Liver cirrhosis, *n* (%)	
Absent	94 (11.6)
Present	715 (88.4)
Child–Pugh score, *n* (%)	
A	324 (40.0)
B	279 (34.5)
C	112 (13.8)
Extrahepatic spread, *n* (%)	65 (8.0)
Portal vein thrombosis, *n* (%)	167 (20.6)
Treatment, *n* (%)	
OLT	34 (4.2)
Ablation	26 (3.2)
Resection	105 (12.9)
TACE	475 (58.7)
Systemic therapy	65 (8.0)
BSC	83 (10.2)
Others	21 (2.5)
AFP_1	
Median	26 (1–411,417)
Positive, *n* (%)	562 (69.5)
Negative, *n* (%)	247 (30.5)
>400 ng/ml, *n* (%)	224 (27.7)
<400 ng/ml, *n* (%)	585 (72.3)
AFP_2, *n* (%)	
Positive	576 (71.2)
Negative	233 (28.8)
>400 ng/ml	253 (31.3)
<400 ng/ml	556 (68.7)
AFP-slope	
Median	0.051 (−676 to 9228)
≥0.051	405 (50.1)
<0.051	404 (49.9)
Median interval between AFP_1 and AFP_2 in days	
Interval 7–90 days	717 (88.6)
Interval 91–180 days	61 (7.5)
Interval 181–365 days	31 (3.8)
Median interval between AFP_1 and start of treatment in days (range)	60 (7–461)
OLT	128 (10–374)
Ablation	68 (9–256)
Resection	55 (8–361)
TACE	56 (7–394)
Systemic therapy	54 (12–397)
BSC	72 (9-461)
Others	58 (8-327)
Median interval between AFP_2 and start of treatment in days (range)	15 (−1 to 361)
OLT	50 (0–270)
Ablation	18 (0–51)
Resection	12 (−1 to 145)
TACE	13 (0–182)
Systemic therapy	16 (0–257)
BSC	16 (0–363)
Others	16 (0–91)

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; BSC, best supportive care; ECOG PST, Eastern Co-operative Oncology Group performance status; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; OLT, orthotopic liver transplantation; TACE, transarterial chemoembolization.

treatment was retrieved from medical records or records from the clinical registry. Tumour size, extrahepatic spread and presence of portal vein thrombosis was documented based on resected specimen or radiological assessment as applicable.

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AFP-slope improves prediction for subgroup of HCC-patients with preserved liver function and without PVTT

In order to determine whether high static and/or dynamic AFP variables prior to therapy are independent predictors for OS of HCC patients, multivariate analyses were performed including (i) a full model with all parameters as well as models, (ii) without any AFP variable and (iii) with only AFP_1, AFP_2 or the AFP-slope (Tables S2a,b and S3a–e).

First, we investigated the whole study cohort ($N = 809$). Several well-known clinical parameters such as the Child–Pugh score B (HR: 1.49; CI: 1.13–2.00, $p < 0.01$) and C (HR: 2.71; CI: 1.87–3.97, $p < 0.001$), portal vein tumour thrombosis (HR: 3.67; CI: 2.964.52, $p < 0.001$) and extrahepatic spread (HR: 1.87; CI: 1.39–2.42,

TABLE 2 Univariate analyses of prognostic factors for overall survival of hepatocellular carcinoma patients

