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ORIGINAL RESEARCH

Comparison of Hematologic and Other Prognostic Markers in Metastatic Colorectal Cancer

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Abstract

Background Associations of thrombocytosis, neutrophilia, and lymphopenia with prognosis have been confirmed in many cancers. This study aims at comparing various prognostic indices based on blood counts in metastatic colorectal adenocarcinomas.

Patients and Methods Records from 152 patients with metastatic colorectal cancer who were treated in our center were reviewed. Demographic and disease characteristics and hematologic parameters data were extracted and patients were stratified according to their scores of several hematologic ratios. Hematologic ratios and parameters considered included the platelet-neutrophil to lymphocyte ratio (PNLR), the platelet to lymphocyte ratio (PLR), the neutrophil to lymphocyte ratio (NLR), the Abnormal Hematological Markers Index (AHMI), and the neutrophil-platelet score (NPS). Optimal cutoffs were defined with the aid of an online tool. Baseline parameters of the two groups derived for each tool were evaluated and compared with the χ^2 test. Univariate and multivariate Cox proportional-hazards regression analyses were performed on variables of interest.

Results Progression-Free Survival (PFS) hazard ratios (HR) between the high-risk and low-risk groups derived from the multivariate analyses for each index were as follows: for PNLR 2.0 (95% CI 1.28–3.13), for PLR 1.74 (95% CI 1.13–2.67), for NLR 1.54 (95% CI 1.04–2.29), for AHMI 1.62 (95% CI 1.06–2.46), and for NPS 1.47 (95% CI 1.1–1.96). Overall Survival (OS) hazard ratios (HR) derived from the multivariate analyses for each index were as follows: for PNLR 2.23 (95% CI 1.36–3.66), for PLR 1.68 (95% CI 1.03–2.75), for NLR 1.62 (95% CI 1.06–2.49), for AHMI 1.7 (95% CI 1.07–2.69), and for NPS 1.53 (95% CI 1.11–2.11). Another prognostic index called PRONOPALL, which is based on ECOG PS (0–1 versus 2–3 versus 4), number of metastatic sites (≤ 1 versus ≥ 2), LDH (< 600 U/L versus ≥ 600 U/L), and albumin (≥ 33 g/L versus < 33 g/L), had HRs of 1.75 and 2.20 for PFS and OS, respectively, with a cutoff of < 4 versus ≥ 4. This score has a range of 0 to 10 and points are attributed for the presence of each of the four prognostic factors.

Conclusion In this analysis of metastatic colorectal cancer patients, several ratios and other prognostic tools had prognostic value for both OS and PFS. While other variables held significance for poorer prognosis, PNLR had the highest HR and the highest significance in multivariate analysis for both PFS and OS. Thus, it represents a valid prognostic tool in metastatic colorectal cancer among the spectrum of hematologic parameter-constructed tools.

Keywords Colorectal cancer · Prognosis · Metastatic · Neutrophilia · Lymphopenia · Thrombocytosis · Ratio

Introduction

Colorectal cancer is the most common gastrointestinal malignancy in the western world. Advanced and metastatic disease remains a prominent cause of cancer morbidity and mortality, despite progress in its management [1]. It is therefore clinically important to discover and validate prognostic markers for the disease that are practical, reliable, and inexpensive.

The cancer micro-environment is recognized to play a significant role in cancer progression [2]. Immune and other cells originating from the peripheral blood and recruited to the tumor environment can shape the tumor behavior both directly and indirectly through production of cytokines. More recently, immune cells have come to the forefront of cancer research

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with the successful introduction of immune blockade inhibitors, drugs that potentiate anti-cancer immune function by blocking inhibitory receptors expressed in lymphocytes (e.g., CTLA4, PD-1) [3]. Perturbations in the number of immune cells in peripheral blood may be the result of cytokines produced in the tumor which may in turn affect tumor progression. Lymphocytosis, for example, has been associated with positive prognosis in various cancers [4]. Increased numbers of circulating lymphocytes may be a marker of increased cytokine signals from the tumor that would mobilize and attract marrow or tissue lymphocytes to the tumor microenvironment where they could attack tumor cells under the right conditions.

Neutrophils, on the other hand, have a more controversial role in cancer. Certain subsets of these pro-inflammatory cells may have a pro-tumorigenic effect by induction of immune suppression [2]. Due to this effect, neutrophilia has generally been found to be a negative prognostic factor in several malignancies [5, 6]. Cancer is also often associated with thrombocytosis, as cytokines that stimulate thrombopoiesis, such as IL-6 and thrombopoietin, are elevated in the circulation of some cancer patients [7, 8]. As a result, thrombocytosis has been found to be an adverse prognostic factor in many common cancers, including gastrointestinal cancers [9].

Many studies have reported the use of the platelet to lymphocyte and/or the neutrophil to lymphocyte ratio (PLR and NLR, respectively), culminating in recent systematic reviews and meta-analyses of both these ratios as prognostic markers of cancers [10, 11]. These ratios consider the pro-tumorigenic properties of either platelets or neutrophils respectively while considering the protective effects of lymphocytes in the denominator. Recently, we have proposed a new prognostic tool called the platelet-neutrophil to lymphocyte ratio (PNLR) which included all three circulating cell types together, and we have shown that it is a valuable addition in the prognostic toolkit of metastatic colorectal cancer patients [12]. The current paper aims to compare the different hematologic markers previously proposed for the prognostication of colorectal cancer with the PNLR and with another published prognostic tool called PRONOPALL [13]. Correlations of these prognosticators with an additional published survival prediction tool, the clinical calculator [14], are additionally presented.

Materials and Methods

Records of metastatic colorectal cancer patients diagnosed and treated between 2008 and 2014 in our center were retrospectively reviewed. A total of 152 patients had complete follow-up data on progression and survival and were thus included in the analyses. Data extracted for each patient included sex, age, clinical presentation (high risk presentation defined as obstruction, perforation, or a change in bowel habits and low risk presentation defined as diagnosed with screening or bleeding/anemia), site of the primary tumor in the colon, pathologic grade, previous treatment with adjuvant chemotherapy as well as number of different lines of metastatic treatment, whether the patient was diagnosed with de novo metastatic disease (diagnosed with metastatic disease without a previous history of localized disease) or relapsed from a previous localized disease, organs involved, blood hematologic and biochemical markers (CEA, LDH, albumin, platelets, neutrophils, lymphocytes), ECOG performance status, and whether the patient had a metastasectomy. Oligometastatic disease was defined as metastatic disease contained in one organ, with up to three tumor masses, none above 2 cm in major diameter. All hematologic and biochemical analyses used values obtained before the start of any treatment in the metastatic setting.

Overall survival (OS) was defined as the time from diagnosis of metastatic disease to death or censored to last follow-up. Progression-free survival (PFS) was defined as the time from diagnosis of metastatic disease to documented disease progression or death, whichever occurred first, or censored to last follow-up without progression. Cutoffs for hematologic and biochemical parameters were as follows: for platelets $\geq 350 \times 10^9$/L, neutrophils $\geq 7.5 \times 10^9$/L, lymphocytes $\leq 1.4 \times 10^9$/L, CEA ≥ 5 μg/L, LDH > 210 U/L, and albumin < 35 g/L. The platelet-neutrophil to lymphocyte ratio (PNLR) was calculated by multiplying the pre-treatment platelet count ($\times 10^9$/L) by the neutrophil count ($\times 10^9$/L) divided by the lymphocyte count ($\times 10^9$ /L). The platelet to lymphocyte ratio (PLR) was calculated by dividing the pre-treatment platelet count ($\times 10^9$/L) by the lymphocyte count ($\times 10^9$/L). The neutrophil to lymphocyte ratio (NLR) was calculated by dividing the pre-treatment neutrophil count ($\times 10^9$/L) by the lymphocyte count ($\times 10^9$/L). The Abnormal Hematological Markers Index (AHMI) was defined as the number of abnormal platelet ($\geq 350 \times 10^9$/L), neutrophil ($\geq 7.5 \times 10^9$/L), and lymphocyte ($\leq 1.4 \times 10^9$/L) counts and could thus take a value of 0, 1, 2, or 3 if any or none of those values were abnormal. The patient cohort was dichotomized in the group of AHMI of 0 or 1 (low risk) and the group of AHMI of 2 or 3 (high risk). The NPS score was similarly defined as the number of abnormal counts of neutrophils and platelets (with the same cutoffs), and thus could receive a value of 0, 1, or 2.

