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Utility of neutrophil-to-lymphocyte ratio to identify long-term survivors among HCC patients treated with sorafenib

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Abstract

Sorafenib is the first multikinase inhibitor demonstrating a survival benefit for patients suffering from advanced hepatocellular carcinoma (HCC). However, 1 issue remains open: what is the factor able to predict which patients will be long survivors?

In the present study, we harnessed the potential of conditional survival, aiming at estimating the probability that a patient receiving sorafenib survives for more than 3 years.

The present multicentric study was conducted on a cohort of 438 HCC patients. The primary end point was conditional overall survival. Kaplan–Meier survival analysis was used to calculate conditional overall survival probabilities at 3 years.

The 3-year conditional survival of patients without disease progression highlights that NLR and ECOG are the factors that most accurately predict the probability of long survival. The 3-year conditional survival of patients with disease progression showed a medium effect size for HCV status, alpha-fetoprotein and NLR at all time-points. Macro-vascular portal vein invasion, extra hepatic disease, and BCLC we have a large effect size at 6 months and a medium effect size at 12 and 24 months.

Our findings support the use of baseline NLR for the identification of patients with a higher probability of long-survival. NLR should be used as a stratification factor in the forthcoming clinical trials on the drugs for the advanced HCC now in pipeline.

Abbreviations: AFP = alpha-fetoprotein, BCLC = Barcellona Clinic Liver Cancer, CS = conditional survival, DP = disease progression, EHD = extra-hepatic disease, HCC = hepatocellular carcinoma, HCV = Hepatitis C virus, IQR = interquartile range, MaVI = macro-vascular portal vein invasion, mRECIST = modified Response Evaluation Criteria in Solid Tumors, NLR = neutrophil-to-lymphocyte ratio, OS = overall survival, PFS = progression-free survival.

Keywords: alpha-fetoprotein, extra hepatic disease and BCLC, hepatitis C, macro-vascular portal vein invasion, NLR, prognostic factor, survival

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1. Introduction

Sorafenib is the first multikinase inhibitor demonstrating a survival benefit for patients suffering from advanced hepatocellular carcinoma (HCC) and is the standard of care as first-line treatment in this setting.[1,2] The expected median overall survival (OS) range of patients treated with sorafenib is around 10 to 12 months, with a wide variation between patients with the likelihood of long-term survival (>3 years being reported around 2%–7%).[3]

It should be observed that, after the beginning of sorafenib treatment, most of the negative outcomes converge in the early period, being the median progression-free survival (PFS) of only about 4 to 5 months.[1,2] Despite the fact that the disease progression (DP) unfortunately represents an expected event, leading to death, some patients may experience a delayed progression of the disease and/or a slower tumor growth, resulting in the possibility of achieving a long-term goal. Thus, for patients receiving sorafenib it is important to question how survival probability evolves over time. Such information is important not only to answer to patients needs and concerns, but also to provide clinical indications for the continuation of the treatment.

A methodological approach able to address this need is represented by the *conditional survival* (CS). This survival measure considers the time that the patient has already survived to compute the future probabilities of survival at specified endpoints. Representing the changing likelihood of demise over time, the conditional survival analysis offers meaningful prognostic information for patients who survive beyond the early phase of sorafenib treatment, by providing more reliable and updated estimates of their survival probability.[4]

In the present study, we harnessed the potential of conditional survival, aiming at:

1. estimating the probability that a patient receiving sorafenib survives for more than 3 years, given that he/she is alive and without tumor progression during follow-up;
2. estimating the probability of the individual to become a long-term survivor, provided that he/she is alive with tumor progression during follow-up.

When considering these 2 aims, we mainly focused our attention on markers of subclinical inflammation.

2. Patients and methods

The present multicentric study was conducted on a cohort of 438 HCC patients consecutively treated between 2008 and 2018 at University of Modena, Istituto Oncologico Veneto-IRCCS, S. Orsola – Malpighi Hospital of the University of Bologna and University of Palermo.

Patients with histologically or radiologically proven[1,2] advanced- or intermediate-stage (refractory or unsuitable for locoregional therapies) HCC treated with sorafenib were eligible for our analysis. Patients who had received previous systemic therapies were excluded. Eligibility criteria were the same as those of the SHARP study:[1,2] Eastern Cooperative Oncology Group (ECOG) performance status score of ≤2; Child-Turcotte-Pugh liver function class A; adequate hematologic function (platelet count $\geq 60 \times 10^9$/L; hemoglobin ≥8.5 g/dl; and prothrombin time international normalized ratio ≤2.3 or prothrombin time ≤6 seconds above control, adequate hepatic function (albumin ≥2.8 g/dl; total bilirubin ≤3 mg/dl [51.3 μmol/L]; alanine aminotransferase and aspartate aminotransferase ≤5 times the upper limit of the normal range); and adequate renal function (serum creatinine ≤1.5 times the upper limit of the normal range).