Variables	Details	Hazard ratio	95% CI	*p*-Value
Child–Pugh score	Child–Pugh A vs. no LCI	0.88	0.69–1.13	0.302
	Child–Pugh B vs. no LCI	1.23	0.96–1.60	0.103
	Child–Pugh C vs. no LCI	**2.24**	**1.68–3.01**	**<0.001**
AFP-slope	**AFP-slope > median**	**1.87**	**1.60–2.18**	**<0.001**
AFP_1 > 400 ng/ml	**AFP_1 > 400 ng/ml**	**1.85**	**1.57–2.18**	**<0.001**
AFP_2 > 400 ng/ml	**AFP_2 > 400 ng/ml**	**1.96**	**1.67–2.29**	**<0.001**
Aetiology	Alcohol	0.97	0.82–1.16	0.759
	Viral	**0.78**	**0.65–0.93**	**0.005**
	NASH	0.97	0.68–1.35	0.866
Tumour size	Per maximum tumour size increase 1 mm	1.00	1.00–1.01	0.023
	Per maximum tumour size increase 10 mm	1.03	1.00–1.05	0.023
PST > 0/1		**2.12**	**1.53–2.86**	**<0.001**
PVTT		**4.16**	**3.43–5.01**	**<0.001**
Extrahepatic spread		**2.58**	**1.96–3.32**	**<0.001**

Abbreviations: AFP, alpha-fetoprotein; CI, confidence interval; LCI, liver cirrhosis; NASH, nonalcoholic steatohepatitis; PST, performance status; PVTT, portal vein tumour thrombosis.

$p < 0.001$) could be confirmed as strong independent factors for OS of patients with HCC in multivariate analyses (Table S3a). Adding pretreatment static and/or dynamic AFP variables to the models resulted in higher time-dependent AUCs and higher values for Harrell's concordance index (HC), indicating an improved predictive ability for OS (Figure 4 and Table S2a,b). However, difference between static and dynamic AFP variables were not remarkable (Table S2a,b: HC_{AFP1} 0.694 ± 0.009; HC_{AFP2} 0.697 ± 0.009; HC_{AFP}-slope 0.702 ± 0.009).

We next excluded strong independent predictors such Child–Pugh C liver cirrhosis and PVTT in order to analyse patients with more favourable prognoses ($n = 569$), that is, patients with preserved liver function and without PVTT (Tables S4a,b and S5a–e). In this subgroup of patients, we observed slightly higher time-dependent AUCs for the model including AFP-slope in comparison with models including only static AFP values (Table S4a,b; HC_{AFP1} 0.617 ± 0.014; HCafp2 HC 0.621 ± 0.014; HCAFP-slope HC 0.631 ± 0.014). Therefore, in selected patients an AFP-slope prior to therapy is a stronger predictor for OS compared with static AFP values (Figure 5a,b).

DISCUSSION

In the presented study, we evaluated the predictive value of static and dynamic AFP variables prior to therapy. We demonstrate that across all commonly used treatment modalities covering the full range of BCLC stages, high pretreatment AFP variables are associated with worse clinical outcome for HCC patients. Notably, integration of AFP-slopes rather than static AFP values in multivariate models are reliable predictors for OS of a subgroup of patients with HCC without PVTT and with preserved liver function.

AFP is a well-established and widely used biomarker for HCC.[21] However, its use in clinical practice for HCC diagnosis and surveillance is still a matter of ongoing discussion due to limited accuracy of static AFP values.[8,22] Universal agreements of AFP cut-off values do not exist and the use of AFP is currently not recommended by major clinical associations for surveillance or for treatment stratification.[4,5,16,23]

However, over recent years the use of AFP as a predictor for prognosis, recurrence and survival with respect to OLT gained increasing attention.[10] Due to the shortage of liver donors, strong selection criteria are required to stratify patients before OLT. Until now only tumour size and number according to Milan criteria are used as a selection tool for OLT in most Western countries.[24] However, these criteria inaccurately predict the tumour biology and aggressiveness[25,26] and a combination with additional factors might be superior in selecting the most suitable patients. Therefore, identification of novel, noninvasive and reproducible serum biomarkers is highly desirable.[16] Recent studies that focused on AFP demonstrated a shorter OS after OLT for patients with high preoperative AFP values. However, a wide range from 8.5 to 1000 ng/ml has been observed to be associated with OS or recurrence rate and a common threshold has not been established yet.[27–31]

We here evaluated the prognostic impact of pretreatment static and dynamic AFP variables across several treatment modalities and disease stages and demonstrate that indeed high static AFP values as well as a dynamic AFP-slope prior to therapy have prognostic value in uni- and multivariate analyses. Patients with high AFP values over 400 ng/ml as well as with high pretreatment AFP-slopes (>median)

