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Targeted Oncology

ISSN 1776-2596

Targ Oncol
DOI 10.1007/s11523-020-00757-3

Springer

Targeted Oncology
https://doi.org/10.1007/s11523-020-00757-3

ORIGINAL RESEARCH ARTICLE

Prognostic Role of Blood Eosinophil Count in Patients with Sorafenib-Treated Hepatocellular Carcinoma

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Abstract

Background Inflammation is a long-established hallmark of liver fibrosis and carcinogenesis. Eosinophils are emerging as crucial components of the inflammatory process influencing cancer development. The role of blood eosinophils in patients with hepatocellular carcinoma receiving systemic treatment is an unexplored field.

Objective The objective of this study was to analyse the prognostic role of the baseline eosinophil count in patients with sorafenib-treated hepatocellular carcinoma.

Patients and Methods A training cohort of 92 patients with advanced- or intermediate-stage sorafenib-treated hepatocellular carcinoma and two validation cohorts of 65 and 180 patients were analysed. Overall survival and progression-free survival in relation to baseline eosinophil counts were estimated by the Kaplan–Meier method. Univariate and multivariate analyses were performed.

Results A negative prognostic impact of low baseline eosinophil counts ($<50*10^9$/L) was demonstrated in all cohorts (training cohort: hazard ratio = 50.1, 95% confidence interval 11.6–216.5, $p<0.0001$ for low vs high eosinophil counts; first validation cohort: hazard ratio = 4.55, 95% confidence interval 1.24–16.65, $p=0.022$; second validation cohort: hazard ratio = 3.21, 95% confidence interval 1.83–5.64, $p<0.0001$). Moreover, low eosinophil counts had a negative prognostic role in patients progressing on or intolerant to sorafenib who received second-line regorafenib, but not capecitabine or best supportive care.

Conclusions Our analysis identified baseline blood eosinophil counts as a new prognostic factor in patients with sorafenib-treated hepatocellular carcinoma. Concerning second-line therapies, eosinophil counts were associated with survival outcomes only in regorafenib-treated patients, suggesting a possible predictive role in this setting.

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Published online: 12 October 2020

Key Points

Eosinophils are emerging as crucial components of the inflammatory process influencing the development of cancer.

Low baseline blood eosinophil count ($<50*10^9$/L) has a negative prognostic value for overall survival in patients with hepatocellular carcinoma treated with sorafenib.

Low eosinophil counts also have a negative prognostic role in patients with hepatocellular carcinoma previously treated with sorafenib, who receive a second-line treatment with regorafenib, but not with capecitabine or best supportive care.

1 Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, representing the second leading cause of cancer-related death [1, 2]. Patients with HCC either at an advanced stage or at an intermediate stage refractory to loco-regional therapy benefit most from systemic therapy, which still focuses on the tyrosine kinase inhibitor sorafenib for first-line treatment according to international guidelines [3–6]. Lenvatinib has also emerged as a possible alternative to sorafenib in the front-line setting [7]. The second-line therapeutic armamentarium has been expanded with the introduction of regorafenib, ramucirumab and cabozantinib [8–10]. Recently, immune checkpoint inhibitors have become a part of the HCC therapeutic repertoire [11–15]. In the Imbrave 150 Trial, the combination of the anti-programmed death-ligand 1 antibody atezolizumab and the anti-vascular endothelial growth factor antibody bevacizumab was associated with increased overall survival compared with sorafenib in patients with advanced HCC who had not previously received systemic therapies [16].

Neoangiogenesis and immune escape are historical hallmarks of carcinogenesis and also the main targets of the currently available agents for HCC treatment [17]. Inflammation, a more recently identified additional hallmark of carcinogenesis, is the common mechanism underlying the different aetiologies of HCC [18]. The pro-inflammatory response promotes the cross-talk between cancer cells and the tumour microenvironment, including cancer-associated fibroblasts, endothelial cells and immune cells [19, 20]. Inflammatory cytokines such as interleukins and chemokines orchestrate the transition from fibrosis to carcinoma, supporting hepatocarcinogenesis [21]. Furthermore, inflammation retains a prognostic role in HCC [18]. Pro-inflammatory modifications of the peritumoural tissue are associated with earlier recurrence and worse survival after HCC resection [22, 23].

In this light, many efforts have been made to assess indirect biomarkers of the inflammatory status in patients with HCC, aiming at identifying potential prognostic and predictive factors [18]. In the pooled analysis of the SHARP and Asia–Pacific trials, Bruix and colleagues demonstrated that not only does a high neutrophil-to-lymphocyte ratio (NLR) has a negative prognostic value, but also low NLR is a predictive factor for greater sorafenib benefit in patients with HCC [24]. Many other studies support the inverse correlation between NLR and survival outcomes in patients with HCC [25–28]. Other immune-inflammatory indicators assessed for their prognostic and predictive role in HCC include: platelet-to-lymphocyte ratio, systemic immune-inflammation index (SII), the prognostic nutritional index, the prognostic index and the inflammation-based index [18, 28].

In addition to lymphocytes, neutrophils and macrophages, eosinophils are emerging as crucial components of the inflammatory process that influence the development of cancer [29]. The prognostic role of eosinophils has been evaluated in many different tumours, leading to divergent findings depending on the type of cancer analysed [30–38]. Steel and colleagues investigated the role of eosinophils in HCC for the first time, identifying a correlation between lower median eosinophil counts and worse survival outcomes in patients with hepatobiliary cancer, including 84% of patients with HCC treated with local procedures [39]. Nonetheless, the prognostic role of blood eosinophils in patients with HCC receiving systemic treatment remains an open and unexplored field. For this reason, in the present study, we analysed the impact of baseline eosinophil counts on survival outcomes in different cohorts of patients with HCC treated with sorafenib.

2 Methods

2.1 Patients and Treatment

This multi-centric Italian study was conducted on a training cohort of 92 patients with HCC consecutively treated at the University of Modena from 2007 to 2018. The data were then validated in two independent cohorts, including a first prospective validation cohort of 65 patients with HCC derived from the INNOVATE study [40] and a second retrospective validation cohort of 180 patients consecutively recruited at the University of Bologna from 2008 to 2017.

