Cancer Treatment and Research Communications 29 (2021) 100464

Contents lists available at ScienceDirect

Cancer Treatment and Research Communications

journal homepage: www.sciencedirect.com/journal/cancer-treatment-and-research-communications

Mortality in cutaneous malignant melanoma and its association with Neutrophil-to-Lymphocyte ratio.☆

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ARTICLE INFO

Keywords:
Cutaneous malignant melanoma
Neutrophil-lymphocyte ratio
Prognosis
Progression

ABSTRACT

Introduction: Cutaneous malignant melanoma (CMM) incidence has risen rapidly in the last 50 years. Poor progression and high mortality characterize CMM, making a thorough understanding of progression and associated factors essential for optimizing care.
Aims: We assessed the association between the Neutrophil-to-Lymphocyte Ratio (NLR) and mortality in adults with CMM from an entirely mixed-race Hispanic population during 12 consecutive years of extensive follow-up.
Material & Methods: We performed a retrospective cohort study in a tertiary hospital in Peru. NLR was categorized with a cutoff value higher or equal than 3. We collected demographic variables, laboratory results and treatments at baseline of follow-up. Cox regression analysis was performed, and we calculated crude and adjusted hazard ratios (HR) and their 95% confidence interval (95%CI).
Results: The analysis was from 615 CMM cases, and there were 378 deaths. Most melanomas (63.6%) were acral lentiginous. The crude analysis showed that high NLR is a risk factor for mortality, HR = 2.52; 95%CI (2.03–3.14). High NRL ratio remains statistically significant after adjusting for confounding variables, aHR = 1.61; 95%CI (1.16–2.24). Other risk factors for mortality were clinical stages III and IV, older than 60 years, females and greater Breslow thickness.
Conclusions: We concluded that high NRL ratio is a risk factor for mortality and should be monitored in every patient who is diagnosed with malignant melanoma during their first blood count. It should then be carried out in follow-up controls for patients of clinical stage III and IV only, or in patients who present a relapse.

Abbreviation used: CMM, INEN, NLR, RENIEC

1. Introduction

The incidence of cutaneous malignant melanoma (CMM) has increased rapidly in the last 50 years[1]. Annual incidence has risen as rapidly as 4–6% in much white skin populations like North America[2], Northern Europe[3], New Zealand[4], and Australia[5]. It represents 5.2% of new cancer cases and 1.6% of cancer deaths in the US[6]. The five-year survival rate in stage I is 98%, contrasting dramatically with stage IV, which is as low as 10%[7].' [8] As a consequence of the higher mortality rates, several factors have been reported to predict unfavorable/poor progression[9]. These well-known factors are age [10], gender, Breslow thickness, ulceration, and mitotic rate[11]. However, it is necessary to find novel prognostic markers to improve patients' outcomes.

The evidence shows that the inflammatory reaction plays an important role in tumor development[12]. Some hematologic parameters to measure the systemic inflammation include C-reactive protein [13], Platelet-to-lymphocyte ratio[14], baseline neutrophil[15], lactate dehydrogenase[16], and neutrophil-to-lymphocyte ratio (NLR)[17].

NLR has proved to be a helpful prognosis marker because of the neutrophil modulation of T-cell effector function and T cells' role in immune surveillance and probable destruction of tumor cells in different

☆ **Funding Statement:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors
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https://doi.org/10.1016/j.ctarc.2021.100464

Available online 25 September 2021

kinds of cancer[18]. Recent papers revealed an elevated NLR value associated with mortality in different cancer types[19–23].

The five-year relative survival rate varies by race and clinical stage of the disease upon diagnosis. Patients with light colored skin are usually diagnosed at an earlier stage as compared to darker colored patients [24].

Several studies have indicated that the NLR is associated with a bad prognosis, though the majority of them have been conducted in countries with a predominance of white, black or asian race. Even for those studies that did include hispanic populations, they were not the focus of research. Consequently, it remains unclear whether the high pretreatment NLR is associated with mortality in this population.