All patients received sorafenib according to standard schedule (400 mg bid continuously); dose reduction was applied as clinically indicated. Follow-up consisted of CT/MRI scan every 8 weeks or as clinically indicated. Tumor response was evaluated by modified Response Evaluation Criteria in Solid Tumors (mRECIST). Treatment with sorafenib was continued until disease progression, unacceptable toxicity or death. The study protocol was reviewed and approved by the local Ethics Committee (CEIIAV: comitato etico IRST IRCCS AVR). Study number IRST B041 protocol.

2.1. Statistical analysis

Descriptive characteristics were reported as percentages or median and interquartile range (IQR). Overall survival (OS) was computed from the time since sorafenib starts until the death of the patient. Time-to-progression (TTP) was computed until the clinical and radiological evidence of tumor progression. Finally, Progression-free Survival (PFS) was computed until tumor progression or death. The effect of disease progression on the patient outcome is important and can be studied through the ordered multivariate event time data of time-to-event from enrolment, to progression and to death. Results obtained from the estimation of the conditional survival probabilities, $S(y \mid x) = P(T > y | T1 > x)$, can be used to understand which individuals without disease progression after Sorafenib start at time x, are most likely to survive from their disease at time y. Conditional survival probabilities were calculated based on Kaplan–Meier weights and the Landmark approaches. The package condSURV for R-project software was used for this analysis.

The effect size between estimated conditional survival probabilities were measured through the application of standardized differences as proposed by Austin et al.[5] Effect size is a simple way of quantifying the difference between 2 groups and is commonly interpreted as follows: values around 0.2 indicated small differences; values around 0.5 indicated moderate differences; and values around 0.8 or more indicated considerable differences.

3. Results

Baseline characteristics of the study population are reported in Table 1. During a median follow-up of 9.8 months, 412 (87.3%) patients showed disease progression, and 331 (70.0%) patients died. Median OS was 14.1 months, whereas TTP and PFS were 4.7 and 4.5, respectively. At 3 years after sorafenib beginning, 32 of the initial 438 patients (7.3%) were alive. Of these, 18 patients had experienced disease progression within the 3 years.

Neutrophil-to-lymphocyte ratio showed significant relationship with OS (Supplementary Fig. 1, http://links.lww.com/MD/E137), with a median of 18.1 months in patients with NLR ≤3 and of 8.8 in patients with NLR >3 ($P < .001$). The median PFS of patients with NLR ≤3 was 5.4 months and 3.3 for those with NLR >3 ($P < .001$). Other details regarding OS and PFS for the various clinical conditions here analyzed are reported in the Supplementary Table 1, http://links.lww.com/MD/E137.

4. Probability of become long-term survivors

Results from conditional survival in the whole study population are reported in the Figure 1. The probability of being alive at 3

Table 1

Baseline characteristics of 438 patients treated with sorafenib.

Feature	n = 438
Age [years; (median, IQR)]	67 (60–74)
> 67 years (%)	214 (48.9)
Male (%)	361 (82.4)
Hepatitis C infection (%)	227 (51.8)
Hepatitis B infection (%)	85 (19.4)
Alcohol (%)	70 (20.0)
ECOG (%)	
0	267 (61.0)
1	155 (36.4)
2	16 (3.6)
Macro-vascular portal vein invasion (%)	163 (37.2)
Extra-hepatic disease (%)	161 (36.8)
BCLC Stage (%)	
B	102 (23.3)
C	336 (76.7)
Alpha-fetoprotein [ng/mL; (median, IQR)]	51.8 (7.5–950)
> 400 ng/ml (%)	143 (32.7)
NLR (median, IQR)	2.76 (1.93–4.1)
> 3 (%)	199 (42.1)
Albumin (g/L)	37 (34–40)
Survival [months; (median, 95%C.I.)]	
Overall Survival	14.1 (6.4–25.3)
Time-to-progression	4.7 (2.4–10.1)
Progression-free survival	4.5 (2.4–9.4)

NLR = Neutrophil-to-lymphocyte ratio.

years since sorafenib start was conditional to the time elapsed without disease progression (Fig. 1, Panel A). As an example, if a patient had not experienced disease progression 6 months since sorafenib start, his/her probability to become a long-term survivor was of about 30%. Then, the more is the time to disease progression, the greater are the chances of becoming a long-term survivor. Differently, when patients had disease progression within 12 months their constant probability of being alive at 3 years decreased to 10%. Thereafter, it started to slowly increase (Fig. 1, Panel B).