Patients with histologically or radiologically (according to the American Association for the Study of Liver Diseases 2005 guidelines) proven 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 Llovet et al.'s pivotal study on sorafenib in HCC [3].

The standard treatment protocol included the start of sorafenib at the usual dose of 400 mg twice daily; dose reduction was applied as clinically indicated. Follow-up consisted of a computed tomography/magnetic resonance imaging scan approximately every 8 weeks or as clinically indicated. Tumour response was evaluated by modified Response Evaluation Criteria in Solid Tumors (mRECIST) in all centres. Treatment with sorafenib was continued until disease progression, unacceptable toxicity or death.

Patients progressing on sorafenib were treated either with second-line therapies (regorafenib, capecitabine) or best supportive care, according to Performance Status and liver function. Although not recommended by the European Association for the Study of the Liver guidelines as a second-line treatment option [5], capecitabine, which was administered outside a study setting, was considered an active and safe option for patients with advanced HCC progressing on sorafenib on the basis of retrospective clinical studies [41, 42].

2.2 Statistical Analysis

The aim of this analysis was to examine the association between baseline eosinophil counts and overall survival (OS) and progression-free survival (PFS) in patients with HCC treated with sorafenib. Eosinophil count as well as all other laboratory examinations were retrieved from the medical records provided that available laboratory examinations had been performed within 1 week before sorafenib treatment start. X-tile 3.6.1 software (Yale University, New Haven, CT, USA) was used to determine the cut-off value for baseline levels. From the result of a receiver operating characteristic curve, eosinophil $< 50*10^9$/L was considered an elevated level. Categorical variables were compared with the Fisher's exact test [43].

Overall survival was defined as the time interval between the first day of treatment with sorafenib until the day of death or the last follow-up visit. Overall survival and PFS were estimated by the Kaplan–Meier method and curves were compared by the log-rank test. Unadjusted and adjusted hazard ratios (HRs) by baseline characteristics positive at the univariate analysis were calculated using the Cox proportional hazards model. The same model of the training cohort was applied to the two validation cohorts. Then, we performed a second model of the multivariate analysis, where we added the positive parameters identified at the univariate analysis of each validation cohort. The predictive value and the discrimination ability of the final model were assessed with Harrell's concordance index (*C*-index). MedCalc package (MedCalc® version 16.8.4, Bruges, Belgium) was used for the statistical analysis.

2.3 Ethics Approval

The present research was performed in accordance with the Declaration of Helsinki (6th revision, 2008) and the study protocol was reviewed and approved by the local ethics committee (CEIIAV: comitato etico IRST IRCCS AVR), study number IRST B041, protocol number 5482/v.1 intern code: L3P1192.

The study did not involve direct human participants, but only medical records of patients. All participants provided written informed consent to authorise the use of their medical data.

3 Results

A total of 92 patients with HCC consecutively treated with sorafenib at the University of Modena between June 2007 and August 2018 constituted the training cohort. Median follow-up was 30.9 months (95% CI 26.8–55.0). Median OS was 12.0 months (95% CI 8.2–15.6) and median PFS was 2.6 months (95% CI 2.2–3.2).

Sixty-five patients with HCC treated with sorafenib between March 2015 and June 2018 within the INNOVATE study [40] were analysed as the first validation cohort. Median follow-up was 18.1 months (95% CI 17.0–20.6). Median OS was 13.0 months (95% CI 11.4–18.2).

Finally, 180 patients with HCC treated with sorafenib at the University of Bologna between 2008 and 2017 were analysed as the second validation cohort. Median follow-up was 53.7 months (95% CI 39.4–53.7). Median OS was 10.3 months (95% CI 8.7–12.7). Patient characteristics are shown in Table 1.

3.1 Clinical Outcome of Eosinophil Count in the Training Cohort

Median OS was 2.7 months (95% CI 1.7–4.5) and 13.9 months (95% CI 11.2–15.8) for patients in the training cohort with low ($\leq 50*10^9$/L, 11 patients) and high ($> 50*10^9$/L, 81 patients) eosinophil counts, respectively (HR = 50.1, 95% CI 11.6–216.5, $p < 0.0001$) (Fig. 1, Table 2). Median PFS was 1.7 months (95% CI 0.9–2.4)

Table 1 Patient characteristics

Characteristics	Training cohort (%)	First validation cohort (%)	Second validation cohort (%)	*P* value
Median age, years (range)	71 (25–87)	61 (25–72)	68 (20–85)	0.03
Sex				0.95
Male	79 (86)	55 (85)	156 (87)	
Female	13 (14)	10 (15)	24 (13)	
Aetiology				0.20
HCV	38 (41)	25 (38)	90 (50)	
HBV	10 (11)	10 (15)	29 (16)	
Others	44 (48)	30 (47)	61 (34)	
BCLC stage				0.12
B	28 (30)	18 (28)	40 (22)	
C	64 (70)	47 (72)	140 (78)	
Child–Pugh				0.09
A (all patients)	81 (88)	59 (91)	169 (94)	
5	43 (46.7)	37 (56.9)	92 (51.1)	
6	38 (41.3)	28 (43.0)	77 (42.7)	
B7	11 (12)	6 (9)	11 (6)	
ECOG				0.09
0	56 (61)	42 (65)	127 (71)	
Others	36 (39)	23 (35)	53 (29)	
Extrahepatic disease				< 0.0001
No	65 (71)	40 (59)	81 (45)	
Yes	27 (29)	25 (41)	99 (55)	
Portal vein thrombosis				0.49
No	58 (63)	40 (61)	106 (59)	
Yes	34 (37)	25 (39)	74 (41)	
Alpha-fetoprotein				0.13
< 400	62 (67)	44 (68)	136 (76)	
> 400	30 (33)	21 (32)	44 (24)	
Macrovascular invasion				0.30
Yes	12 (13)	12 (18)	33 (18)	
No	80 (87)	53 (82)	147 (82)	
Bilirubin				0.18
< NV	65 (71)	50 (77)	116 (64)	
> NV	27 (29)	15 (23)	64 (36)	
Albumin				0.0051
< 35	31 (34)	10 (15)	65 (36)	
> 35	61 (66)	55 (85)	115 (64)	
NLR				0.65
< 3	54 (59)	34 (52)	85 (53)	
> 3	38 (41)	31 (48)	75 (47)	
Data missing			20	
SII				0.79
< 360	40 (43)	24 (37)	74 (41)	
> 360	52 (57)	41 (63)	106 (59)	
Eosinophil				0.49
< 50	11 (12)	9 (154	31 (17)	
> 50	81 (88)	56 (86)	149 (83)	
ALBI				0.95
1	81 (88)	58 (89)	158 (88)	