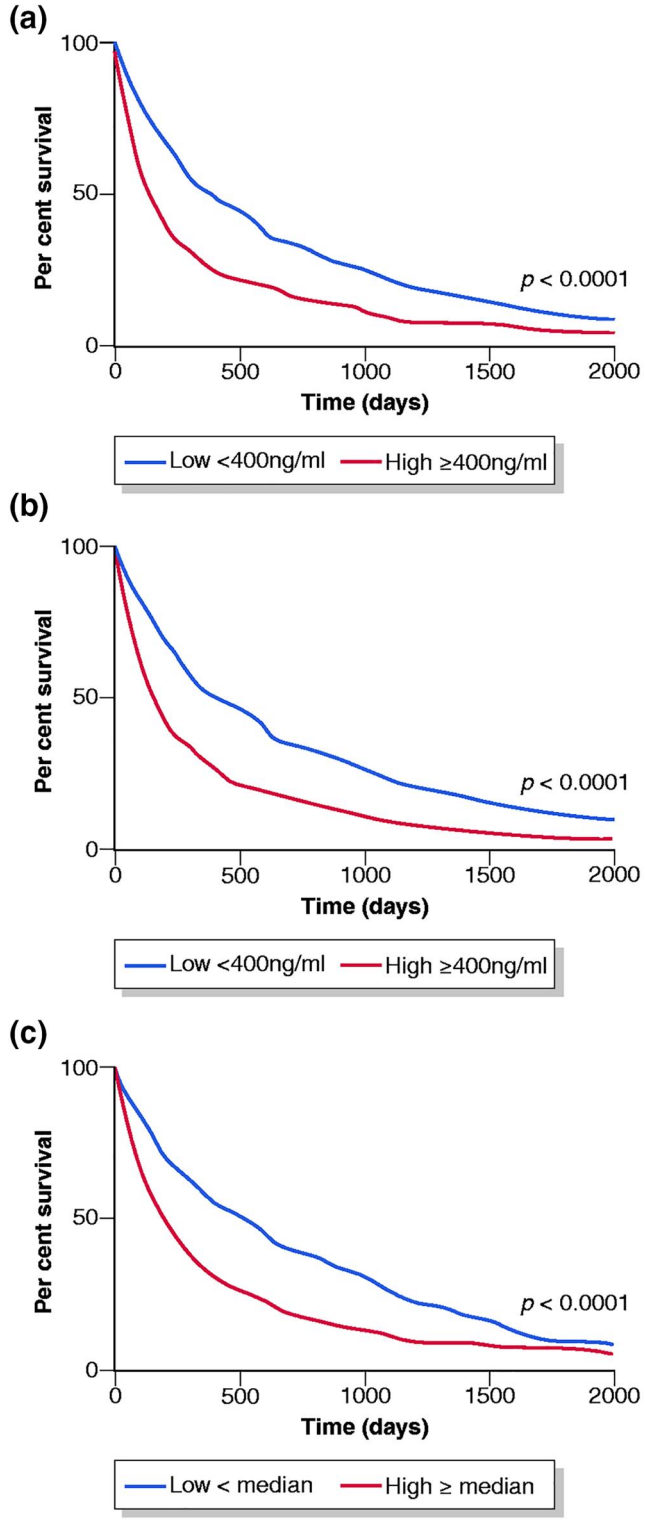

FIGURE 2 Survival probability of 809 patients with low (<400 ng/ml) or high (>400 ng/ml) AFP values at timepoint 1 (a) and timepoint 2 (b) and with low or high AFP-slope divided by the median in (c); log-rank test: $p < 0.001$; time in days. AFP, alpha-fetoprotein

had significantly reduced survival rates compared with patients below the cut-offs (Figure 2). We further confirmed the significance of other well-known clinical parameters such as Child–Pugh