4.1. Stratification of chances of long-term survival

The 3-year conditional survival of patients without disease progression highlights that NLR and ECOG are the factors that most accurately predict the probability of long survival (Table 2). In patients with NLR ≤3 the probability of long survival is 20% higher compared to patients with NLR > 3 (at 6 months 34.5% vs 14.3%, effect size 0.503; at 12 months 51.1% vs 27.2%, effect size 0.505) and increases up to 50% at 24 months (84.8% vs 30.0%, effect size 1.331). The same results were observed when evaluating the performance status of patients. In patients with ECOG = 0 the probability of long survival is 20% higher

Table 2

Probabilities of become a long-term survivor conditional that the patient is alive and without disease progression at different time points by covariates.

	Temporal end-point considered since Sorafenib start		
Features	**Month #6**	**Month# 12**	**Month #24**
HCV			
Negative	24.8% (11.7–37.9)	39.9% (19.6–63.8)	46.7% (0.0–85.7)
Positive	30.7% (19.7–42.6)	46.9% (33.2–63.4)	79.2% (57.1–94.7)
Effect size	0.132	0.142	0.715†
ECOG			
0	37.7% (27.6–49.4)	52.8% (36.5–67.5)	78.6% (56.3–95.2)
1–2	14.4% (4.3–25.5)	28.6% (11.3–49.9)	52.5% (0.0–88.9)
Effect size	0.551†	0.508†	0.571†
MaVI			
Absent	31.9% (15.9–47.2)	45.9% (31.3–59.8)	76.9% (54.1–94.7)
Present	27.6% (16.9–36.7)	43.7% (23.5–64.4)	60.0% (25.0–88.9)
Effect size	0.094	0.044	0.370 *
EHD			
Absent	29.1% (19.4–41.6)	44.9% (30.3–58.7)	69.9% (49.4–88.5)
Present	27.9% (13.7–42.0)	44.5% (24.1–65.6)	71.4% (40.0–100)
Effect size	0.027	0.008	0.033
BCLC			
Stage B	29.3% (11.5–45.0)	46.4% (31.7–59.3)	74.1% (53.8 - 100)
Stage C	28.7% (20.6–37.3)	42.3% (19.9–68.4)	66.3% (41.7–88.2)
Effect size	0.013	0.083	0.171
AFP			
≤400 ng/mL	31.9% (12.1–50.8)	48.8 (23.9–80.2)	71.4% (24.9–100)
>400 ng/mL	28.1% (19.2–38.4)	44.1% (28.9–57.0)	70.7% (50.5–90.0)
Effect size	0.083	0.094	0.015
NLR			
≤3	34.5% (23.2–43.6)	51.1% (35.3–63.9)	84.8% (61.5–100)
>3	14.3% (2.8–26.8)	27.2% (6.9–50.6)	30.0% (0.0–66.7)
Effect size	0.503†	0.505†	1.331†

* medium effect size = between 0.2 and 0.5.

† large effect = above 0.5 (effect size value < 0.2 was considered as negligible).