Table 1 (continued)

Characteristics	Training cohort (%)	First validation cohort (%)	Second validation cohort (%)	*P* value
2	11 (12)	7 (11)	22 (12)	
Prior RFA				0.22
Yes	9 (10)	7 (11)	30 (17)	
No	83 (90)	58 (89)	150 (83)	
Prior surgery				0.78
Yes	21 (23)	16 (25)	48 (27)	
No	71 (77)	49 (75)	132 (73)	
Prior TACE				0.60
Yes	33(36)	25 (38)	75 (42)	
No	59 (64)	40 (62)	105 (58)	

ALBI albumin-bilirubin, *BCLC* Barcelona-Clinic Liver Cancer, *ECOG* Eastern Cooperative Oncology Group, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *NLR* neutrophils/lymphocytes ratio, *NV* Normal Value, *RFA* radiofrequency, *SII* systemici Inflammatory index, *TACE* transarterial chemoembolisation

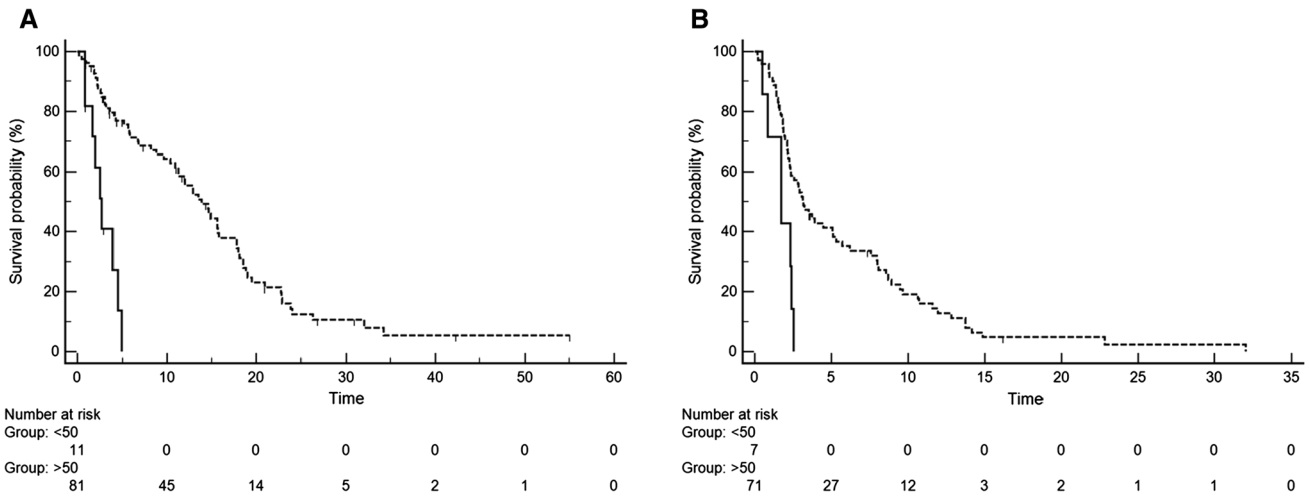

Fig. 1 Kaplan–Meier curves for overall survival (**a**) and progression-free survival (**b**) in the training cohort

and 3.2 months (95% CI 2.3–5.1) for patients with low and high eosinophil counts, respectively (HR = 7.56, 95% CI 2.05–27.93, $p = 0.0024$) (Fig. 1). The two groups of patients, namely low and high eosinophil counts, were comparable for all clinical characteristics investigated, except for Barcelona-Clinic Liver Cancer and portal vein thrombosis (Table 3).

At the univariate analysis, the other parameters associated with prognosis were: Eastern Cooperative Oncology Group (ECOG) [median OS was 14.7 months (95% CI 11.3–18.0) and 5.6 months (95% CI 3.2–12.9) for patients with ECOG 0 and ECOG > 0, respectively (ECOG > 0 vs 0; HR = 1.75, 95% CI 1.03–2.96, $p = 0.0357$)], portal vein thrombosis [median OS was 3.9 months (95% CI 2.7–5.82) and 15.6 months (95% CI 12.9–18.1) for patients with and without portal vein thrombosis, respectively (yes vs no; HR = 2.68, 95% CI 1.52–4.73, $p = 0.0006$)], albumin [median OS was 4.2 months (95% CI 2.6–11.28) and 14.6 months (95% CI 10.3–18.5) for patients < normal value and > normal value, respectively (< normal value vs > normal value; HR = 2.07, 95% CI 1.01–4.23, $p = 0.0452$)], and albumin-bilirubin (ALBI) grade [median OS was 2.0 months (95% CI 0.9–17.8) and 12.9 months (95% CI 8.7–18.1) for patients with grade 2 and grade 1, respectively (HR = 5.44, 95% CI 1.58–18.78, $p = 0.0073$)].

Following adjustment for clinical covariates positive in the univariate analysis, the multivariate analysis confirmed eosinophil counts (HR = 3.51, 95% CI 1.25–9.87, $p = 0.0168$), ALBI grade (HR = 3.24, 95% CI 1.38–7.59, $p = 0.0066$), extra-hepatic disease (2.12, 95% CI 1.02–4.38, $p = 0.0419$) and portal vein thrombosis (2.01, 95% CI 1.07–3.79, $p = 0.0297$) as independent prognostic factors for OS (Table 4).