The optimal cutoff values for PNLR, PLR, and NLR were calculated using the online X-tile tool (http://www.yalepath.org/edu//PathCamp/x-tile/) [15], and the whole cohort of patients was dichotomized in each case to two cohorts that were used for the various prognostic comparisons. The tool compares survivals by the Log-Rank test after assigning members of the population to a high- and a low-risk group and creating all possible groupings. The cutoff producing the highest p value among the comparisons is retained as the optimal cutoff. Baseline characteristics of the two groups in the case of each prognostic tool (PNLR, PLR, NLR, AHMI,

Table 1 Baseline patient characteristics of the whole group of 152 patients and according to the high- and low-risk groups of the five hematologic tools and the PRONOPALL

	All patients	PNLR					PLR					NLR					Abnormal Hematological Markers					NPS score					PRONOPALL				
		< 2088.92		≥ 2088.92		*P*	< 330		≥ 330		*P*	< 5.62		≥ 5.62		*P*	< 2		≥ 2		*P*	Score = 0		Score ≥ 1		*P*	Score < 4		Score ≥ 4		*P*
		N	%	*N*	%		*N*	%	*N*	%		*N*	%	*N*	%		*N*	%	*N*	%		*N*	%	*N*	%		*N*	%	*N*	%	
N	152	103	*67.8*	49	*32.2*		112	*73.7*	40	*26.3*		90	*59.2*	62	*40.8*		105	*69.1*	47	*30.9*		102	*67.1*	*50*	*32.9*		112	*73.7*	37	*24.3*	
Age																															
< 65	48	30	*29.1*	18	*36.7*	0.346	32	*28.6*	16	*40.0*	0.182	29	*32.2*	19	*30.6*	0.837	29	*27.6*	19	*40.4*	0.116	29	*28.4*	*19*	*38.0*	0.233	38	*33.9*	10	*27.0*	0.436
≥ 65	104	73	*70.9*	31	*63.3*		80	*71.4*	24	*60.0*		61	*67.8*	43	*69.4*		76	*72.4*	28	*59.6*		73	*71.6*	*31*	*62.0*		74	*66.1*	27	*73.0*	
Sex																															
M	95	64	*62.1*	31	*63.3*	0.893	68	*60.7*	27	*67.5*	0.447	49	*54.4*	46	*74.2*	0.013	64	*61.0*	31	*66.0*	0.556	66	*64.7*	*29*	*58.0*	0.422	71	*63.4*	23	*62.2*	0.893
F	57	39	*37.9*	18	*36.7*		44	*39.3*	13	*32.5*		41	*45.6*	16	*25.8*		41	*39.0*	16	*34.0*		36	*35.3*	*21*	*42.0*		41	*36.6*	14	*37.8*	
Clinical presentation																															
Low risk	75	59	*57.8*	16	*32.7*	0.004	60	*54.1*	15	*37.5*	0.073	49	*55.1*	26	*41.9*	0.113	57	*54.8*	18	*38.3*	0.060	57	*55.9*	*18*	*36.7*	0.028	63	*56.8*	11	*29.7*	0.004
High risk	76	43	*42.2*	33	*67.3*		51	*45.9*	25	*62.5*		40	*44.9*	36	*58.1*		47	*45.2*	29	*61.7*		45	*44.1*	*31*	*63.3*		48	*43.2*	26	*70.3*	
ECOG																															
≤ 1	92	73	*70.9*	19	*38.8*	0.000	74	*66.1*	18	*45.0*	0.019	64	*71.1*	28	*45.2*	0.001	69	*65.7*	23	*48.9*	0.050	70	*68.6*	*22*	*44.0*	0.004	91	*81.3*	0	*0.0*	
> 1	60	30	*29.1*	30	*61.2*		38	*33.9*	22	*55.0*		26	*28.9*	34	*54.8*		36	*34.3*	24	*51.1*		32	*31.4*	*28*	*56.0*		21	*18.8*	37	*100.0*	
Adjuvant chemo																															
Y	46	36	*35.0*	10	*20.4*	0.068	38	*33.9*	8	*20.0*	0.100	28	*31.1*	18	*29.0*	0.784	36	*34.3*	10	*21.3*	0.107	36	*35.3*	*10*	*20.0*	0.054	42	*37.5*	3	*8.1*	0.001
N	106	67	*65.0*	39	*79.6*		74	*66.1*	32	*80.0*		62	*68.9*	44	*71.0*		69	*65.7*	37	*78.7*		66	*64.7*	*40*	*80.0*		70	*62.5*	34	*91.9*	
Line of palliative chemo																															
0–1	109	76	*73.8*	33	*67.3*	0.410	83	*74.1*	26	*65.0*	0.272	64	*71.1*	45	*72.6*	0.843	79	*75.2*	30	*63.8*	0.149	74	*72.5*	*35*	*70.0*	0.743	76	*67.9*	30	*81.1*	0.124
> 1	43	27	*26.2*	16	*32.7*		29	*25.9*	14	*35.0*		26	*28.9*	17	*27.4*		26	*24.8*	17	*36.2*		28	*27.5*	*15*	*30.0*		36	*32.1*	7	*18.9*	
De novo metastatic																															
Y	62	31	*30.1*	31	*63.3*	< 0.001	35	*31.3*	27	*67.5*	< 0.001	33	*36.7*	29	*46.8*	0.213	32	*30.5*	30	*63.8*	<0.001	33	*32.4*	*29*	*58.0*	0.003	37	*33.0*	23	*62.2*	0.002
N	90	72	*69.9*	18	*36.7*		77	*68.8*	13	*32.5*		57	*63.3*	33	*53.2*		73	*69.5*	17	*36.2*		69	*67.6*	*21*	*42.0*		75	*67.0*	14	*37.8*	
Oligometastatic																															
Y	34	27	*28.1*	7	*14.9*	0.081	31	*29.5*	3	*7.9*	0.007	24	*28.2*	10	*17.2*	0.129	23	*23.7*	11	*23.9*	0.979	25	*26.3*	*9*	*18.8*	0.316	34	*32.7*	0	*0.0*	
N	109	69	*71.9*	40	*85.1*		74	*70.5*	35	*92.1*		61	*71.8*	48	*82.8*		74	*76.3*	35	*76.1*		70	*73.7*	*39*	*81.3*		70	*67.3*	36	*100.0*	
Metastatectomy																															
Y	30	26	*26.8*	4	*8.5*	0.011	28	*26.4*	2	*5.3*	0.006	24	*27.9*	6	*10.3*	0.011	23	*23.5*	7	*15.2*	0.256	25	*26.0*	*5*	*10.4*	0.030	30	*28.6*	0	*0.0*	
N	114	71	*73.2*	43	*91.5*		78	*73.6*	36	*94.7*		62	*72.1*	52	*89.7*		75	*76.5*	39	*84.8*		71	*74.0*	*43*	*89.6*		75	*71.4*	36	*100.0*	

For some characteristics, the total number of patients included is less than 152 because of missing data

Table 2 Baseline hematologic and biochemical markers of the whole group of 152 patients and according to the high- and low-risk groups of the five hematologic tools and the PRONOPALL

	All patients	PNLR					PLR					NLR					Abnormal Hematological Markers					NPS score					PRONOPALL				
		< 2088.92		≥ 2088.92		P	< 330		≥ 330		P	< 5.62		≥ 5.62		P	< 2		≥ 2		P	Score = 0		Score ≥ 1		P	Score < 4		Score ≥ 4		P
		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%		N	%	N	%	
CEA																															
< 5	49	36	*38.3*	13	*28.9*	0.277	37	*37.0*	12	*30.8*	0.490	31	*38.3*	18	*31.0*	0.378	39	*40.2*	10	*23.8*	0.063	41	*43.2*	8	*18.2*	0.004	39	*37.1*	10	*30.3*	0.474
≥ 5	90	58	*61.7*	32	*71.1*		63	*63.0*	27	*69.2*		50	*61.7*	40	*69.0*		58	*59.8*	32	*76.2*		54	*56.8*	36	*81.8*		66	*62.9*	23	*69.7*	
LDH																															
< 210	86	67	*65.7*	19	*39.6*	0.003	66	*60.0*	20	*50.0*	0.273	58	*65.2*	28	*45.9*	0.019	70	*67.3*	16	*34.8*	< 0.001	68	*67.3*	18	*36.7*	< 0.001	72	*64.3*	14	*37.8*	0.005
≥ 210	64	35	*34.3*	29	*60.4*		44	*40.0*	20	*50.0*		31	*34.8*	33	*54.1*		34	*32.7*	30	*65.2*		33	*32.7*	31	*63.3*		40	*35.7*	23	*62.2*	
Albumin																															
< 35	26	12	*11.9*	14	*29.2*	0.009	16	*14.7*	10	*25.0*	0.141	10	*11.4*	16	*26.2*	0.019	15	*14.6*	11	*23.9*	0.165	14	*14.0*	12	*24.5*	0.113	11	*9.8*	15	*40.5*	< 0.001
≥ 35	123	89	*88.1*	34	*70.8*		93	*85.3*	30	*75.0*		78	*88.6*	45	*73.8*		88	*85.4*	35	*76.1*		86	*86.0*	37	*75.5*		101	*90.2*	22	*59.5*	
Platelets																															
< 350	112	91	*88.3*	21	*42.9*	< 0.001	99	*88.4*	13	*32.5*	< 0.001	72	*80.0*	40	*64.5*	0.033	95	*90.5*	17	*36.2*	< 0.001	90	*88.2*	22	*44.0*	< 0.001	87	*77.7*	22	*59.5*	0.030
≥ 350	40	12	*11.7*	28	*57.1*		13	*11.6*	27	*67.5*		18	*20.0*	22	*35.5*		10	*9.5*	30	*63.8*		12	*11.8*	28	*56.0*		25	*22.3*	15	*40.5*	
Neutrophils																															
< 7.5	113	95	*92.2*	18	*36.7*	< 0.001	92	*82.1*	21	*52.5*	< 0.001	85	*94.4*	28	*45.2*	< 0.001	100	*95.2*	13	*27.7*	< 0.001	102	*100.0*	11	*22.0*		92	*82.1*	19	*51.4*	< 0.001
≥ 7.5	39	8	*7.8*	31	*63.3*		20	*17.9*	19	*47.5*		5	*5.6*	34	*54.8*		5	*4.8*	34	*72.3*		0	*0.0*	39	*78.0*		20	*17.9*	18	*48.6*	
Lymphocytes																															
< 1.4	98	53	*51.5*	45	*91.8*	< 0.001	60	*53.6*	38	*95.0*	< 0.001	38	*42.2*	60	*96.8*	< 0.001	53	*50.5*	45	*95.7*	0.000	61	*59.8*	37	*74.0*	0.086	69	*61.6*	27	*73.0*	0.211
≥ 1.4	54	50	*48.5*	4	*8.2*		52	*46.4*	2	*5.0*		52	*57.8*	2	*3.2*		52	*49.5*	2	*4.3*		41	*40.2*	13	*26.0*		43	*38.4*	10	*27.0*	