This study specifically aims to characterize patients of mixed-race hispanic populations that have CMM to determine whether the NLR is a risk factor related to mortality in an extensive follow-up during 12 consecutive years.

2. Methods

2.1. Study design

We carried out a retrospective cohort study in patients diagnosed with CMM during the period from Jan 1, 2005, to Dec 31, 2012. Participants were followed up from the beginning of the disease's diagnosis to death and those who survived until May 30, 2016, at Peruvian Institute of Neoplastic Diseases (INEN), in Lima, Peru. To this end, variables from the hospital record data sets that served as potential associations included demographics, laboratory results, surgeries performed, medical treatments and pathology results.

2.2. Population and sample

INEN is part of the top leading cancer hospitals in Latin America. It is the main cancer hospital in Peru, receiving 1600 new cases and 54,000 consultations/visits per year[25]. Our study included all patients with a confirmed CMM diagnosis (ICD-10 C43) in the Department of Breast and Soft Tissue Surgery. We included all cases with 1) patients with CMM diagnosis confirmed by histopathology; 2) With complete medical history (study variables) - Diagnosed with this disease from Jan 1, 2005, to Dec 31, 2012. The exclusion criteria were: 1) preoperative blood cells count not available; 2) patients with an active infection, multiple primary melanomas, concurrent tumor malignancies, or previous systemic chemotherapy; 3) patients with less than six months follow-up and 4) Patients with inflammatory connective tissue diseases, pregnant patients.

2.3. Definitions

Patients were divided into mixed-race and white skin population. The NLR was calculated as the absolute neutrophil count divided by absolute lymphocyte count. The NLR cutoff value applied was three, as previously reported by Davis et al.[26].. We assessed the NLR using the value of the patient's first admission blood count. Neutrophil versus lymphocyte ratio was greater or equal to 3 or less than 3 in the different clinical stages[17][20]..

The clinical stage of the patients was determined with an adequate medical history, preferential examination of the lesion and drainage area, complemented with ultrasound images of the drainage area and thoracic, abdominal, and pelvic tomography with contrast. In some cases, depending on the symptoms, brain tomography with contrast was performed. The definitive clinical stage was given by the result of anatomopathology of the main lesion, the lymph node study, and the images. The follow-up controls of the patients with melanoma in the INEN were during the first two years after diagnosis, every three months. Then for the third and fourth year, they were every six months. And from the fifth year onwards, the controls are yearly. The controls were clinical evaluations of the region where the lesion was located, as well as the corresponding lymph node region; and of laboratory tests with a follow-up blood count. Depending on the symptomatology presented by the patient at the time of the control or clinical findings that were found in the evaluation, an ultrasound, tomography, or radiography were requested. The clinical evaluations of the different controls carried out were recorded in the clinical history and consequently reviewed for this study. The result of the ultrasound or tomography requested from all patients diagnosed with this disease for follow-up was used. We determined the metastasis site and number, which was divided into visceral and non-visceral according to TNM[8,12].

The survival of the patients was reviewed in the clinical history. Follow-up was defined by the start date of the first selected case and the last observation for the closing date. We revised the last consultation as recorded, in which service it was performed, if they continue in their controls until May 30, 2016, or whether they died or are lost from sight. Additionally, it was verified whether the patient continued to be alive until the study's final date or was deceased with information obtained from the RENIEC. (National Registry of Identification and Civil Status from Peru).

Favorable evolution is defined as those patients who after treatment and in the follow-up carried out in their respective controls did not present recurrences of the disease nor metastasis, and unfavorable to those patients who after their surgery and complementary treatments in their follow-up controls presented local or regional recurrence of the disease, or metastasis.

2.4. Data collection and statistical analysis

We collected the study data by accessing the hospital admission database and identifying subjects with the CMM diagnosis between the seven years of the study: Jan 1, 2005, to Dec 31, 2012. Patients' alive status were censored on the last follow-up date or on May 30, 2016, consulting with the National Registry from Peru (RENIEC). After reviewing the medical chart, especially demographics, treatment plan, progress notes, pathology and laboratory and test results. We use the double data entry method, value limits, pre-coded categories, and character limits for data quality control.