Table 2 Univariate analysis of overall survival in the training cohort

Characteristics	Univariate analysis		
	HR	95% CI	*P* value
Sex	1.04	0.55–1.97	0.8944
Alpha-fetoprotein			
< 400	1		
> 400	1.15	0.68–1.93	0.5952
Age at start of therapy, years			
< 75	1		
> 75	1.12	0.68–1.84	0.6401
ECOG			
0	1		
> 0	1.75	1.03–2.96	0.0357
Child–Pugh			
A	1		
B	1.77	0.68–4.63	0.2404
Aetiology			
Hepatitis C	1		
Hepatitis B	1.16	0.46–2.91	
NASH	1.04	0.57–1.88	
Others	1.09	0.60–1.97	0.9809
Extra-hepatic disease			
No	1		
Yes	1.41	0.80–2.49	0.2335
BCLC			
B	1		
C	1.52	0.94–2.48	0.086
Portal vein thrombosis			
No	1		
Yes	2.68	1.52–4.73	0.0006
Bilirubin, mg/dL			
< Normal value	1		
> Normal value	1.43	0.82–2.49	0.1979
Albumin g/L			
> Normal value	1		
< Normal value	2.07	1.01–4.23	0.0452
ALBI			
1	1		
> 1	5.44	1.58–18.78	0.0073
NLR			
< 3	1		
> 3	1.13	0.67–1.88	0.6345
SII			
< 360	1		
> 360	1.08	0.67–1.73	0.7436
Eosinophils			
> 50	1		
< 50	50.1	11.6–216.5	< 0.0001

ALBI albumin-bilirubin, *BCLC* Barcelona-Clinic Liver Cancer, *CI* confidence interval, *ECOG* Eastern Cooperative Oncology Group, *HR* hazard ratio, *NASH* non-alcoholic steato-hepatitis, *NLR* neutrophil/lymphocyte ratio, *SII* systemic inflammatory index

Differences were also found between eosinophil count $\leq 50*10^9$/L and $> 50*10^9$/L in terms of disease control rate (9.0% vs 54.8%; $p = 0.007$) and best response ($\leq 50*10^9$/L: Complete response 0%, Partial response 10.0%, Stable disease 0%, Progression disease 90.0%; $> 50*10^9$/L: Complete response 0%, Partial response 17.7%, Stable disease 37.0%, Progression disease 45.1%; $p = 0.01$). Moreover, blood eosinophil count was significantly correlated with lymphocytes ($p = 0.011$) and NLR ($p = 0.005$), but not with Alpha-fetoprotein (AFP), ALBI grade, neutrophils, PCR and SII.

In terms of toxicity, eosinophil count was significantly associated with all toxicity ($\leq 50*10^9$/L: 18.1%; $> 50*10^9$/L: 77.5%; $p < 0.0001$) and hand-foot syndrome ($\leq 50*10^9$/L: 0%; $> 50*10^9$/L: 43.7%; $p = 0.005$), but not with diarrhoea ($\leq 50*10^9$/L: 0%; $> 50*10^9$/L 16,3%; $p = 0.14$) or other toxicity.

3.2 Clinical Outcome of Eosinophil Count in the Two Validation Cohorts

In the first validation cohort, median OS was 5.3 months (95% CI 4.0–11.5) and 15.1 months (95% CI 12.3–9.6) for patients with low ($\leq 50*10^9$/L) and high ($> 50*10^9$/L) eosinophil counts, respectively (HR = 4.55, 95% CI 1.24–16.65, $p = 0.022$) (Fig. 2). Median PFS was 1.9 months (95% CI 1.4–4.5) and 3.2 months (95% CI 2.4–5.6) for patients with low and high eosinophil counts, respectively (HR = 3.63, 95% CI 1.23–10.68, $p = 0.019$) (Fig. 2). At the univariate analysis, the other parameter associated with prognosis was portal vein thrombosis ($p = 0.004$) [Table 1 of the Electronic Supplementary Material (ESM)]. The two groups of patients, namely low and high eosinophil counts, were comparable for all the clinical characteristics investigated, except for extra-hepatic disease (Table 3).

Performing the same multivariate analysis of the training cohort, eosinophil count (HR = 3.57, 95% CI 1.19–10.67, $p = 0.0224$), portal vein thrombosis (HR = 5.78, 95% CI 2.28–14.65, $p = 0.0002$), ECOG (HR = 2.46, 95% CI 1.03–5.82, $p = 0.0407$) and extra-hepatic disease (3.28, 95% CI 1.40–7.66, $p = 0.0060$) were confirmed as independent prognostic factors for OS (Table 4). The predictive value of eosinophil counts remained statistically significant even after applying the second multivariate model (HR = 3.85, 95% CI 1.48–9.99, $p = 0.0055$). The model had a C-index of 0.75. As in the training cohort, eosinophils significantly correlated with lymphocytes ($p = 0.006$) and NLR ($p = 0.04$), but not with AFP, ALBI grade, neutrophils, PCR and SII.