For some characteristics, the total number of patients included is less than 152 because of missing data

Table 3 Median (95% CI) PFS and OS comparison of all predictors

		PFS		OS	
All patients		10.80	(8.67–13.07)	16.88	(12.84–20.71)
PNLR	< 2088.92	13.33	(11.60–16.30)	21.75	(17.16–25.37)
	≥ 2088.92	6.46	(4.59–8.74)	9.63	(6.38–12.54)
PLR	< 330	12.85	(9.73–15.17)	20.27	(14.65–23.95)
	≥ 330	7.40	(4.92–9.03)	10.73	(7.96–14.45)
NLR	< 5.62	13.66	(11.72–16.60)	21.75	(17.84–24.76)
	≥ 5.62	6.77	(4.69–9.02)	9.99	(5.92–12.95)
Abnormal Markers	< 2	12.85	(9.70–15.03)	20.32	(14.52–24.00)
	≥ 2	8.25	(4.92–10.03)	10.82	(8.44–16.95)
NPS	0	13.33	(11.27–15.55)	21.75	(16.12–25.25)
	≥ 1	7.33	(3.32–8.87)	9.21	(4.79–11.98)
PRONOPALL score	< 4	13.41	(11.53–15.93)	21.86	(17.83–24.80)
	≥ 4	2.89	(2.03–7.55)	3.32	(2.16–9.63)

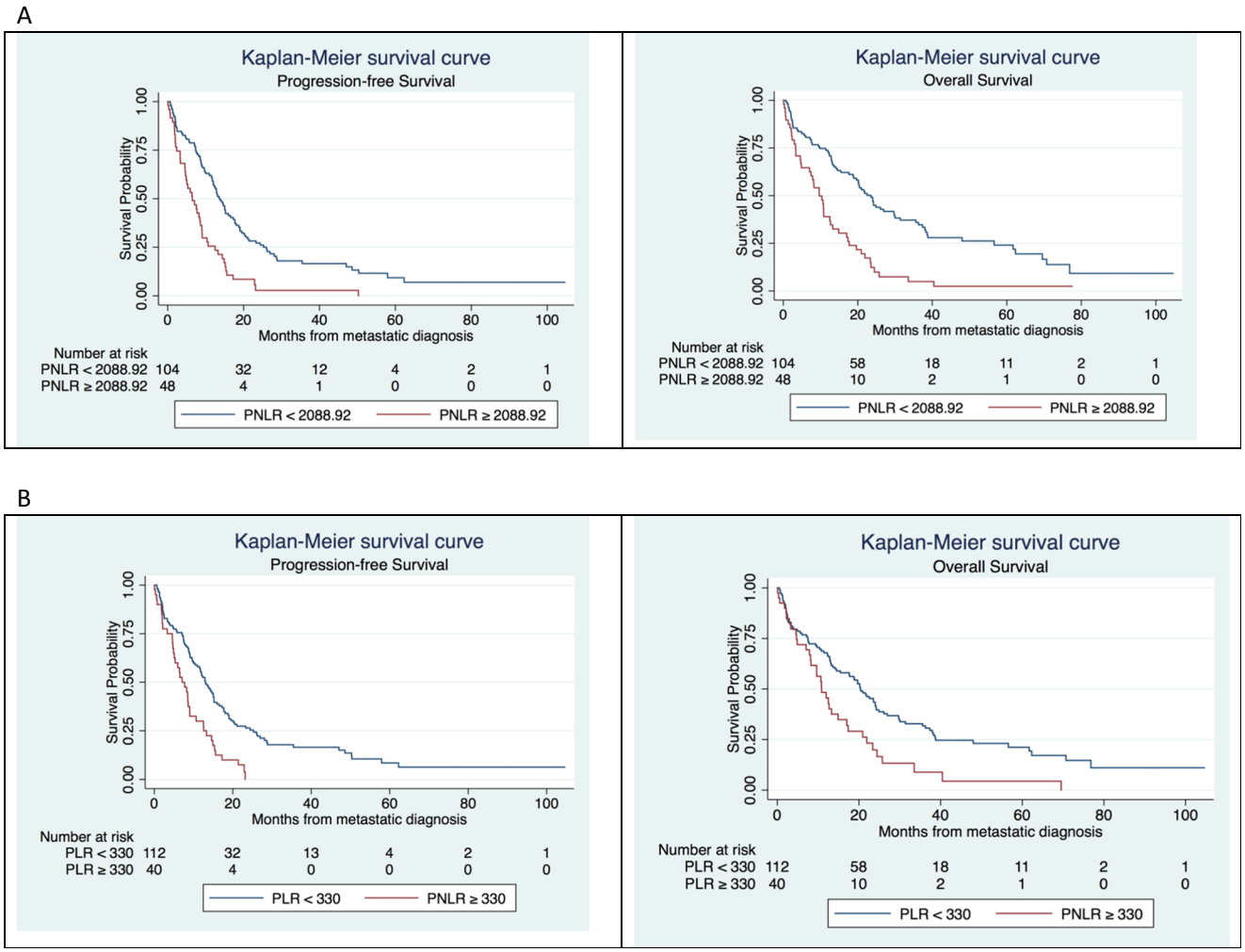
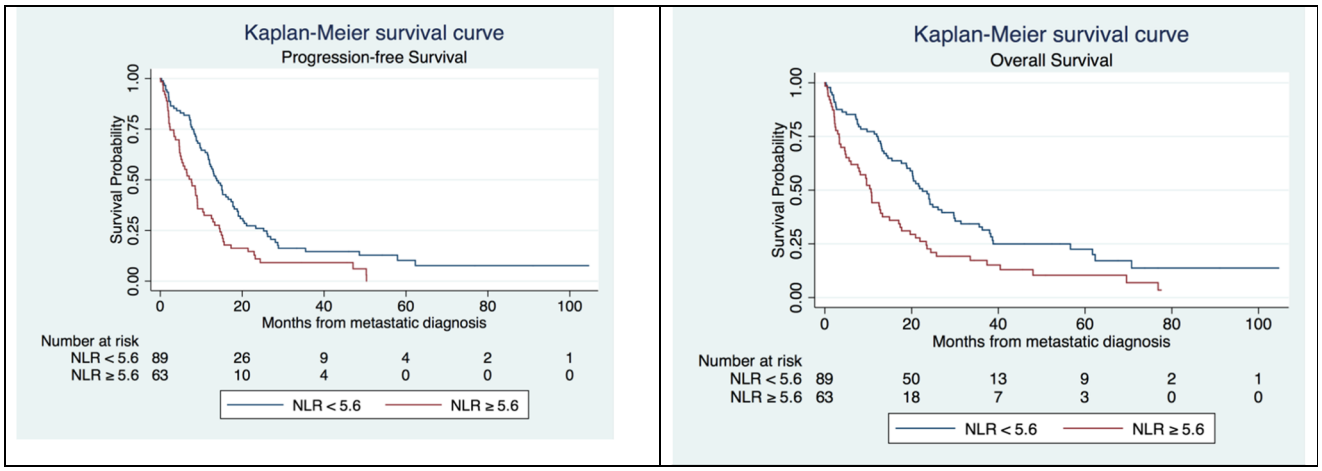