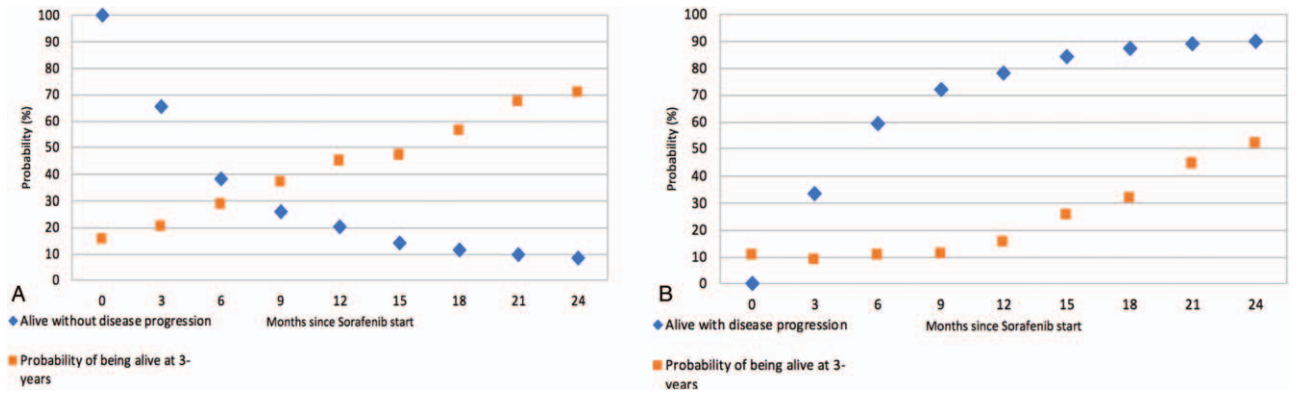

Figure 1. Survival probabilities of being alive at 3 years since sorafenib start conditional that the patient is alive and without disease progression /panel A) or with disease progression (panel B) at different time points.

Table 3

Probabilities of become a long-term survivor conditional that the patient is alive and with disease progression at different time points by covariates.

	Temporal end-point considered since sorafenib start		
	Month# 6	Month #9	Month #12
HCV			
Negative	5.9% (1.5–11.9)	7.7% (2.6–13.7)	11.9% (5.6–18.9)
Positive	14.5% (8.2–21.4)	16.1% (8.3–23.1)	19.3% (12.4–25.6)
Effect size	0.287*	0.261*	0.205*
ECOG			
0	12.3% (6.2–19.1)	13.4% (7.6–18.8)	20.1% (12.8–26.7)
1–2	8.7% (3.4–13.8)	7.5% (2.8–13.1)	9.4% (5.2–16.1)
Effect size	0.117	0.193	0.305*
MaVI			
Absent	14.3% (8.6–21.0)	15.6% (9.6–22.2)	20.4% (13.8–27.5)
Present	0.0%	5.8% (1.4–12.0)	7.1% (1.6–12.7)
Effect size	0.578†	0.321*	0.393*
EHD			
Absent	15.0% (9.0–21.7)	14.3% (8.1–19.4)	20.4% (14.2–27.2)
Present	0.0%	5.6% (1.2–10.3)	7.1% (2.6–13.0)
Effect size	0.594†	0.293*	0.394*
BCLC			
Stage B	28.2% (12.1–46.1)	22.7% (8.6–36.0)	28.8% (15.9–42.9)
Stage C	7.2% (3.8–11.5)	8.4% (4.8–12.3)	12.2% (7.7–17.7)
Effect size	0.572†	0.403*	0.420*
AFP			
≤ 400 ng/mL	14.2% (7.9–20.5)	14.7% (9.6–20.6)	20.2% (14.1–25.8)
> 400 ng/mL	5.7% (0.0–13.0)	4.6% (0.0–10.2)	6.3% (1.6–12.8)
Effect size	0.287*	0.347*	0.419*
NLR			
≤ 3	15.5% (9.2–22.9)	16.5% (8.4–24.9)	20.6% (13.3–26.4)
> 3	5.6% (1.2–10.6)	6.6% (2.3–11.4)	10.1% (5.4–15.8)
Effect size	0.327*	0.313*	0.294*

* medium effect size = between 0.2 and 0.5.

† large effect = above 0.5 (effect size value < 0.2 was considered as negligible).

compared to patients with ECOG 1–2 (at 6 months 37,7% vs 14.4%, effect size 0.551; at 12 months 52.8% vs 28.6%, effect size 0.508; at 24 months 78.6% vs 52.5%, effect size 0.571).

The 3-year conditional survival of patients with disease progression showed a medium effect size for HCV status, alpha-fetoprotein (AFP) and NLR at all time-points (Table 3). On the other hand, for macro-vascular portal vein invasion (MaVI), EHD and BCLC we have a large effect size at 6 months (0.578, 0.594 and 0.572) and a medium effect size at 12 and 24 months. In particular, patients with MaVI and EHD who had disease progression within the first 6 months of treatment have 0% chance of being long-term survivors.

4.2. Stratification by neutrophil-to-lymphocyte ratio

Stratifying the patients without disease progression for NLR (cut-off 3). In patients with NLR ≤3, the 3-year conditional survival underscored that ECOG is the factor that most accurately predicts the probability of long survival at all time-points (at 6 months 44.5% vs 16.1%, effect size 0.650; at 12 months 60.2% vs 29.6%, effect size 0.647; at 24 months 93.0% vs 64.3%, effect size 0.748). In patients with NLR > 3 we found that the negativity for HCV is the major factor able to identify patients with the lowest probability of long survival (at 3 months 2.0% vs 13.8%, effect size 0.448; at 6 months 0.0% vs 22.5%, effect size 0.762; at 9 months 0.0% vs 31.1% effect size 0.950) (Table 4).