In the second validation cohort, median OS was 5.5 months (95% CI 4.4–8.3) and 12.5 months (95% CI 9.7–14.9) for patients with low ($\leq 50*10^9$/L) and high ($> 50*10^9$/L) eosinophil counts, respectively (HR = 3.21, 95% CI 1.83–5.64, $p < 0.0001$) (Fig. 3). At the

Table 3 Patients characteristics in relation to the cut-off value of eosinophils

Characteristics	Training cohort			First validation			Second validation		
	< 50	> 50	*P* value	< 50	> 50	*P* value	< 50	> 50	*P* value
Median age, years (range)	68 (62–74)	71 (66–81)	0.97	69 (63–76)	72 (65–79)	0.67	68 (41–85)	65 (37–84)	0.07
Sex			1.00			0.17			0.26
Male	10 (90.9)	69 (85.2)		9 (100)	46 (81.4)		25 (80.6)	131 (87.9)	
Female	1 (9.1)	12 (14.8)		0 (0)	10 (18.6)		6 (19.4)	18 (12.1)	
Aetiology			0.17			0.78			0.42
HCV	4 (36.4)	34 (42.0)		3 (33.3)	22 (39.3)		15 (48.4)	75 (50.3)	
HBV	3 (27.3)	7 (8.6)		1 (11.1)	3 (5.3)		3 (9.7)	26 (17.4)	
Others	4 (36.3)	40 (49.4)		5 (55.6)	31 (55.4)		13 (41.9)	48 (32.3)	
BCLC stage			0.01			1.00			0.81
B	0 (0)	28 (34.6)		2 (22.2)	16 (28.6)		6 (19.4)	34 (22.8)	
C	11 (100)	53 (65.4)		7 (77.8)	40 (71.4)		25 (80.6)	115 (77.2)	
Child–Pugh			0.29			0.19			0.26
A	9 (81.8)	74 (91.3)		7 (77.8)	52 (92.8)		27 (87.1)	139 (93.3)	
B	2 (18.2)	7 (8.7)		2 (22.2)	4 (7.2)		4 (12.9)	10 (6.7)	
ECOG			0.10			0.70			0.55
0	4 (36.4)	52 (64.2)		5 (55.6)	37 (66.1)		19 (61.3)	80 (53.7)	
> 0	7 (63.6)	29 (35.8)		4 (44.4)	19 (33.9)		12 (38.7)	69 (46.3)	
Extra-hepatic disease			0.28			0.02			1.00
No	6 (54.5)	59 (72.8)		2 (22.2)	36 (62.3)		16 (51.6)	76 (51.0)	
Yes	5 (45.5)	22 (27.2)		7 (77.8)	20 (37.7)		15 (48.4)	73 (49.0)	
Portal vein thrombosis			0.001			1.00			0.84
No	2 (18.2)	56 (69.1)		6 (66.7)	34 (60.7)		19 (61.3)	87 (58.4)	
Yes	9 (81.8)	25 (30.9)		3 (33.3)	22 (39.3)		12 (38.7)	62 (41.6)	
ALBI grade			0.61			0.45			0.22
Grade 1	9 (81.8)	72 (88.8)		8 (88.9)	53 (94.6)		25 (80.6)	133 (89.3)	
Grade 2	2 (18.2)	9 (11.2)		1 (11.1)	3 (5.4)		6 (19.4)	16 (10.7)	

ALBI albumin-bilirubin, *BCLC* Barcelona-Clinic Liver Cancer, *ECOG* Eastern Cooperative Oncology Group, *HBV* hepatitis B virus, *HCV* hepatitis C virus

univariate analysis, the other parameters associated with prognosis were portal vein thrombosis ($p = 0.0132$), ECOG ($p = 0.0174$), Barcelona-Clinic Liver Cancer ($p = 0.0196$) and NLR ($p = 0.008$) (Table 1 of the ESM). The two groups of patients, namely low and high eosinophil counts, were comparable for all clinical characteristics investigated.

Performing the same multivariate analysis of the training cohort, eosinophil count (HR = 1.82, 95% CI 1.13–2.94, $p = 0.0171$) and ECOG (HR = 1.58, 95% CI 1.04–2.40, $p = 0.0306$) were confirmed as independent prognostic factors for OS (Table 4). The predictive value of eosinophil counts remained statistically significant even after applying the second multivariate model (HR = 1.88, 95% CI 1.17–3.03, $p = 0.0087$). The model had a *C* index of 0.50. As in the other cohorts, eosinophils were correlated with lymphocytes ($p = 0.004$) and NLR ($p < 0.001$), but not with AFP, ALBI grade, neutrophils and SII.

3.3 Clinical Outcome of Eosinophil Count in Second-Line Treatment

Among the three cohorts analysed, we considered 116 patients who progressed or were intolerant to sorafenib, for whom data on second-line treatment (capecitabine, regorafenib or best supportive care) and eosinophil counts before starting the subsequent therapy were available. Seventy (60%) patients received capecitabine, 13 (11%) were treated with regorafenib and 33 (29%) were given best supportive care. Eosinophil count had a significant prognostic value among patients receiving regorafenib [median OS was 5.1 months (95% CI 2.7–7.6) and 11.0 months (95% CI 4.2–14.5) for patients with low ($\leq 50*10^9$/L) and high ($> 50*10^9$/L) eosinophil counts, respectively (HR = 11.13, 95% CI 1.67–74.05, $p = 0.0126$) (Fig. 4). On the contrary, for patients treated with capecitabine (HR = 1.38; $p = 0.45$) or best supportive care (HR = 1.78; $p = 0.1954$) the impact

Table 4 Multivariate analysis of all cohorts

	Multivariate analysis for training cohort			Multivariate analysis of first validation cohort			Multivariate analysis of second validation cohort		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Eosinophils									
> 50	1			1			1		
< 50	3.51	1.25–9.87	0.0168	3.57	1.19–10.67	0.0224	1.82	1.13–2.94	0.0171
Portal vein thrombosis									
No	1			1			1		
Yes	2.01	1.07–3.79	0.0297	7.78	2.28–14.65	0.0002	1.44	0.98–2.12	0.0580
ECOG									
0	1			1			1		
> 0	1.61	0.84–3.07	0.1474	2.46	1.03–5.82	0.0407	1.58	1.04–2.40	0.0306
ALBI									
1	1			1			1		
> 1	3.24	1.38–7.59	0.0066	1.83	0.84–3.43	0.1016	1.26	0.72–2.20	0.4022
Extra-hepatic disease									
No	1			1			1		
Yes	2.12	1.02–4.38	0.0419	3.28	1.40–7.66	0.0060	1.05	0.71–1.55	0.4022