Fig. 1 Kaplan-Meier curves of PFS (left) and OS (right) of the high-risk and low-risk groups for each of the hematologic tools. Curves were compared with the Log-Rank test. **a** PNLR PFS, $x^2 = 21.86$; $p < 0.0001$; OS, $x^2 = 23.21$; $p < 0.0001$. **b** PLR PFS, $x^2 = 18.86$; $p < 0.0001$; OS, $x^2 = 15.72$; $p = 0.0001$. **c** NLR PFS, $x^2 = 12.03$; $p = 0.0005$; OS, $x^2 = 10.97$; $p = 0.0009$. **d** Abnormal Markers (AHMI) PFS, $x^2 = 8.47$; $p = 0.0036$; OS, $x^2 = 6.82$; $p = 0.009$. **e** NPS PFS, $x^2 = 16.41$; $p = 0.0001$; OS, $x^2 = 17.99$; $p < 0.0001$

C



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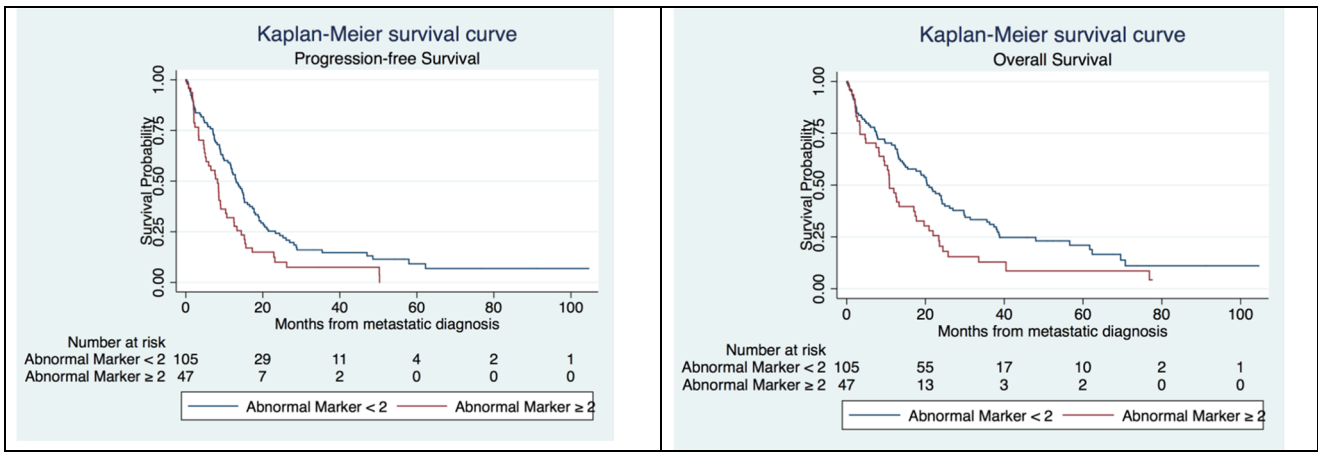

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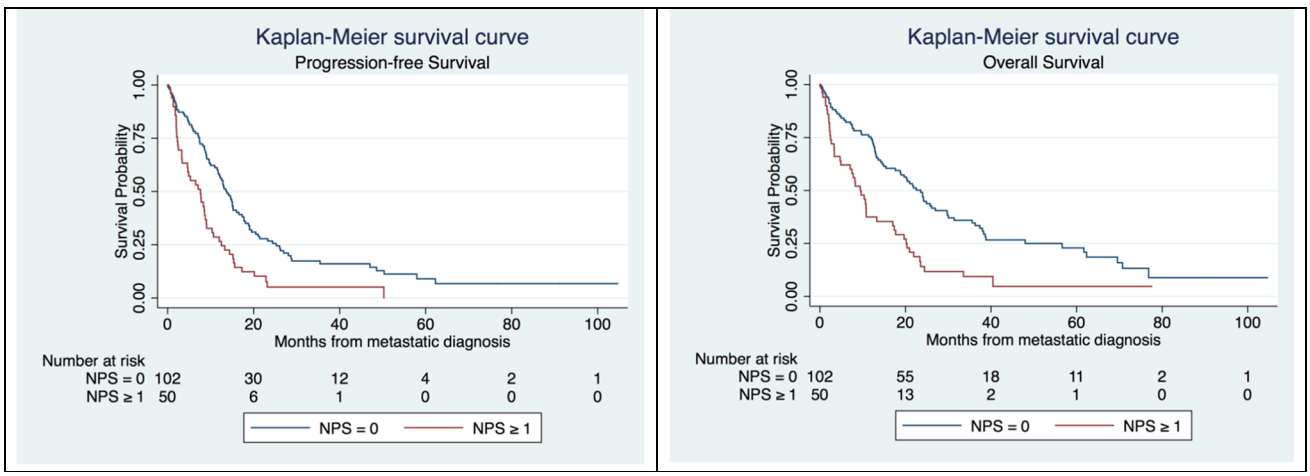


Fig. 1 (continued)

and NPS) were compared, and significance between the groups was tested using the χ^2 test. OS and PFS Kaplan-Meier curves of groups were constructed and compared with the Log-Rank test. Individual univariate analyses were completed on all extracted variables, and significant variables were included in the multivariate analysis model. The Cox proportional-hazards model was used for regression analysis, using in each occasion the prognostic tool and other

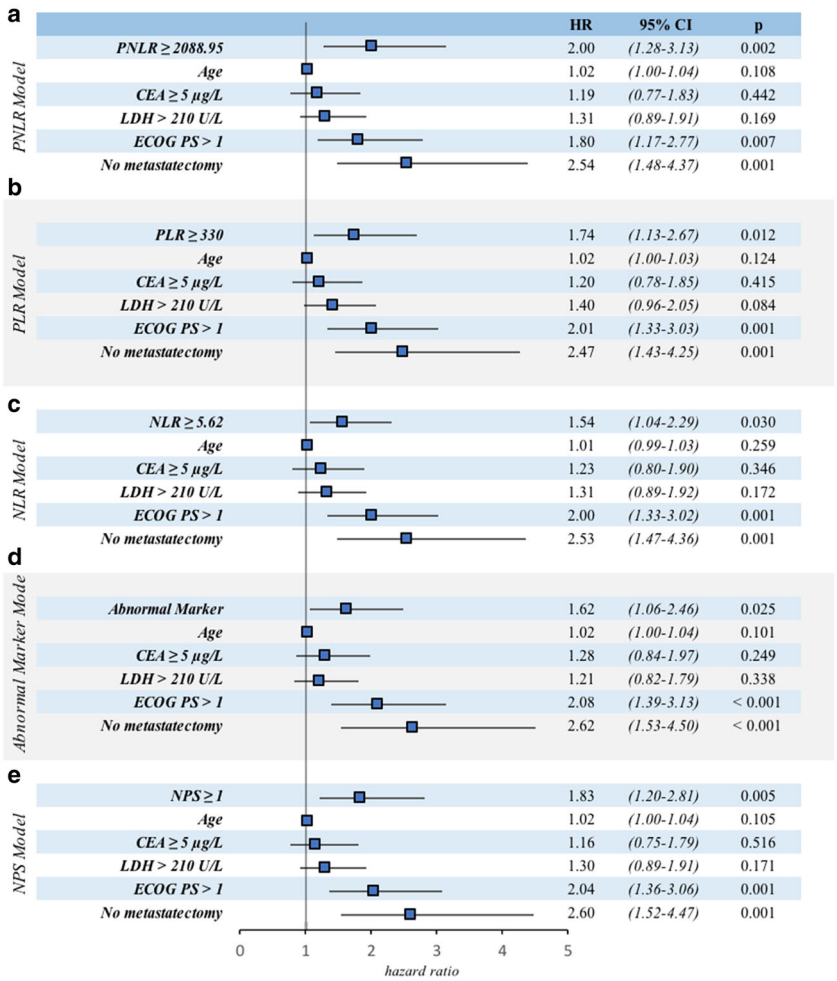


Fig. 2 **a**–**e** Multivariate analyses for PFS of the five hematologic markers. **a** PNLR, **b** PLR, **c** NLR, **d** Abnormal Markers (AHMI), **e** NPS

prognostic variables that were significant in the univariate analysis. It was also used to compute hazard ratios (HR). All *p* values were considered significant at values of < 0.05.