Table 4

Probabilities of become a long-term survivor conditional that the patient is alive and without disease progression in different neutrophil-to-lymphocyte ratio subgroups.

	Temporal end-point considered since sorafenib start		
NLR ≤ 3	Month #6	Month# 12	Month #24
HCV negative	34.4% (17.8–50.7)	49.6% (24.0–70.8)	64.3% (19.5–100)
HCV positive	34.3% (21.3–47.7)	51.4% (29.9–69.9)	92.9% (79.9–100)
Effect size	0.002	0.036	0.744†
ECOG 0	44.5% (28.6–60.2)	60.2% (43.8–74.6)	93.0% (75.0–100)
ECOG 1–2	16.1% (3.7–32.3)	29.6% (10.3–57.1)	64.3% (10.0–100)
Effect size	0.650†	0.647†	0.748†
MaVI Absent	33.0% (21.6–45.8)	51.2% (34.1–68.4)	86.7% (66.7–100)
MaVI Present	39.3% (20.2–61.5)	51.6% (27.5–79.0)	83.3% (50.0–100)
Effect size	0.131	0.008	0.095
EHD Absent	34.8% (22.3–48.6)	55.1% (35.2–79.3)	79.5% (53.5–100)
EHD Present	34.2% (18.1–53.4)	48.5% (30.8–66.9)	72.0% (46.8–100)
Effect size	0.013	0.132	0.176
AFP ≤400 ng/mll	41.6% (21.4–64.3)	50.9% (34.4–68.3)	87.5% (71.4–100)
AFP >400 ng/ml	33.1% (21.4–43.8)	53.9% (19.9–83.1)	82.3% (39.8–100)
Effect size	0.176	0.060	0.146
NLR > 3	**Month #3**	**Month# 6**	**Month #9**
HCV negative	2.0% (0.0–10.1)	0.0% (0.0–0.0)	0.0% (0.0–0.0)
HCV positive	13.8% (3.0–26.1)	22.5% (5.2–40.5)	31.1% (8.9–55.6)
Effect size	0.448*	0.762†	0.950†
ECOG 0	9.9% (0.0–19.8)	18.2% (0.0–40.2)	25.4% (0.0–52.6)
ECOG 1–2	7.3% (0.0–16.0)	13.3% (0.0–33.3)	22.5% (0.0–59.3)
Effect size	0.093	0.135	0.068
MaVI Absent	8.7% (0.0–21.1)	18.4% (0.0–44.4)	28.6% (0.0–62.6)
MaVI Present	7.5% (0.0–19.7)	13.2% (0.0–29.3)	19.4% (0.0–42.9)
Effect size	0.044	0.143	0.217*
EHD Absent	10.2% (2.1–19.0)	18.7% (3.7–36.7)	28.2% (7.4–52.9)
EHD Present	6.2% (0.0–23.8)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Effect size	0.146	0.678†	0.886†
AFP ≤400 ng/ml	9.4% (0.0–18.7)	14.4% (0.0–30.0)	19.8% (0.0–40.4)
AFP > 400 ng/ml	4.6% (0.0–14.8)	12.5% (0.0–43.0)	17.2% (0.0–55.3)
Effect size	0.189	0.056	0.067

* medium effect size = between 0.2 and 0.5.

† large effect = above 0.5 (effect size value < 0.2 was considered as negligible).

Analyzing patients with disease progression, we showed that the presence of MaVI and EHD are the major factors associated with the lowest probability of being long-term survivor, in particular at 6 months (0.0% vs 21.0%, effect size 0.729; 0.0% vs 27.3% respectively) (Table 5). Conversely, for patients with NLR > 3 we detected only a moderate effect size for HCV, ECOG, and EHD (Table 5). It should be noted that in all categories and at any time point the patients had a less than 10% probability of being long-term survivors.

5. Discussion

In our analysis, we evaluated 438 HCC patients treated with sorafenib, observing that a non-negligible proportion of them (7.3%) experienced a long-term survival (≥36 months). This finding confirms the possibility of achieving long-term survival with sorafenib in clinical practice.[6] However, 1 issue remains open: what is the factor able to predict which patients will be long survivors? Our analysis suggests that the baseline level of neutrophil-to-lymphocyte ratio (NLR) is the factor that can detect long-term survivors in both cohorts of patients with and without progression. To our knowledge, this is the first study to

Table 5

Probabilities of become a long-term survivor conditional that the patient is alive and with disease progression in different neutrophil-to-lymphocyte ratio subgroups.