ALBI albumin-bilirubin, *CI* confidence interval, *ECOG* Eastern Cooperative Oncology Group, *HR* hazard ratio

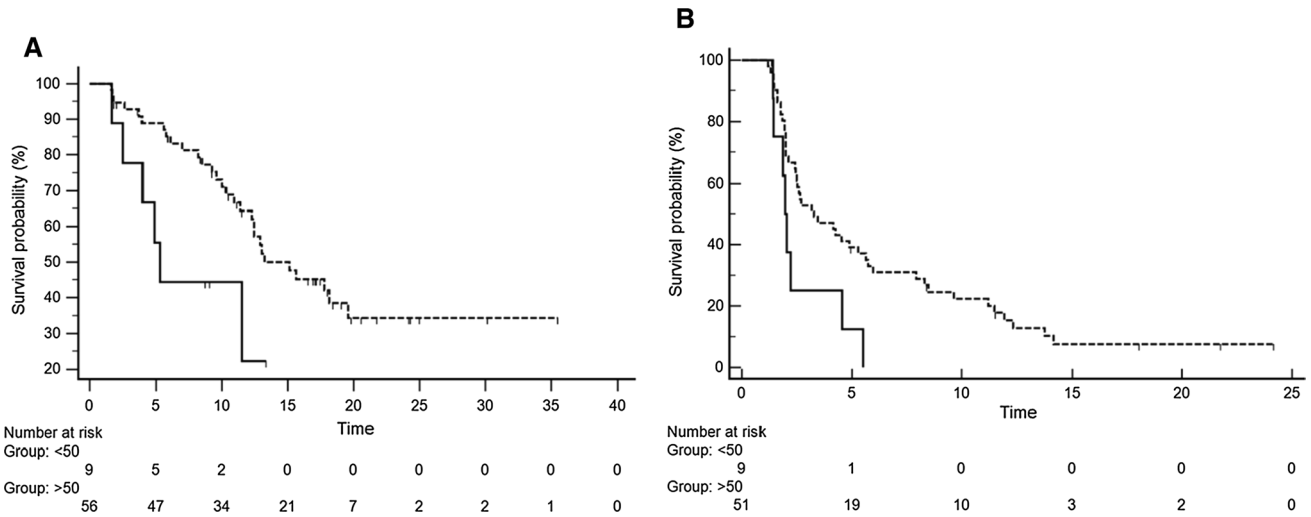

Fig. 2 Kaplan–Meier curves for overall survival (**a**) and progression-free survival (**b**) in the first validation cohort

of an eosinophil count on OS was not statistically significant (Fig. 4).

4 Discussion

In this study, a negative prognostic impact of a low baseline blood eosinophil ($\leq 50*10^9$/L) count was demonstrated both in a training cohort and in two validation cohorts of patients with HCC treated with sorafenib. Indeed, lower pre-treatment levels of eosinophils were significantly associated with worse OS and PFS in all three cohorts examined, as well as with lower DCR and best response rate in the training cohort. In addition, eosinophil count was the only clinical covariate retaining a prognostic value for OS at the multivariate analysis across the whole study population, outperforming other well-established prognostic factors, such as ALBI grade, portal vein thrombosis, Barcelona-Clinic Liver Cancer stage, Child–Pugh, ECOG, NLR, and extra-hepatic disease.

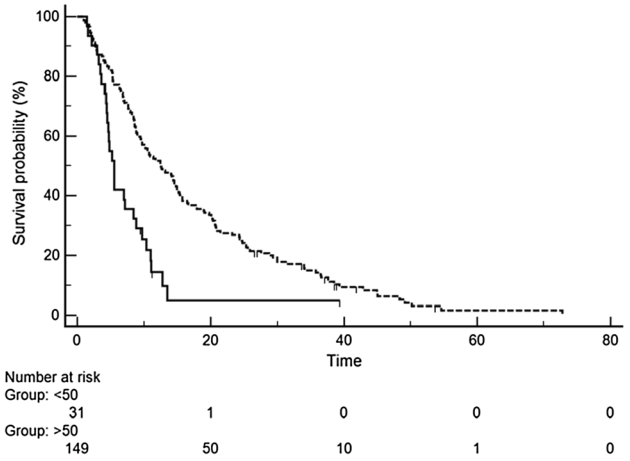

Fig. 3 Kaplan–Meier curves for overall survival in the second validation cohort

To our knowledge, this is the first time the baseline eosinophil count is described as a prognostic factor in patients with sorafenib-treated HCC. In a unique previous report, Steel et al. investigated the role of eosinophils in a cohort of 206 patients with hepatobiliary cancer treated with transarterial chemoembolisation (84% HCC), showing a correlation not only between eosinophil increase over time and cancer-related symptoms, especially pain, but also between lower baseline eosinophil count and shorter survival, regardless of the vascular invasion status [39].

The prognostic significance of blood eosinophils has been demonstrated in several other types of tumours. Generally, a high baseline eosinophil count was significantly associated with better outcomes in metastatic renal cell carcinoma, metastatic melanoma, stage I–III colorectal cancer, non-small-cell lung cancer, cervical cancer and pancreatic cancer [30–35, 44–46]. Interestingly, several studies have