Two other published prognostic tools, the PRONOPALL score and the clinical calculator for early mortality, were considered for their value as prognosticators in our cohort. The PRONOPALL score is a prognostic tool that was proposed by Bourgeois et al. [13] for use in patients receiving palliative treatment for a solid tumor and is calculated from ECOG PS (0–1 versus 2–3 versus 4), number of metastatic sites (≤ 1 versus ≥ 2), LDH (< 600 versus ≥ 600 U/L), and albumin (≥ 33 versus < 33 g/L). The PRONOPALL score range is between 0 and 10, and a high score is associated with poor outcomes. Patients were assigned to two groups according to their PRONOPALL score (< 4 or ≥ 4) and compared for survival outcomes, in a manner similar to the comparisons in the case of hematologic ratios.

The clinical calculator for early mortality was proposed specifically as a predictor of mortality in metastatic colorectal cancer patients and attributes points for calculation of 90-day mortality from age, BMI, ECOG PS, BRAF status, number of metastatic sites, total bilirubin, hemoglobin, WBC, and neutrophil count [14]. A modified version of the clinical calculator was used without consideration of the BRAF status of tumors, as this information was available for only a few patients in our cohort. The accuracy of the clinical calculator for predicting mortality at 90 days was examined in our population. Correlation of the clinical calculator score with the hematologic ratio prognosticators and the PRONOPALL score was evaluated by the Pearson R or the Spearman rho test.

Hematologic parameters and the PRONOPALL tool contain different non-overlapping data for their predictions. A combination of the best hematologic predictor (proved to be PNLR) with PRONOPALL was thus devised and assayed as a potential new predictor of outcomes in metastatic colorectal cancer. According to this combined PNLR-PRONOPALL predictor, patients were classified in the high-risk group if they had both a high PNLR score and a PRONOPALL score of ≥ 4. All other patients with either or both scores below the prespecified limits were included in the low-risk group.

Data analysis was performed in Microsoft Excel (Microsoft corp., Redmond, WA), and all statistical calculations were performed in STATA 13.1 (StataCorp.).

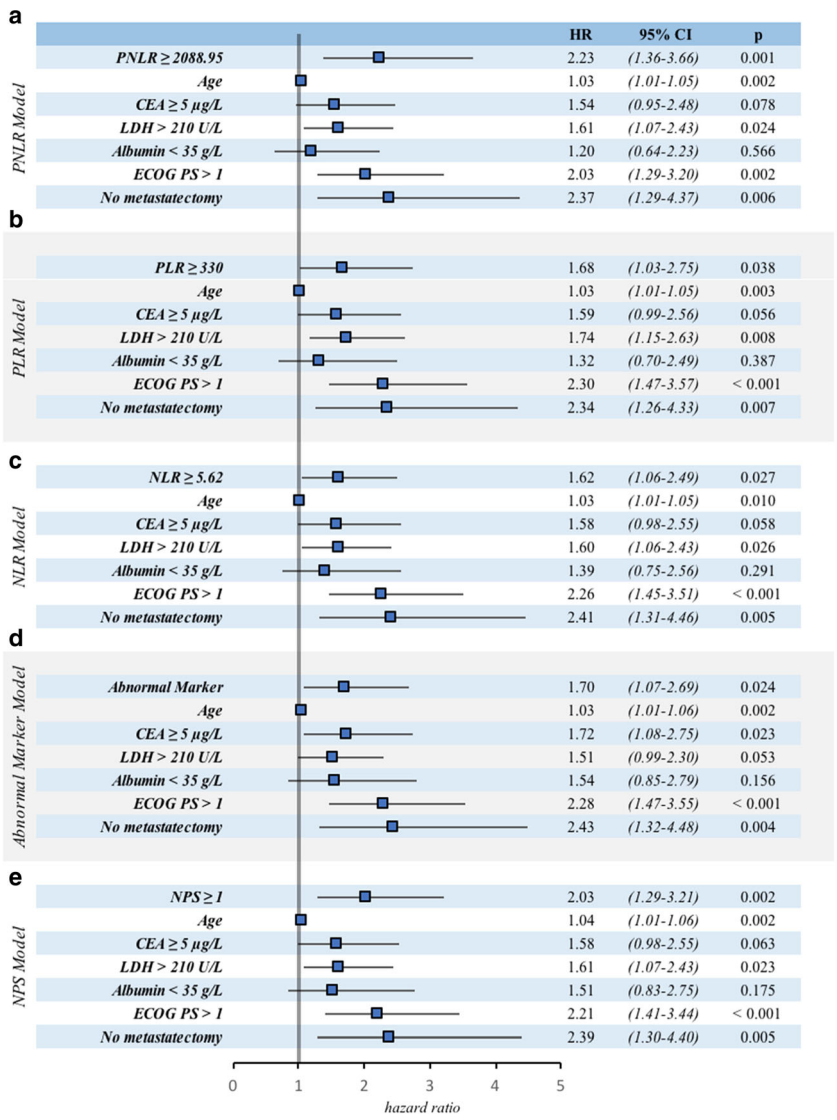


Fig. 3 **a**–**e** Multivariate analyses for OS of the five hematologic markers. **a** PNLR, **b** PLR, **c** NLR, **d** Abnormal Markers (AHMI), **e** NPS

Results

Hematologic markers examined included three ratios (PNLR, PLR, and NLR) and two indexes (AHMI and NPS). Optimal cutoff values for PNLR, PLR, and NLR were determined to be 2088.92, 330, and 5.62 respectively. For the AHMI analysis, the cutoff was set to < 2 or ≥ 2. For the analysis regarding the NPS score, the two groups were with scores 0 (low risk) and 1 or 2 (high risk). For the PRONOPALL analysis, the intermediate and bad prognostic groups were lumped together and compared with the good prognosis group.

Among the total cohort of 152 patients included in the study, about two thirds of the patients were older than 65 years old, 60% had an ECOG PS of 0 or 1, and 40% presented with de novo metastatic disease. Tables 1 and 2 show the baseline clinical and hematologic parameters of the low- and high-risk groups for each of the six studied prognostic tools. The number of patients in the low-risk groups varied slightly in the different tools and ranged from 59.2% in the NLR to 73.7% in the PLR and the PRONOPALL (Table 1).

Several baseline patient characteristics and hematologic values had statistically significant differences in the two groups produced by each of the six prognostic tools (Tables 1 and 2). Most notably, this statistically significant difference in baseline characteristics was most consistent with ECOG PS (≤ 1 versus > 1) and de novo metastatic presentation, being present in five of the six tools.

Median PFS of the whole cohort was 10.8 months (95% CI 8.67–13.07 months) and median OS was 16.9 months (95% CI 12.84–20.71 months). Table 3 shows the median PFS and median OS of the two groups in each predictive tool. The low-risk groups had a similar median PFS, all ranging in a 13–14-month window. Conversely, the high-risk groups had a median PFS of around 7 to 8 months with all tools except with the PRONOPALL, where the high-risk group had a median PFS of 2.9 months. Similarly, in the OS analysis, all six tools