Afterwards, we performed a descriptive analysis summarizing numerical variables with their mean and standard deviation and categorical variables with their absolute and relative frequencies. Then, we compared the NLR greater than or equal to 3 and the NLR less than 3 using their demographic, clinical and treatment characteristics. The chi-square test compared the categorical variables. Finally, the categorical variables are presented as frequencies and the continuous variables as means with SD.

Survival analysis was performed according to the Kaplan-Meier method to describe survival according to the variables of interest. The non-parametric Log-rank test was used to inferentially compare groups' survival, determining the value of the relationship NLR greater or equal than three or less in the different clinical stages as a possible new prognostic value. Univariate and multivariate analyses were performed using the Cox proportional hazards model to determine associations between potential prognostic factors and survival outcomes. Hazard ratios estimated from the Cox analyses were reported as relative risk for all-causes of mortality with a corresponding 95% confidence interval (CI). We reported crude and adjusted hazard ratios (HR). In addition, we evaluated the survival function at 5 and 10 years of follow-up. Statistically significant differences were considered within each period when the 95% CI for the strata in the variables evaluated did not overlap. An epidemiological criterion was used to enter confounders to the adjusted Cox regression, verifying compliance with the proportional hazards assumption and the absence of collinearity bias. Additionally, we calculated the area under the curve with its 95% CI for the NLR using ROC analysis. Likewise, we calculated the best cut-off point for our data using Youden's method and for the 95% CIs, a bootstrap with 1000

repetitions. All statistical analyses were performed using STATA 14. The p-value <0.05 was considered statistically significant.

2.5. Ethical aspects

The INEN Human Research Ethics Committee reviewed and approved the study protocol (Cod 047–2015-CRP-DI-DICON/INEN). The participants' data were coded to protect personal information. Also, the study forms and codes were protected and handled only by the study researchers.

3. Results

3.1. Patient population

During the study period, we identified 1228 eligible subjects and excluded a total of 613 cases. Most cases were excluded because they did not complete a minimum of 6 months of follow-up, (550; 88.1%), followed up by exclusion from one time attendance for a second opinion (73; 11.8%). Hence, we analyzed a total of 615 cases of malignant cutaneous melanoma, with a mean of 54.2 ± 40.9 months of follow-up (follow-up, month-person). The main demographics and clinical characteristics of these patients were summarized in Table A.1. The mean age was 62 years old (interquartile range, 49–72), and most study subjects were male patients (50.9%), and 76.1% were of mixed race. Upper and lower extremity's location (78.7%) and the acral lentiginous melanoma subtype (63.6%) were frequent. Regarding the thickness of the Melanoma (Breslow), 51.9% ($n = 280$) had a thickness greater than 4 mm and 10.9% ($n = 59$) equal to 1 mm, with a median of 4.2 mm (range 0 to 95). Ulceration was present in 348 cases, accounting for 62.5%, and no case of regression was found. Two hundred fifty patients (46%) had a Clark IV, and 136 (25%) had a Clark V. Concerning the inflammatory infiltrate, and this was present in a mild and moderate form in 309 patients (82%). In 116 (21.4%) patients diagnosed with melanoma, it was found that they showed a high mitotic index, that is, between 5 and 10 mitosis / mm^2 and 51 patients (9.4%) presented a mitotic index greater than 11 or more mitosis / mm^2. In this study population, 327 patients were found in clinical stage I (14.2%) and II (39%), 323 for the clinical stage III (40.8%), which was the most frequent, and 34 patients for the clinical stage IV (6%). For the patients in clinical stages I and II treatment was surgical (radical resection of the lesion plus flap or amputation and sentinel node biopsy) and were under observation. Those patients whose sentinel node biopsy was positive (115), were reclassified as clinical stage III. In clinical stage III patients underwent surgery of the lesion and lymph node dissection from the lymph node drainage region plus interferon and chemotherapy. While in clinical stage 4 they received palliative chemotherapy (Dacarbazine "DTIC", Temozolamide, paclitaxel, carboplatin / paclitaxel, Cisplatin / Vinblastine / Dacarbazine "CVD"), radiotherapy, and some of them only palliative care. Patients in clinical stages III and IV did not receive neither immunotherapy nor targeted therapy as these treatments were not considered as standard treatment when the study was performed, so they were not used for this group of patients. In the clinical stages regarding the NLR, it was found that 149 patients (24.2%) had a ratio equal to or greater than 3. In our study, the patients' follow-up window, from the first enrolled patient until the end of the study, was 125 months, and in this period, there were 378 deaths.