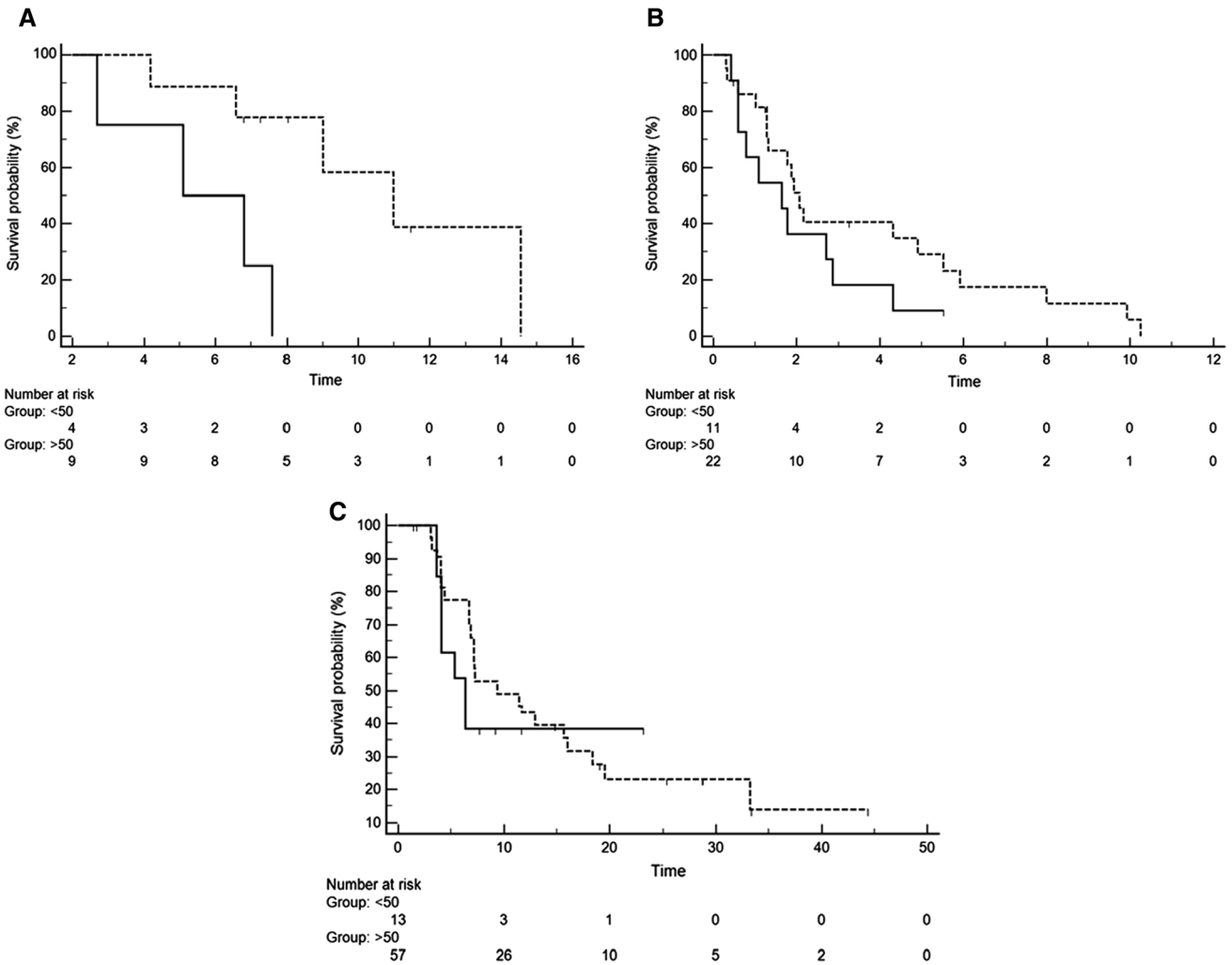

Fig. 4 Kaplan–Meier curves for overall survival in the regorafenib cohort (**a**), metronomic capecitabine (**b**) and best supportive care (**c**)

highlighted that an elevation of eosinophils during treatment was associated with better outcomes in metastatic renal cell carcinoma and metastatic melanoma [47, 48].

Eosinophils represent a minor proportion (≈1%) of circulating leukocytes, canonically associated with allergic disorders and host immune and inflammatory response against parasites [29]. Cytokines and chemokines that play a pivotal role for eosinophil development and activity include interleukin-3, interleukin-5, granulocyte macrophage colony-stimulating factor, eotaxin-1 (CCL11), eotaxin-2 (CCL24) and eotaxin-3 (CCL26) [49]. However, eosinophils exert their cytotoxic activity through several mediators such as eosinophil cationic protein, major basic protein, eosinophil peroxide and eosinophil-derived neurotoxin, included in their secretory granules, but they also produce cytokines, chemokines, angiogenic and lipid factors, through which they participate in immunological and angiogenetic processes [29, 49, 50]. Furthermore, eosinophils also interact with T lymphocytes and other immune cells, either stimulating hypersensitivity reactions or ensuring the balance between T-helper and T-regulatory responses [50]. Moreover, eosinophils play a crucial role in cancer, either as part of the tumour microenvironment or as circulating effectors. Preclinical evidence supports the idea of direct cancer cell killing by eosinophils through the release of cytotoxic granules in the tumour microenvironment [29, 49]. However, Carretero et al. demonstrated that the anti-cancer effect of these cells in melanoma mouse models is only to a lesser extent related to their cytotoxic activity [51], as tumour-infiltrating eosinophils acted mainly by recruiting CD8+ effector T cells inside the tumour, thus promoting T-cell-mediated tumour rejection. In addition, eosinophils exerted an anti-angiogenic and vasculature normalisation activity, promoting the polarisation of tumour-associated macrophages toward a M1-like phenotype, which produces a smaller amount of vascular endothelial growth factor [51].

In our analysis, the cooperation of eosinophils with the immune system was indirectly suggested by the significant correlation highlighted between eosinophils and lymphocytes and NLR, both in the training cohort and in the first validation cohort. On the contrary, this association was not confirmed with other factors, such as neutrophils, inflammatory parameters (PCR and SII), ALBI grade or AFP.