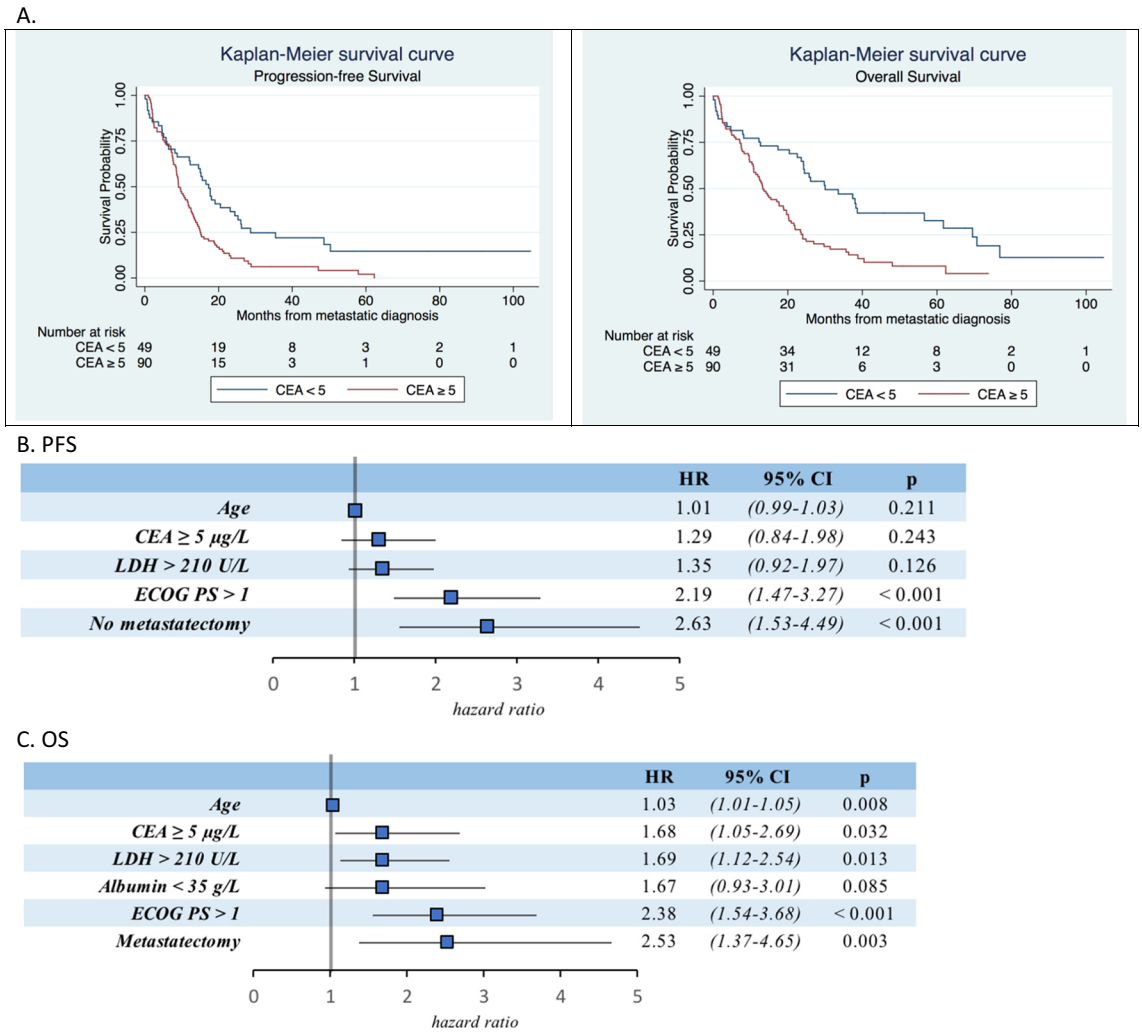


Fig. 4 **a** Kaplan-Meier curves of PFS (left) and OS (right) of the high-risk and low-risk groups for CEA. Log-Rank test for PFS $x^2 = 11.36$, $p = 0.0007$ and for OS $x^2 = 15.58$, $p = 0.0001$. **b** Multivariate analyses for PFS and OS. CEA shows no statistical significance for PFS ($p = 0.2$) but retains statistical significance for OS ($p = 0.032$)

produced low-risk groups with a median OS of 21 to 22 months and high-risk groups with median OS of 9 to 10 months. The exception once again held true for the PRONOPALL high-risk group: the median OS was 3.32 months. The PRONOPALL thus had the best discriminatory power for both median PFS and median OS.

In univariate analysis for PFS, the five hematologic tools showed statistically significant discriminatory power with the PNLR being the strongest. PFS K-M curves with Log-Rank tests of each of the five hematologic parameters tools are given in Fig. 1a–e, left panels. In multivariate analysis for PFS, all hematologic tools retained statistical significance with HR ranging from 1.54 for the NLR to 2.0 for the PNLR (Fig. 2a–e). PNLR had the most robust HR 2.00 (95% CI 1.28–3.13, $p = 0.002$) (Fig. 2a).

In univariate analysis for OS, the five hematologic tools showed similarly statistically significant discriminatory power (Fig. 1a–e). OS K-M curves and Log-Rank tests of each of the five hematologic parameters tools are shown in Fig. 1a–e, right panels. In multivariate analysis for OS, all hematologic tools also remained statistically significant (Fig. 3a–e), with PNLR once again having the highest HR (2.23, 95% CI 1.36–3.66, $p = 0.001$) (Fig. 3a).

CEA is a serum tumor marker that is used for evaluation of treatment response in colorectal cancer and has been proposed as a prognostic marker. In our series, a CEA ≥ 5 µg/L was associated with both a worse PFS and OS (Fig. 4a). However, in multivariate analysis models, CEA retained statistical significance only for OS, losing its significant prognostic discrimination for PFS (Fig. 4b, c). In these multivariate models, which did not include hematologic ratios, ECOG PS and whether metastatectomy was part of a patient's treatment were the most significant factors for both PFS and OS (Fig. 4b, c).

A univariate analysis for the PRONOPALL tool showed significantly worse PFS and OS for the group of patients with scores ≥ 4 (Log-Rank $p < 0.0001$ for both PFS and OS,

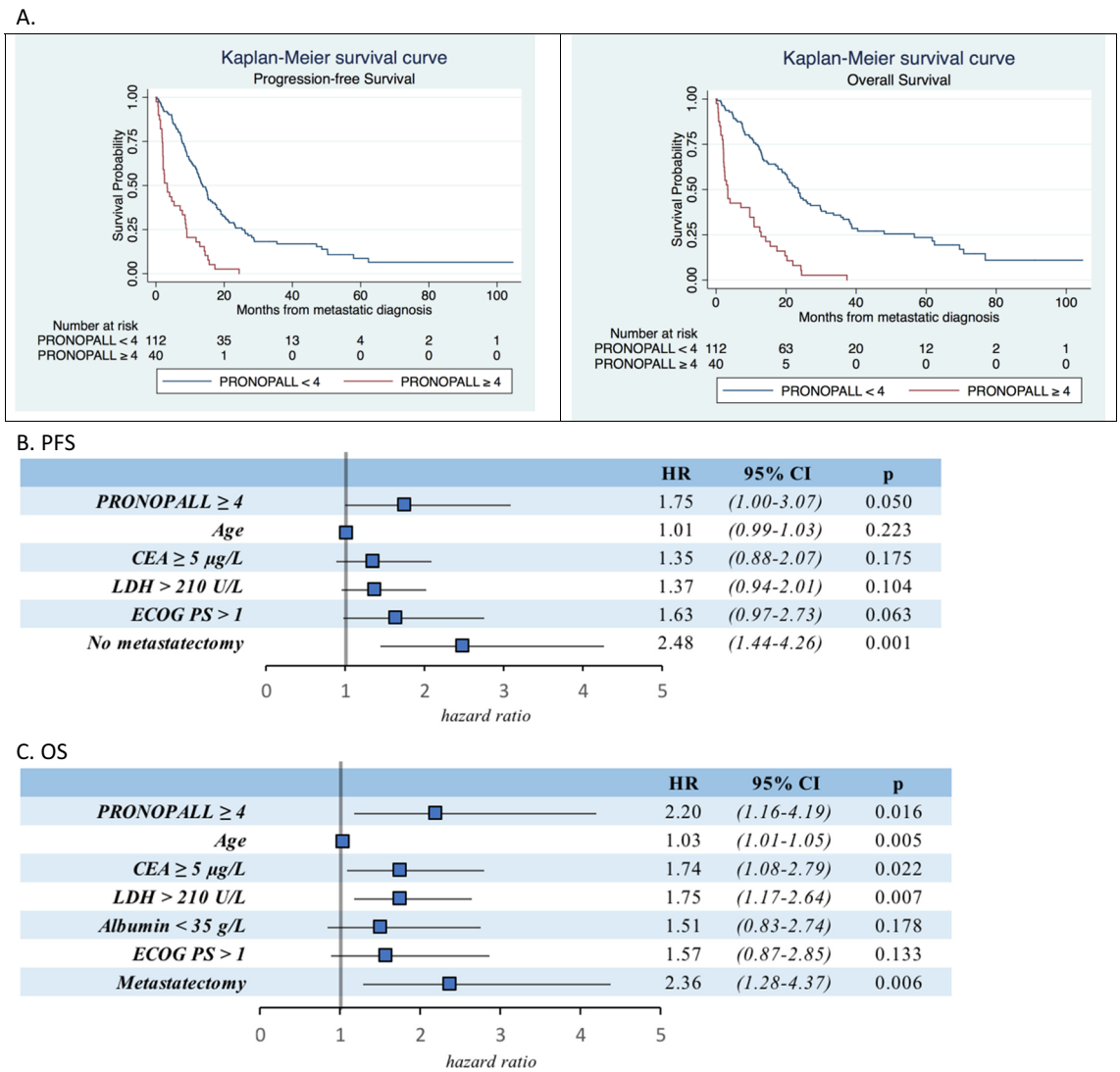


Fig. 5 **a** Kaplan-Meier curves of PFS (left) and OS (right) of the high-risk and low-risk groups for PRONOPALL. Log-Rank test for PFS $x^2 = 43.68$, $p < 0.0001$ and for OS $x^2 = 54.4$, $p < 0.0001$. **c** Multivariate analyses for PFS and OS. PRONOPALL shows borderline significance for PFS ($p = 0.05$) and statistical significance for OS ($p = 0.016$)

Fig. 5a). In multivariate analyses, although the PRONOPALL retained significance for both PFS and OS, it was borderline in the former ($p = 0.05$) (Fig. 5b, c). It is important to note, however, that the multivariate models have included several of the components used in the PRONOPALL score calculations, as they had been significant in univariate analyses. This may have resulted in reducing the significance of the PRONOPALL score under these models.