3.2. Association of neutrophil lymphocytes with the study variables

The study variables age, sex, number of involved nodes, positive sentinel node, metastasis, clinical stage, Breslow thickness, uncompromised margins, microsatellitosis, lymphovascular infiltrate and perineural infiltration was associated with NLR ($p < 0.05$) (Table A.2). Fig. 1

Table A.1
Demographic description of the study's population (n = 615).

Variable	Number (%)
Age (years)	
≤ 60	290 (47.1)
> 60	325 (52.9)
Median: 62	
Interquartile range: 49–2	
Sex	
Male	313 (50.9)
Female	302 (49.1)
Race	
Mixed race 468 (76.1)	
White 147 (23.9)	
Anatomic localization	
Head / neck	64 (10.5)
Trunk	66 (10.8)
Extremities	68 (11.1)
Hand / foot	413 (67.6)
Histological type	
SSM	36 (6.6)
NM	137 (25.0)
MLM	27 (4.9)
ALM	349 (63.6)
Breslow thickness (mm)	
≤ 1	59 (10.9)
1.01–2	74 (13.7)
2.01–4	127 (23.5)
> 4	280 (51.9)
Median: 4.2	
Range: 0–95	
Ulceration	
Absent	209 (37.5)
Present	348 (62.5)
Invasion level (Clark)	
I	20 (3.7)
II	45 (8.3)
III	93 (17.1)
IV	250 (46.0)
V	136 (25.0)
Inflamatory infiltrate	
Absent	57 (15.1)
Light /moderate	309 (82.0)
Abundant	11 (2.9)
Mitotic index (mitosis/mm^2)	
0	115 (21.2)
1–4	260 (48.0)
5–10	116 (21.4)
≥ 11	51 (9.4)
Sentinel node	
Negative	212 (64.8)
Positive	115 (35.2)
Clinical stage	
I	81 (14.2)
II	223 (39.0)
III	233 (40.8)
IV	34 (6.0)
Neutrophil / lymphocyte ratio	
< 3	466 (75.8)
≥ 3	149 (24.2)
Deaths	378 (62.2)

SSM: Superficial Spreading Melanoma, NM: Nodular Melanoma, MLM Malignant Lentiginous Melanoma, ALM: Acral Lentiginous Melanoma,.

3.3. Survival analysis

The median follow-up for the sample was 3.91 years (IQR 1.32 - 7.37) and the incidence rate across the sample was 6.13 deaths from all causes per 100 person-years. The incidence rate was higher in the group with NLR ≥ 3 (27.97 deaths from all causes per 100 person-years) compared to those with NLR <3 (11.19 deaths from all causes per 100 person-years). There are significant differences in the survival function between participants with NLR ≥ 3 compared to those with NLR <3 (log-rank test $p < 0.01$) (Fig. 2).

Table A.2
Association of Neutrophil Lymphocytes with the study variables.