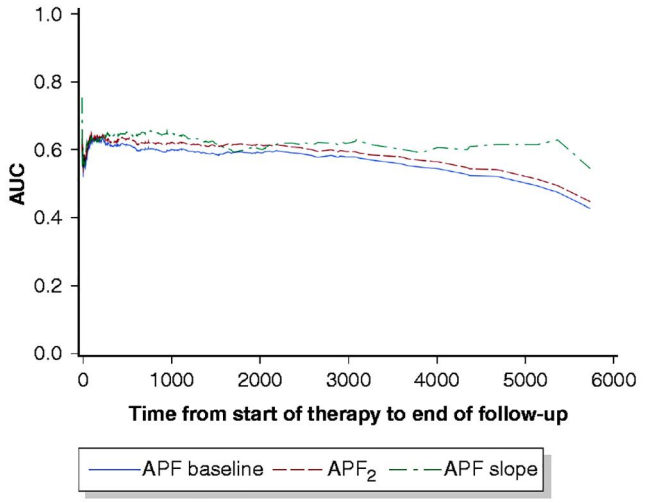

FIGURE 3 Time-dependent area under the curve for pretreatment static AFP values of univariate analyses. AFP, alpha-fetoprotein; AUC, area under the curve

scoring, PVTT and extrahepatic tumour spread for OS of patients with HCC.

Previously studies mainly on dynamic AFP variables focused only on OLT. Lai et al.[14] recently demonstrated that an AFP-slope greater than 15 ng/ml/month and radiological progression according to mRECIST were unique independent risk factors for HCC recurrence and death after OLT. Survival rates of patients outside the Milan criteria without risk factors showed similar outcome compared with patients inside the MC without risk factors and were even significantly superior to patients inside the MC with risk factors. The authors conclude that integration of *dynamic* biological and morphological tumour characteristics into classic HCC staging tools could be more effective to accurately select patients for OLT.[14] Furthermore, Vibert et al.[12] also showed that an AFP progression (>15 ng/ml/month) was more relevant than a static AFP value in predicting OLT outcomes in a cohort of 153 HCC patients. A Canadian study ($N = 144$) confirmed that a rising AFP-slope (>0.1 ng/ml/day) was a faithful and independent predictor of microvascular invasion and HCC recurrence after OLT.[13] Our results are in agreement with the aforementioned studies. We confirmed that an AFP-slope is associated with OS of HCC patients before major therapeutic approaches including OLT, HCC-resection, TACE and sorafenib therapy, that is, across all BCLC stages. To address clinical relevance of static as well as dynamic AFP values, we performed several multivariate models and showed that, if a strong negative predictor such as a Child–Pugh C cirrhosis or a PVTT is present, incorporation of AFP values does only slightly improve the AUC over time (Figure 4 and Tables S2 and S3). In this context, AFP-slopes were not superior to static AFP values for patients harbouring a poor clinical outcome. We next investigated a subgroup of patients with more favourable prognosis, that is, preserved liver function without PVTT (Figure 5 and Tables S4 and S5). In these selected patients inclusion of AFP--slopes in multivariate models reached higher time-dependent AUCs