	Temporal end-point considered since sorafenib start		
NLR ≤ 3	**Month #6**	**Month# 12**	**Month #24**
HCV negative	7.4% (0.0–17.5)	13.4% (3.1–23.6)	68.2% (58.2–76.7)
HCV positive	22.6% (14.0–34.9)	25.4% (15.4–35.4)	63.9% (53.9–72.9)
Effect size	0.436*	0.307*	0.091
ECOG 0	17.4% (7.4–28.2)	25.1% (14.9–35.8)	75.2% (67.8–82.7)
ECOG 1–2	14.1% (3.4–24.2)	14.0% (5.1–23.1)	51.2% (38.8–62.0)
Effect size	0.091	0.283*	0.514†
MaVI Absent	21.0% (8.6–32.1)	26.0% (16.0–34.0)	72.9% (65.6–80.1)
MaVI Present	0.0%	0.0%	52.8% (41.3–66.5)
Effect size	0.729†	0.838†	0.425*
EHD Absent	27.3% (14.6–38.9)	28.9% (18.5–42.8)	74.2% (65.4–82.7)
EHD Present	0.0%	10.0% (2.8–19.4)	55.8% (44.4–65.6)
Effect size	0.867†	0.492*	0.393*
AFP ≤400 ng/ml	20.3% (9.7–30.4)	25.7% (15.8–35.1)	72.3% (64.0–79.1)
AFP > 400 ng/ml	10.4% (0.0–20.7)	11.5% (0.0–22.1)	54.0% (42.8–66.4)
Effect size	0.277*	0.371*	0.386*
NLR > 3	**Month #3**	**Month# 6**	**Month #9**
HCV negative	0.0%	0.0%	6.7% (1.6–12.9)
HCV positive	5.9% (0.0–17.9)	8.6% (0.0–17.2)	7.4% (0.0–17.0)
Effect size	0.354*	0.433*	0.027
ECOG 0	7.3% (0.0–16.2)	7.4% (1.5–14.5)	9.3% (3.3–17.9)
ECOG 1–2	0.0%	3.2% (0.0–9.4)	4.7% (0.0–8.6)
Effect size	0.397*	0.188	0.181
MaVI Absent	7.3% (0.0–16.7)	9.5% (2.2–16.5)	9.9% (2.0–17.0)
MaVI Present	0.0%	0.0%	2.4% (0.0–8.7)
Effect size	0.396*	0.458*	0.316*
EHD Absent	3.6% (0.0–10.9)	5.6% (0.0–12.6)	9.7% (0.0–21.1)
EHD Present	0.0%	0.0%	4.9% (0.0–10.1)
Effect size	0.273*	0.344*	0.185
AFP ≤400 ng/ml	6.9% (0.0–18.6)	8.9% (1.9–16.6)	10.7% (4.2–18.9)
AFP > 400 ng/ml	0.0%	0.0%	0.0%
Effect size	0.385	0.442	0.489

* medium effect size = between 0.2 and 0.5.

† large effect = above 0.5 (effect size value < 0.2 was considered as negligible).

highlight that NLR is not only a prognostic factor in HCC patients treated with sorafenib, but it is also the most important factor capable of identifying the long-term survivors within these patients.

The tumor promoting inflammation is a well-established hallmark of cancer, being a paramount driver of liver fibrosis and carcinogenesis. Systemic inflammatory parameters, reflecting the inflamed cancer-induced environment, predict survival in both cirrhosis and HCC.[7] The NLR is one of the biomarkers of systemic inflammatory response and has been identified as an important prognostic factor for several tumors.[8–13] The exact mechanism that explains the poor survival outcomes for patients with elevated blood NLR has not been clearly determined. A possible explanation is that this index may reflect a high level of circulating cytokine including transforming growth factor β, which enhances tumor progression by favoring immunosuppression, angiogenesis, and peritumoral stroma formation.[14]

In the study sub-population without disease progression we found that ECOG was another parameter able to predict long-term survival. Several studies have highlighted the correlation between ECOG > 0 and a poorer overall survival.[15,16] In our study, we highlighted that ECOG > 0 can impact the survival in those patients who have a higher probability of long survival (patients with NLR <3), but not in patients with lower probability of long survival. This underscores the importance of the NLR as a determinant of the probability of long-term survival.