Next, the clinical calculator of 90-day mortality was evaluated in our population and correlations with the hematologic and PRONOPALL tools were explored. The mean probability of death at 90 days obtained by the clinical calculator in the group of patients that were actually alive at 90 days ($n = 124$) was 6% (95% CI 5–8.2%) (Fig. 6). The mean probability of death at 90 days obtained by the clinical calculator in the group of patients that had died at or before 90 days ($n = 27$) was 16% (95% CI 12–20%). This difference in means is highly statistically significant but of limited clinical utility due to the fact that the calculator significantly underestimated the risk of death at 90 days, given that 84% of patients that had died by 90 days had been predicted to be alive. It must be noted that the calculator attributes 20 points in cases with BRAF mutations, and as mentioned previously, this genetic data was lacking for the majority of our cohort. However, these mutations are prevalent in about 2% of colon cancers, implying that only in these cases would the risk have been underestimated by the calculator. In terms of correlation, the points obtained from the clinical calculator prediction had only limited to moderate correlation with the hematologic ratios (Fig. 7a–c). Pearson R coefficients were 0.45, 0.27, and 0.48 for the correlation with PNLR, PLR, and NLR respectively. Similarly, the Spearman correlation of the clinical

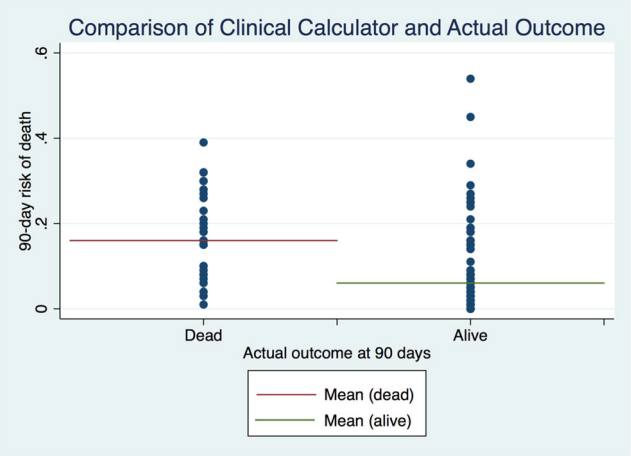


Fig. 6 Percentage prediction of 90-day risk of mortality by the clinical calculator and actual 90-day mortality outcomes in the series of 151 patients. The mean prediction of the group of patients that were actually alive at 90 days ($n = 124$) was 0.06 (95% CI 0.05–0.082), and the mean prediction of the group of patients that were actually dead at 90 days ($n = 27$) was 0.16 (95% CI 0.12–0.20). $t = 5.24$, $p < 0.0001$. Risk could not be calculated in one patient because of missing data

calculator prediction points with the AHMI and the NPS score was 0.32 and 0.46, respectively.

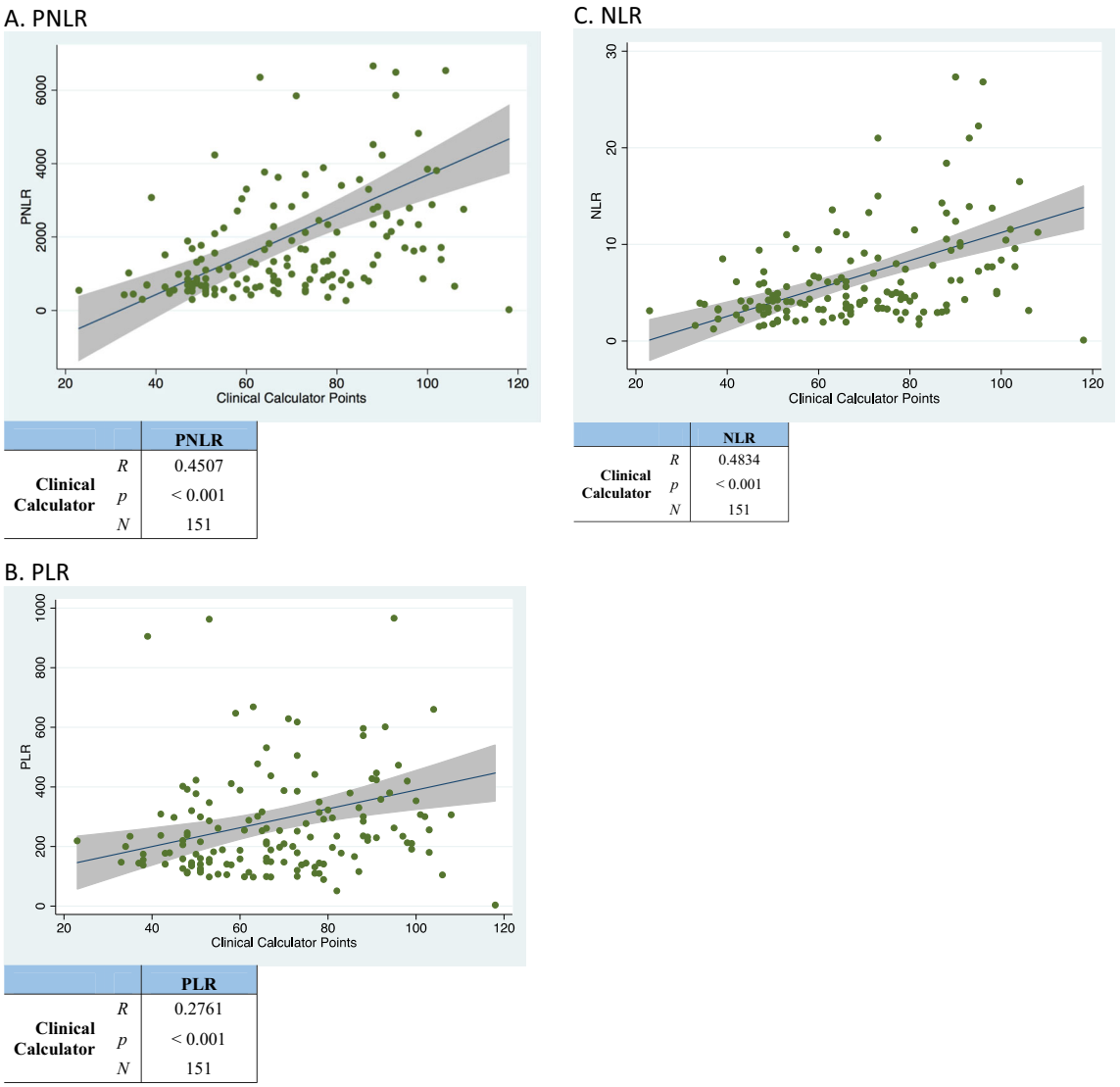
Finally, we investigated whether combining the PRONOPALL tool with the PNLR index could further add to the discriminatory prognostic value of each tool alone (Fig. 8). Although the combined tool had similar discriminatory capacity for PFS and OS as did PRONOPALL alone in univariate analysis (Log-Rank x^2 of 28.65 and 33.97 in the combination versus 43.68 and 54.4 in the PRONOPALL alone for PFS and OS, respectively), the combination became non-significant in the multivariate model. This may be partially due to a redundancy from the inclusion of several components of PRONOPALL in the multivariate model (ECOG PS, LDH, and albumin) as well as the limited numbers of patients ($n = 24$) classified in the high-risk group with the two combined tools.

Discussion

Colorectal cancer is one of the most common cancers in general and the most common gastrointestinal malignancy. It presents in the metastatic stage in about one fourth of patients, and a significant number of patients with locally advanced disease will eventually progress to develop metastatic disease. Although the outcomes of patients with metastatic colorectal cancer have improved in recent years with median survival exceeding 2 years with contemporary therapies, there still exists significant variability in both PFS and OS in these patients. Thus, prediction of survival outcomes in metastatic colorectal cancer is an important clinical need in oncology. Various prognostic tools have been proposed in this setting. Circulating peripheral blood cells such as neutrophils, lymphocytes, and platelets have been proposed as prognostic markers in various cancers. Neutrophils and lymphocytes are important parts of the innate and the adaptive immune systems, and they both participate in the formation and shaping of the tumor micro-environment [16]. In this micro-environment, neutrophils mostly promote a non-specific pro-inflammatory reaction that may be exploited by tumor cells for promotion of their survival [17, 18]. Moreover, specific myeloid cell subsets, such as myeloid-derived suppressor cells, interfere with the anti-tumor function of lymphocytes [19]. In contrast, T lymphocytes, the main effector cells of the adaptive immune system, infiltrate tumor beds of immunogenic tumors, and their presence in histologic sections is usually associated with improved outcomes. Conversely, lymphopenia is a marker indicating worse overall survival in early colorectal cancer [20].