Variables		Neutrophil / lynphocytes ratio < 3 (n = 466)	≥ 3 (n = 149) x^2	value p*
Age (years)			8,2773	0.004
	≤ 60	235 (50.4)	55 (36.9)	
	> 60	231 (49.6)	94 (63.1)	
Sex			5,8361	0.016
	Male	250 (53.7)	63 (42.3)	
	Female	216 (46.3)	86 (57.7)	
Primary tumor			3,8408	0.279
	T1	43 (10.2)	10 (8.3)	
	T2	63 (15.0)	14 (11.7)	
	T3	105 (24.9)	24 (20.0)	
	T4	210 (49.9)	72 (60.0)	
Lymph nodes			15,7625	0.001
	N0	264 (60.1)	57 (46.7)	
	N1	62 (14.1)	18 (14.8)	
	N2	74 (16.9)	21 (17.2)	
	N3	39 (8.9)	26 (21.3)	
Metastasis			7245	0.007
	M0	429 (96.9)	115 (91.3)	
	M1	14 (3.2)	11 (8.7)	
Clinical stage			21,2443	0.001
	I	67 (15.3)	14 (10.5)	
	II	184 (42.0)	39 (29.3)	
	III	170 (38.8)	63 (47.4)	
	IV	17 (3.9)	17 (12.8)	
Breslow thickness (mm)			3,8243	0.001
	≤ 1	46 (10.9)	14 (10.5)	
	1.01 – 2	60 (14.3)	39 (29.3)	
	2.01 – 4	105 (25.0)	63 (47.4)	
	> 4	209 (49.8)	17 (12.8)	
Clark invasion level			6,1263	0.190
	I	13 (3.1)	7 (5.8)	
	II	35 (8.3)	10 (8.3)	
	III	79 (18.7)	14 (11.6)	
	IV	196 (46.3)	54 (44.6)	
	V	100 (23.6)	36 (29.8)	
Mitotic index (mitosis/mm^2)			4,4267	0.219
	0	88 (30.0)	27 (22.1)	
	1–4	208 (49.5)	52 (42.6)	
	5–10	90 (21.4)	26 (21.3)	
	> 10	34 (8.1)	17 (13.9)	
Ulceration			3,2182	0.073
	Absent	171 (39.5)	38 (30.7)	
	Present	262 (60.5)	86 (69.3)	
Committed margins			5,0743	0.024
	Absent	368 (85.2)	99 (76.7)	
	Present	64 (14.8)	30 (23.3)	
Microsatellitosis			5,0539	0.025
	Absent	406 (92.5)	111 (86.0)	
	Present	33 (7.5)	18 (14.0)	
LVI			8,4102	0.004
	Absent	325 (72.4)	80 (59.3)	
	Present	124 (27.6)	55 (40.7)	
PNI			6,6268	0.010
	Absent	352 (78.0)	90 (67.2)	
	Present	99 (22.0)	44 (32.8)	
TIL			2,6623	0.264
	Absent	41 (13.7)	16 (20.5)	
	Light / moderate	250 (83.6)	59 (75.6)	
	Abundant	8 (2.7)	3 (3.9)	
Location			1231	0.746
	Head / neck	47 (10.1)	17 (11.6)	
	Trunk	49 (10.5)	17 (11.6)	
	Upper limb	55 (11.8)	13 (8.9)	
	Lower limb	314 (67.5)	99 (67.8)	
Histological type			0,7483	0.862
	SSM	30 (7.0)	6 (4.9)	
	NM	105 (24.6)	32 (26.2)	
	MLM	21 (4.9)	6 (4.9)	
	ALM	271 (63.5)	78 (63.9)	
Sentinel node			5,3511	0.021
	Negative	185 (67.5)	27 (50.9)	
	Positive	89 (32.5)	26 (49.1)	

* Chi-square test.
LVI: Lymphovascular Invasion, PNI: Perineural Infiltration, TIL: Tumor Infiltration Lymphocyte SSM: Superficial Spreading Melanoma, NM: Nodular Melanoma, MLM Malignant Lentiginous Melanoma, ALM: Acral Lentiginous Melanoma.

3.4. Crude and adjusted cox regression models

Table A.3 shows the crude and adjusted Cox regression models expressed as Hazard's, together with the prognostic survival variables in patients diagnosed with malignant cutaneous melanoma.