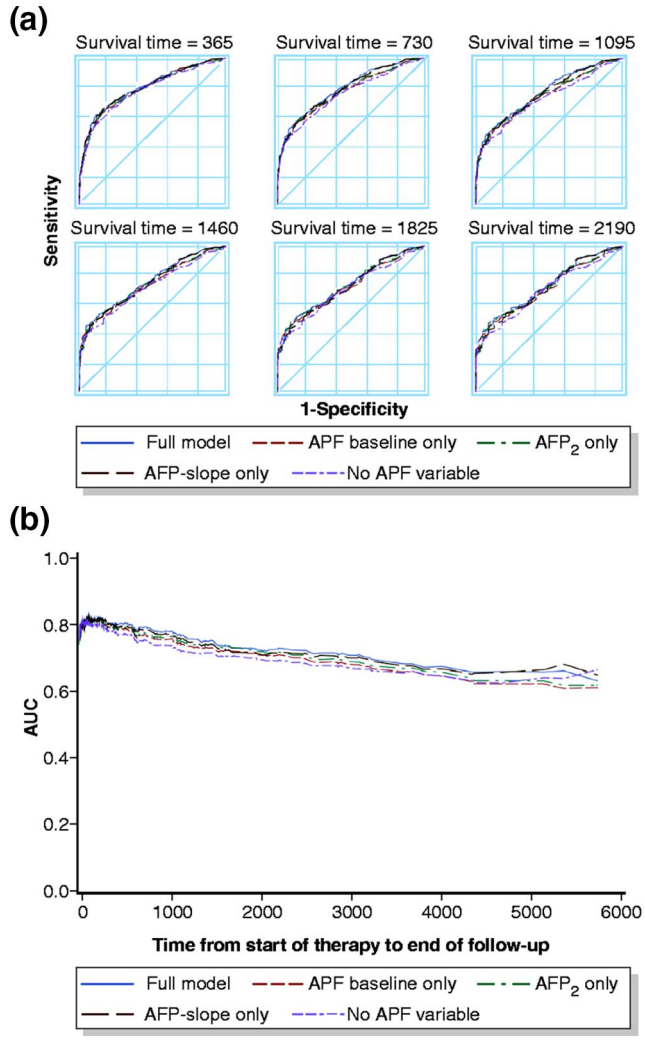

FIGURE 4 Receiver operating curve (a) and time-dependent area under the curve (b) for analysed models of multivariate analyses including all patients (N = 809). AFP, alpha-fetoprotein; AUC, area under the curve

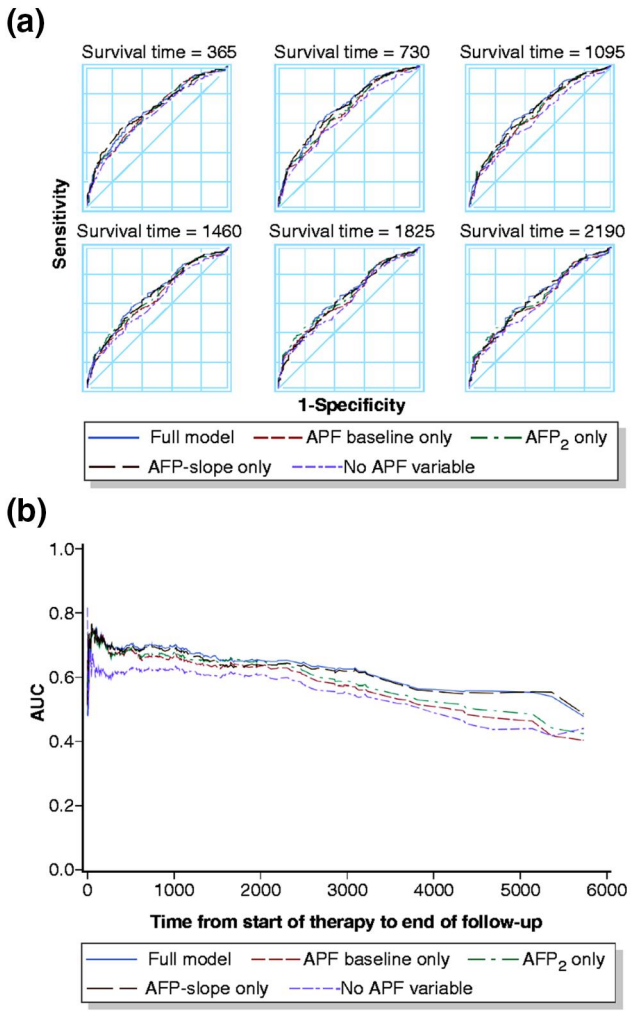

FIGURE 5 Receiver operating curve (a) and time-dependent area under the curve (b) for analysed models of multivariate analyses including patients with preserved liver function and without portal vein tumour thrombosis (n = 569). AFP, alpha-fetoprotein; AUC, area under the curve