Thrombocytosis has been associated with adverse cancer outcomes in cancers from several primary sites, including breast, lung, and gastrointestinal cancers [21–23]. Mechanistically, platelets may promote carcinogenesis in several ways, such as a mechanical protection of tumor cells in transit in the circulation, as well as by enriching the tumor micro-environment for several bioactive pro-tumorigenic molecules transported and released from their granules [24, 25]. Examples of ligands contained in platelet alpha or dense granules that may serve as tumor-promoting signals include vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor ß (TGFß), Interleukin 1ß (IL-1ß), IL-8, and CXC motif-containing ligand 12 (CXCL12) [26, 27].

Given these considerations, various prognostic markers have been proposed using combinations of peripheral blood counts in prognosis of various cancers and in colorectal cancer in particular. Recently, we reported for the first time that an index that considers both the pro-tumorigenic effect of platelets and neutrophils and the protective effect of lymphocytes, called the platelet-neutrophil to lymphocyte ratio (PNLR), could provide prognostic information in this disease. The index provided useful prognostic information for both PFS and OS with a cutoff of 2000 in metastatic colorectal cancer [12]. In this report, we compare this index (with a slightly modified cutoff of 2088 derived from an online marker optimization tool) with other prognostic markers previously proposed [10, 11]. These other ratios include only two of the three hematologic parameters at a time (neutrophil to lymphocyte ratio and platelet to lymphocyte ratio) and have been the subjects of more extensive investigations in the prognostic marker literature [10, 11]. Another marker previously published is the NPS [28]. This index was reported to be prognostic of adverse outcomes in patients with stage I–III colorectal cancer. In this report, a slightly higher platelet cutoff of 400×10^9/L was used [28]. A similar marker, the Abnormal Hematological Marker



		PNLR
Clinical Calculator	*R*	0.4507
	p	< 0.001
	N	151

		NLR
Clinical Calculator	*R*	0.4834
	p	< 0.001
	N	151

		PLR
Clinical Calculator	*R*	0.2761
	p	< 0.001
	N	151

Fig. 7 Correlation of the hematologic ratios with the points obtained from the clinical calculator. **a** PNLR. **b** PLR. **c** NLR. A moderate correlation of the clinical calculator points attribution exists for PNLR and NLR ratios and a weak correlation with PLR

Index (AHMI for short), also takes into consideration abnormalities of all three hematologic markers: neutrophils, lymphocytes, and platelets. However, a shortcoming of this specific tool is that it does not factor in absolute values; it merely indicates whether a value is abnormal (e.g., it inaccurately depicts a thrombocytosis of 400×10^9/L as being equal to a much higher one). In this study, we report that all five hematologic value-based tools had prognostic merit in metastatic colorectal cancer, with PNLR being slightly superior over the other tools. PNLR also produced slightly better discriminatory HRs in multivariate analyses models than the PRONOPALL tool. No additional prognostic value was derived by combining PNLR with the PRONOPALL tool, despite the two tools measuring different parameters for calculation of their scores. Correlation of the hematologic marker tools with another survival prediction tool, the clinical calculator, was poor. This was consistent with the poor performance of this last tool in predicting survival at 90 days in our population. Several other factors may also have contributed to the lack of such correlation, such as the fact that the survival prediction tool was not specifically introduced for colorectal cancer but in general for any cancer.

High-risk and low-risk prognostic groups produced with the cutoffs used in this comparison study all contained at least a fourth of the total patients, suggesting clinical relevance. This is an important consideration, given that, if a marker is only present in a few patients, its prognostic interest is decreased. An example of this in metastatic colorectal cancer is the immunohistochemical marker CDX2 [29]. Absence of this transcription factor is a robust adverse prognostic factor in these patients compared with patients whose tumors express it, but this absence is observed only in 10% of patients.

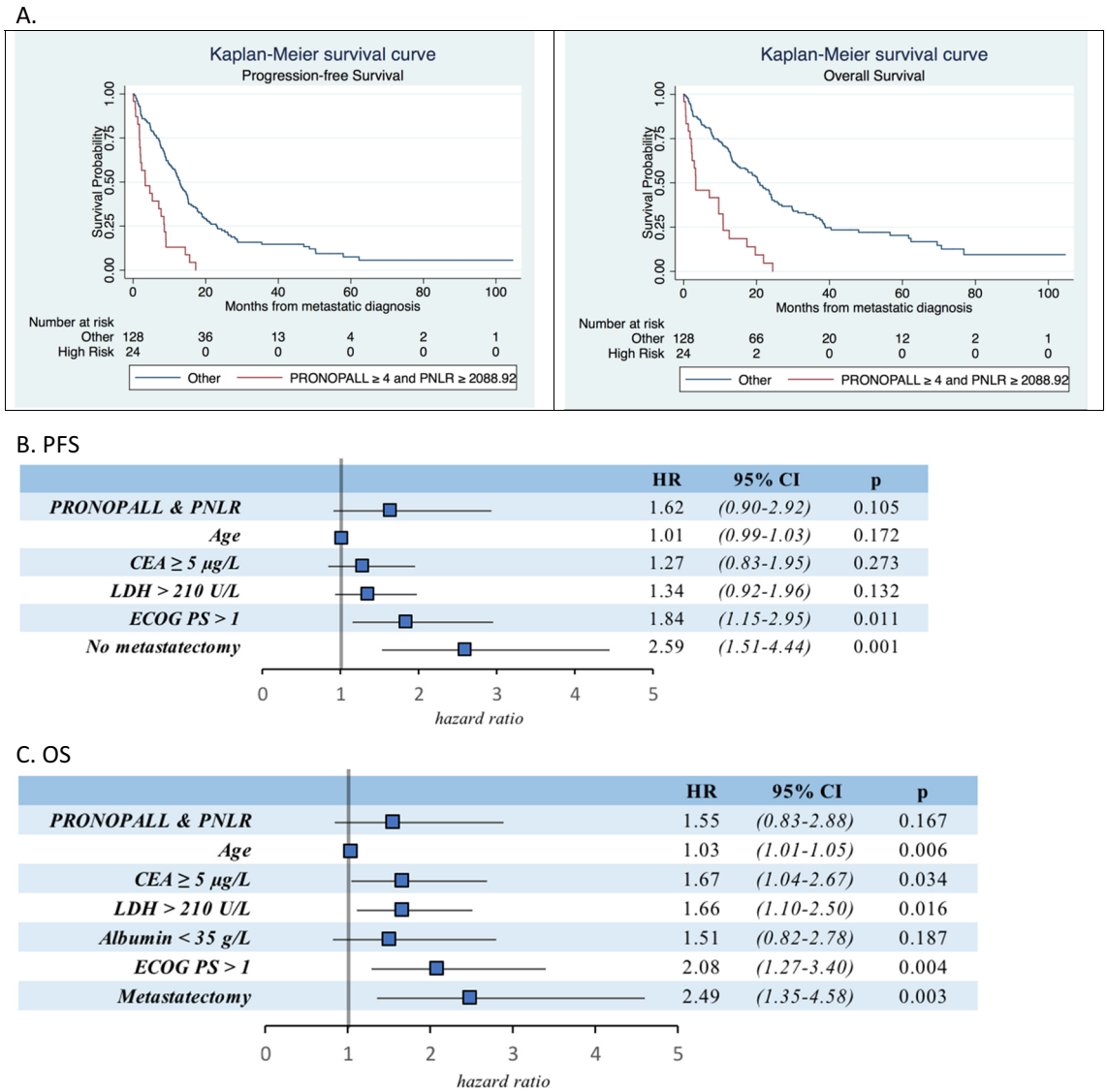


Fig. 8 **a** Kaplan-Meier curves of PFS (left) and OS (right) of the high-risk and low-risk groups with the use of a combined PNLR-PRONOPALL tool. The high-risk group includes patients with a PNLR score of 2088.92 and above and a PRONOPALL score of 4 and above, and the low-risk group includes all other patients with either or both scores below the cutoffs. Log-Rank test for PFS $x^2 = 29.65$, $p = 0.0000$ and for OS $x^2 = 33.97$, $p = 0.0000$. **b** Multivariate analyses for PFS and OS. PNLR-PRONOPALL loses significance for both PFS ($p = 0.10$) and for OS ($p = 0.16$)

Limitations of the current research consist of the retrospective nature of the studied cohort that may introduce bias associated with retrospective studies in general, and the fact that patients included were treated in a single center. In addition, no data on common molecular lesions in colorectal cancer such as mutations in KRAS, BRAF, or microsatellite instability were available in the majority of the cohort to include in the analysis models.

Despite these limitations and if confirmation in additional patient populations is obtained, the PNLR may be the marker of choice among hematologic indexes in metastatic colorectal cancer. Investigation in other stages of colorectal cancer as well as other malignancies may be warranted.

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Compliance with Ethical Standards

Conflicts of Interests The authors declare that they have no conflicts of interest.

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