As we considered only patients with HCC treated with sorafenib and because of the lack of a comparative placebo arm, no conclusions can be drawn on a possible role of blood eosinophil counts in predicting response to sorafenib. However, given the aforementioned activity of eosinophils on cancer vasculature within the tumour microenvironment [51], we can speculate that the dismal outcomes reported in patients with HCC with lower baseline eosinophil counts might be related to a smaller benefit from sorafenib, owing to an interaction between this anti-angiogenic agent and eosinophils. In line with this hypothesis and with the results showed in metastatic renal cell carcinoma and metastatic melanoma [47, 48], we reported a reduction in the median blood eosinophil count at disease progression compared with baseline in the subset of patients with available data. However, this result was not statistically significant, possibly owing to the small number of patients eligible for the sub-analysis. Another indirect sign of this speculative interaction is the significant association between pre-treatment eosinophil count and all type of adverse events and especially skin toxicity (hand-foot syndrome), but not with diarrhoea, which suggests that eosinophils act specifically as mediators of cutaneous adverse effects of sorafenib. This also provides another possible explanation of the association of eosinophil count with survival outcomes. Indeed, it is well established that dermatological toxicity correlates with better outcomes in patients with sorafenib-treated HCC, although molecular mechanisms accounting for this phenomenon are not fully elucidated [52, 53]. Furthermore, given the role of eosinophils in several immune-mediated skin disease [50], it is reasonable to think that these cells could promote and sustain the dermatological effects of TKIs, including sorafenib.

A further analysis of survival in patients who progressed on sorafenib and received either second-line treatment (capecitabine or regorafenib) or best supportive care showed that low eosinophil counts retained a negative prognostic significance only in patients treated with regorafenib and not in those receiving capecitabine or no active treatment. This finding hints at a possible role of eosinophils in predicting the efficacy of regorafenib as second-line therapy. From a biological standpoint, this argues in favour of a close interaction between eosinophils and angiogenesis, which could justify their predictive role in relation to anti-angiogenic agents, including regorafenib.

Among the limitations of our analysis, we must mention its retrospective nature, which may have affected the completeness of data and the uniformity of the study population. This limitation has been partially overcome through the inclusion of two validation cohorts, which, although including a small number of patients, were relatively homogenous. Another drawback is the limited number of patients with low baseline blood eosinophil counts; however, through this variable, we selected a subset of patients with significantly worse survival outcomes. In addition, despite some evidence being provided on the possible role of eosinophils in predicting response to regorafenib, these data need to be confirmed and validated in larger and prospective studies.

5 Conclusions

Inflammation, immune response and neoangiogenesis act as key regulators of tumourigenesis in HCC, thus serving as targets of both well-established and emerging therapeutic agents. In this scenario, our analysis identified blood eosinophils as a new prognostic factor in three cohorts of patients with HCC receiving sorafenib. Moreover, concerning second-line treatment, we showed that eosinophils were associated with survival outcomes only in patients receiving regorafenib, suggesting that they could be not only prognostic, but also predictive of efficacy in this setting. Undoubtedly, prospective trials are warranted to provide further insights on this topic. In addition, it would be of particular interest to address the impact of eosinophils on prognosis in patients with HCC treated with immune checkpoint inhibitors. In conclusion, the low cost, easy determination and reproducibility make eosinophils a promising tool for assessing the prognosis of patients with HCC in clinical practice, which should be extensively explored in future prospective investigations.

Declarations

Funding No external funding was used in the conduct of this study or the preparation of this article.

Conflict of Interest Andrea Casadei-Gardini: consultant for Bayer, EISAI, AstraZeneca and Ipsen. Mario Scartozzi: advisory board for EISAI. Fabio Piscaglia: Esaote (Genova, Italia): Institutional Research Contract; Honoraria: Astrazeneca (Milano, Italia): advisory board, Bayer (Leverkusen, Germania): speaker bureau, advisory board, Bracco (Milano, Italia): speaker bureau, BMS (UK): speaker bureau, La Force Guerbet (Minneapolis, USA): speaker bureau, EISAI (Milano, Italia): speaker bureau, advisory board, GE (Milwaukee, USA): consultant, Tiziana Life Sciences (UK) advisory board, Siemens Healthcare (Germany) advisory board, Alkermes (USA) advisory board, IPSEN (Italy and Greece) speaker bureau). Giulia Orsi, Francesco Tovoli, Vincenzo Dadduzio, Caterina Vivaldi, Oronzo Brunetti, Luca Ielasi, Fabio Conti, Giulia Rovesti, Laura Gramantieri, Mario Domenico Rizzato, Irene Pecora, Antonella Argentiero, Federica Teglia, Sara Lonardi, Francesca Salani, Alessandro Granito, Vittorina Zagonel, Giorgia Marisi, Giuseppe Cabibbo, Francesco Giuseppe Foschi, Francesca Benevento, Alessandro Cucchetti and Stefano Cascinu have no conflicts of interest that are directly relevant to the content of this article.

Ethics Approval The present research was performed in accordance with the Declaration of Helsinki (6th revision, 2008) and the study protocol was reviewed and approved by the local ethics committee (CEIIAV: comitato etico IRST IRCCS AVR), study number IRST B041, protocol number 5482/v.1 intern code: L3P1192.

Consent to Aarticipate The study did not involve direct human participants, but only medical records of patients. All participants provided written informed consent to authorise the use of their medical data.

Consent for Publication The authors declare that the manuscript has not been published previously, in whole or in part, and is not under consideration for publication elsewhere. All authors have approved the manuscript and consent to its publication.

Availability of Data and Material The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions Study concept and design: ACG, GO. Data collection: GO, FT, VD, CV, OB, LI, FC, GR, LG, MDR, IP, AA, FT, SL, FS, AG, VZ, GM, GC, FGF, AC, FP, SC, MS, ACG. Experiments and procedures: ACG, GO. Writing and critical revision of the draft: GO, FT, VD, CV, OB, LI, FC, GR, LG, MDR, IP, AA, FT, SL, FS, AG, VZ, GM, GC, FGF, FB, AC, FP, SC, MS, ACG.Study concept and design: ACG, GO. Data collection: GO, FT, VD, CV, OB, LI, FC, GR, LG, MDR, IP, AA, FT, SL, FS, AG, VZ, GM, GC, FGF, AC, FP, SC, MS, ACG. Experiments and procedures: ACG, GO. Writing and critical revision of the draft: GO, FT, VD, CV, OB, LI, FC, GR, LG, MDR, IP, AA, FT, SL, FS, AG, VZ, GM, GC, FGF, FB, AC, FP, SC, MS, ACG.

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