Evaluating the differences between patients with low (≤3) and high (>3) value of NLR, HCV negative status was the most important factor predicting the low probability of long survival in patients with NLR > 3. Conflicting data can be found in the literature about the different efficacy of sorafenib in HCV positive and negative patients.[17–19] In our study we showed that, in the whole population, the association between NLR > 3 and HCV negative status, rather than HCV status alone, is able to detect patients with the lowest chance of long surviving. The different cytokine profiles of T cells between HBV and HCV infection could explain this difference.[20]

Concerning the population with disease progression, our study showed that, in addition to NLR, macro-vascular portal vein invasion (MaVI), extra-hepatic disease (EHD) and BCLC are the factors able to predict which patients will be long-survivors. Evaluating the differences between patients with low (≤3) and high (>3) value of NLR, the same factors (MaVI, EHD and BCLC) were predictive of long survival in both groups. These results are not surprising, since they are known survival predictors in this setting of patients.[21]

Our study also highlighted that alpha-fetoprotein (AFP) is not a predictor of long survival in patients without disease progression and is a weaker predictor of survival in patients with disease progression. Sorafenib is the current standard first-line systemic treatment for HCC irrespective of baseline AFP. Nevertheless, it should be outlined that the SHARP trial showed that high baseline AFP levels (> 200 ng/ml) had a negative impact on OS, a finding later confirmed in a pooled analysis of SHARP and Asia-Pacific trials.[1,2] When considering second-line treatments, AFP was used as a stratification factor (<400 ng/ml or ≥ 400 ng/ml) in the RESORCE study,[22] the phase III trial that established regorafenib as the recommended second-line option of treatment. AFP levels play an important role when considering ramucirumab. Indeed, patients with baseline AFP levels ≥400 ng/ml were enrolled and treated with ramucirumab in the REACH-2 study,[23] which became the first positive phase III HCC trial in a biomarker-selected patient population. This trial was based on a *post hoc* analysis of a previous, negative trial that suggested efficacy in this subgroup. In our study, AFP level at disease progression is missing and this might represent a limit of our analysis.

The different results between the 2 cohorts of patients (with and without disease progression) can be explained by the fact that patients with macro-vascular portal vein invasion, EHD and BCLC-C had a higher risk of early progression to sorafenib treatment. It should be noted, for example, that patients with macro-vascular portal vein invasion who progress within 6 months of treatment have a 0% chance of being long-survivors.

Another important concept to point out is that the probability of being long survivors is directly related to the time to progression since starting sorafenib. In fact, as shown in Figure 1B, the probability of being long survivors increases progressively only in patients who do not progress in the first 9 months of treatment with sorafenib. This is a crucial point because, to date, the probability of long survival cannot be changed by second lines. Recent scientific evidence enhanced the opportunities and the future perspectives in HCC treatment,

making patients selection more and more important. Several drugs, including multikinase inhibitors (lenvatinib, regorafenib, cabozantinib),[21–25] monocolonal antibodies (ramucirumab),[22] and immunotherapy (tremelimumab, nivolumab, pembrolizumab)[26] demonstrated activity in HCC. As for first line treatment, lenvatinib proved to be non-inferior to sorafenib in the phase III REFLECT trial.[23] As for second line treatment, regorafenib demonstrated a survival benefit vs placebo after first line sorafenib failure in the phase III RESORCE trial. Similarly, cabozantinib demonstrated a survival improvement vs placebo for sorafenib pre-treated patients in second and third line setting in the phase III CELESTIAL trial.[25] In the context of such a rapidly evolving scenario, in the next years it will become essential to understand whether these new treatments can really change the prognosis of patients.

In conclusion, our findings support the use of baseline NLR for the identification of patients with a higher probability of long-survival. The implications for the clinical practice are relevant since this information might allow a more accurate selection of candidates to sorafenib in order to consider individual therapeutic approaches capable to maximize the clinical benefit, with minimal toxic effects and costs. In addition, this evidence suggests that NLR should be used as a stratification factor in the forthcoming clinical trials on the drugs for the advanced HCC now in pipeline.

Author contributions

Conception and design: A. Casadei Gardini, A. Cucchetti.
Acquisition of data (acquired and managedpatients): All Authors.
Analysis and interpretation of data: A. Casadei Gardini and A. Cucchetti.
Writing, review, and/or revision of the manuscript: A. Casadei Gardini and A. Cucchetti.
Final approval of manuscript: All authors.