compared with inclusion of static AFP values in multivariate analyses (Figure 5 and Tables S4 and S5). These findings emphasise that a significant AFP progression prior to therapy is a valid marker for an aggressive tumour biology and worse outcome for selected HCC patients. Therefore, dynamic AFP-slope measurements might reflect more faithfully than static AFP values the natural cause of the disease in patients with more favourable prognoses. Notably, consistent with previous studies, the predictive ability of static or dynamic AFP variables alone is limited (AUCs ranging from 0.59 to 0.64). Similar data has been obtained in recent publications with AUCs ranging from 0.557 to 0.727, sensitivities and specificities of 30.8%–63% and 58%–92.3%, respectively.[11–14]

Therefore, further refinement and combination with other markers seem necessary. But AFP-slopes might be a helpful addition to other established staging tools. The BALAD-score, a new staging system, which is exclusively based on serum markers (bilirubin, albumin, AFP-L3, AFP and des-y-carboxyprothrombin) has recently been introduced.[9,20] The score is, therefore, not only highly reproducible and noninvasive, but also objective. Overall, the BALAD-score showed good discriminative ability across different populations of HCC patients.[32,33] Nevertheless, our findings as well as aforementioned studies strengthen that *changes* of biomarkers instead of static values might possess superior classification abilities for selected patient subgroups and should be considered as an integrative classification and/or selection tool to refine existing staging systems.

Of note, the retrospective, single centre design of our study as well as limited numbers of patients in some subgroups are important limitations. Prospective studies performed on independent cohorts are needed to validate AFP-slopes prior to therapy as an improved selection and classification tool for the patient's prognosis and treatment selection. Implementation of least-squares-based slopes including sequential AFP measurements over time could further refine AFP-slopes and reduce noise of random fluctuations.

In conclusion, we showed that high static AFP values as well as high AFP-slopes prior to therapy are strongly associated with poor prognosis of HCC patients across different treatment modalities and BCLC stages. Importantly, for patients with preserved liver function and without PVTT, a pretreatment AFP-slope improved prediction for patients' survival in comparison with static AFP values. Therefore, integration of AFP dynamics might be a promising approach to improve prognostic scoring systems for HCC subgroups and help to refine patient selection for most suitable therapies. Prospective evaluation and validation in independent patient cohorts of the concept and of the ideal interval of the sequential AFP tests without interfering with a timely start of therapy seems warranted.

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CONFLICT OF INTERESTS

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Collection of data: Carolin Czauderna, Sandra Koch, Lukas Pilz, Sophia Heinrich, Gerd Otto, Jens Mittler, Hauke Lang, Roman Kloeckner, Christoph Düber, Martin F. Sprinzl, Marcus A. Worns, Peter R. Galle, Jens U. Marquardt, Arndt Weinmann. *Analysed the data*: Carolin Czauderna, Irene Schmidtmann, Sandra Koch, Lukas Pilz, Arndt Weinmann, Jens U. Marquardt. *Wrote the paper*: Carolin Czauderna, Irene Schmidtmann, Jens U. Marquardt, Arndt Weinmann. All authors discussed the results and critically commented on the manuscript. All authors had access to the study data and reviewed and approved the final manuscript.