References

[1] Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–90.
[2] Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34.
[3] Tanaka K, Shimada M, Kudo M, et al. Characteristics of long-term survivors following sorafenib treatment for advanced hepatocellular carcinoma: report of a workshop at the 50th Annual Meeting of the Liver Cancer Study Group of Japan. Oncology 2014;87(Suppl 1):104–9.
[4] Facciorusso A, Del Prete V, Antonino M, et al. Conditional survival analysis of hepatocellular carcinoma patients treated with radio-frequencyablation. Hepatol Res 2015;45:E62–72.
[5] Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009;28:3083–107.
[6] Sacco R, Granito A, Bargellini I, et al. Clinical outcomes with long-term sorafenib treatment of patients with hepatocellular carcinoma: a multicenter real-life study. Future Oncol 2018;14:3049–58.
[7] Howell J, Pinato DJ, Ramaswami R, et al. Integration of the cancer-related inflammatory response as a stratyfing biomarker of survival in hepatocellular carcinoma treated with sorafenib. Oncotarget 2017;8: 36161–70.
[8] Casadei-Gardini A, Scarpi E, Ulivi P, et al. Prognostic role of a new inflammatory index with neutrophil-to-lymphocyte ratio and lactate dehydrogenase (CII: Colon Inflammatory Index) in patients with metastatic colorectal cancer: results from the randomized Italian Trial in Advanced Colorectal Cancer (ITACa) study. Cancer Manag Res 2019;11:4357–69.
[9] Casadei-Gardini A, Montagnani F, Casadei C, et al. Immune inflammation indicators in anal cancer patients treated with concurrent chemoradiation: training and validation cohort with online calculator (ARC: Anal Cancer Response Classifier). Cancer Manag Res 2019; 11:3631–42.
[10] Alberto Lué, Maria Trinidad Serrano. Francisco Javier Bustamante et al. Neutrophil-to lymphocyte ratio predicts survival in European patients with hepatocellular carcinoma administered sorafenib. Oncotarget 2017;8:103077–86.
[11] Casadei Gardini A, Foschi FG, Conti F, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. Liver Dis 2019;51:681–8.
[12] Casadei Gardini A, Marisi G, Canale M, et al. Radiofrequency ablation of hepatocellular carcinoma: a meta-analysis of overall survival and recurrence-free survival. Onco Targets Ther 2018;11:6555–67.
[13] Casadei Gardini A, Scarpi E, Faloppi L, et al. Immune inflammation indicators and implication for immune modulation strategies in advanced hepatocellular carcinoma patients receiving sorafenib. Oncotarget 2016;7:67142–9.
[14] Rochefort P, Lardy-Cleaud A, Sarabi M, et al. Long-term survivors in metastatic pancreatic ductal adenocarcinoma: a retrospective and matched pair analysis. Oncologist 2019;24:1543–8.
[15] Abdel-Rahman O. Impact of baseline characteristics on outcomes of advanced HCC patients treated with Sorafenib: a secondary analysis of a phase III study. J Cancer Res Clin Oncol 2018;144:901–8.
[16] Samawi HH, Sim HW, Chan KK, et al. Prognosis of patients with hepatocellular carcinoma treated with Sorafenib: a comparison of five models in a large Canadian database. Cancer Med 2018.
[17] Andrea Casadei Gardini, Marco Puzzoni, Francesco Montagnani, et al. Profile of lenvatinib in the treatment of hepatocellular carcinoma: design, development, potential place in therapy and network meta-analysis of hepatitis B and hepatitis C in all Phase III trials. Onco Targets Ther 2019;12:2981–8. Published online 2019 Apr 24.
[18] Casadei Gardini A, Frassineti GL, Foschi FG, et al. Sorafenib and Regorafenib in HBV- or HCV-positive hepatocellular carcinoma patients: Analysis of RESORCE and SHARP trials. Dig Liver Dis 2017;49:943–4.
[19] Bruix J, Cheng AL, Meinhardt G, et al. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase III studies. J Hepatol 2017;67:999–1008.
[20] Bertoletti A, D'Elios MM, Boni C, et al. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 1997;112:193–9.
[21] Marisi G, Cucchetti A, Ulivi P, et al. Ten years of sorafenib in hepatocellular carcinoma: are there any predictive and/or prognostic markers? World J Gastroenterol 2018;24:4152–63.
[22] Bruix J, Qin S, Merle P, 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;389:56–66.
[23] Zhu AX, Kang YK, Yen CJ, et al. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased (-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2019;20:282–96.
[24] Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellularcarcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391:1163–73.
[25] Abou-Alfa GK, Meyer T, Cheng AL. Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. N Engl J Med 2018;379:54–63.
[26] Sangro C, Gomez-Martin B, de la Mata C, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinomaand chronic hepatitis. J Hepatol 2013;59:81–8. doi: 10.1016/j.jhep.2013.02.022.