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REFERENCES

1. Njei B, Rotman Y, Ditah I, et al. Emerging trends in hepatocellular carcinoma incidence and mortality. Hepatology. 2015;61:191–9.
2. Global Burden of Disease Cancer Collaboration; Fitzmaurice C, Allen C, et al. Global, regional, and National Cancer Incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life- years for 32 cancer groups, 1990 to 2015: a systematic analysis for the Global Burden of Disease Study. JAMA Oncol. 2016;3:524–48.
3. Fujiwara N, Friedman SL, Goossens N, et al. Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol. 2017;68:526–49.
4. European Association for the Study of the Liver; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56:908–43.
5. Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–2.
6. Worns MA, Galle PR. Hepatocellular carcinoma in 2017: two large steps forward, one small step back. Nat Rev Gastroenterol Hepatol. 2018;15:74–6.
7. Chang TS, Wu YC, Tung SY, et al. Alpha-fetoprotein measurement benefits hepatocellular carcinoma surveillance in patients with cirrhosis. Am J Gastroenterol. 2015;110:836–44.
8. Song PP, Xia JF, Inagaki Y, et al. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. World J Gastroenterol. 2016;22:262–74.
9. Kinoshita A, Onoda H, Fushiya N, et al. Staging systems for hepatocellular carcinoma: current status and future perspectives. World J Hepatol. 2015;7:406–24.
10. Lai Q, Iesari S, Melandro F, et al. The growing impact of alpha-fetoprotein in the field of liver transplantation for hepatocellular cancer: time for a revolution. Transl Gastroenterol Hepatol. 2017;2:72.
11. Han K, Tzimas GN, Barkun JS, et al. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. Can J Gastroenterol. 2007;21:39–45.
12. Vibert E, Azoulay D, Hoti E, et al. Progression of alpha-fetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. Am J Transplant. 2010;10:129–37.
13. Dumitra TC, Dumitra S, Metrakos PP, et al. Pretransplantation alpha-fetoprotein slope and Milan criteria: strong predictors of hepatocellular carcinoma recurrence after transplantation. Transplantation. 2013;95:228–33.
14. Lai Q, Avolio AW, Graziadei I, et al. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. Liver Transplant. 2013;19:1108–18.
15. Clavien PA, Lesurtel M, Bossuyt PM, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. Lancet Oncol. 2012;13:e11–22.
16. Heimbach JK, Kulik LM, Finn R, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2017;67:358–80.
17. Weinmann A, Koch S, Niederle IM, et al. Trends in epidemiology, treatment, and survival of hepatocellular carcinoma patients between 1998 and 2009: an analysis of 1066 cases of a German HCC Registry. J Clin Gastroenterol. 2014;48:279–89.
18. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19:329–38.
19. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28:751–5.
20. Toyoda H, Kumada T, Osaki Y, et al. Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. Clin Gastroenterol Hepatol. 2006;4:1528–36.
21. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis. 2001;5:145–59.
22. Forner A, Reig M, Bruix J. Alpha-fetoprotein for hepatocellular carcinoma diagnosis: the demise of a brilliant star. Gastroenterology. 2009;137:26–9.
23. Omata M, Cheng AL, Kokudo N, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017;11:317–70.
24. Mazzaferro V, Bhoori S, Sposito C, et al. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based

analysis of 15 years of experience. Liver Transpl. 2011;17 (Suppl 2): S44–S57.
25. Mehta N, Dodge JL, Goel A, et al. Identification of liver transplant candidates with hepatocellular carcinoma and a very low dropout risk: implications for the current organ allocation policy. Liver Transpl. 2013;19:1343–53.
26. Lai Q, Avolio AW, Manzia TM, et al. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. Clin Transpl. 2012;26:E125–31.
27. Stuart KE, Anand AJ, Jenkins RL. Hepatocellular carcinoma in the United States. Prognostic features, treatment outcome, and survival. Cancer. 1996;77:2217–22.
28. De Carlis L, Giacomoni A, Pirotta V, et al. Surgical treatment of hepatocellular cancer in the era of hepatic transplantation. J Am Coll Surg. 2003;196:887–97.
29. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology. 2001;33:1394–403.
30. Figueras J, Ibanez L, Ramos E, et al. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. Liver Transpl. 2001;7:877–83.
31. Shetty K, Timmins K, Brensinger C, et al. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. Liver Transpl. 2004;10:911–8.
32. Kitai S, Kudo M, Minami Y, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: a comparison of the biomarker-combined Japan Integrated Staging Score, the conventional Japan Integrated Staging Score and the BALAD Score. Oncology. 2008;75 (Suppl 1):83–90.
33. Berhane S, Toyoda H, Tada T, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016;14: 875–86.e6.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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