In our bivariate Cox regression analysis, we observed that unfavorable evolution was strongly associated with a high NLR (≥3) (HR = 2.52, 95% CI, 2.03–3.14). However, this magnitude of association was reduced when we adjusted for this association for the other predictors in our multivariate Cox regression analysis. This analysis confirmed the strong association between for all-cause mortality and higher NLR (aHR = 1.61; 95% CI, 1.16–2.24). Similarly, older than 60 years have a 1.51 times greater risk of all-cause mortality than those younger than 60 years (aHR = 1.51; 95% CI, 1.11–2.05). The female gender has 1.37 times more risk of all-cause mortality than the male gender; this is in an adjusted analysis since in the crude analysis, the percentage is higher (HR = 1.62; 95% CI, 1.32–1.98). In the crude model, the Breslow thickness >4 mm has a significant risk ($p < 0.05$) of all-cause of mortality the thicker it is, but it loses its importance in the model adjusted with the other confounding variables. (Breslow thickness: aHR = 1.68, 95% CI 0.49–5.74). Clinical stages III and IV have 3.2 and 24 times the risk of all-causes mortality, respectively, compared to clinical stage I (clinical stage III: aHR = 3.2, 95% CI, 1.30–7.85) and (clinical stage IV: aHR = 24.0, 95% CI, 7.01–82.4) in the adjusted analysis. On the other hand, for the level of invasion, ulceration, there is no statistically significant difference ($p > 0.05$) when adjusting for all variables of confusion, unlike the crude model. Regarding the type of melanoma, in the crude model, there is a significant statistical difference to a higher risk of all-cause mortality if an acral lentiginous melanoma was diagnosed, but the difference was lost when adjusting for the other confounding variables.

3.5. Survival to 5 and 10 years

Table A.4 shows that the survival for male, Breslow thickness >4 mm, invasion level V, ulceration, mitotic index >10 mitosis/mm^2, acral lentiginous melanoma (ALM) compared to superficial spreading melanoma (SSM), clinical stage IV and NLR ≥ 3 had worse survival at 5 years. Similarly, these factors remained associated with poorer 10-year survival.

3.5. ROC analysis

The NLR evidenced an area under the curve of 0.61, 95% CI (0.56–0.65) (Fig. 2). Youden's analysis showed the best cut-off point for NLR at 2.85; 95% CI (1.92–3.78).

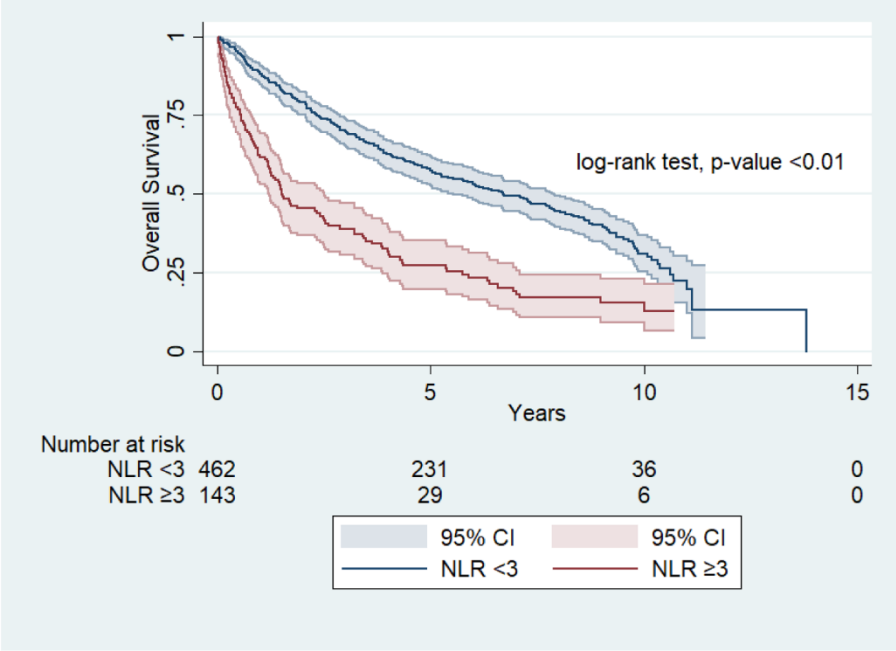

Fig. 1. Overall survival of individuals with NLR $\geq$ 3 (n = 149) compared to NLR < 3 (n = 466). NLR: Neutrophil to lymphocyte ratio.

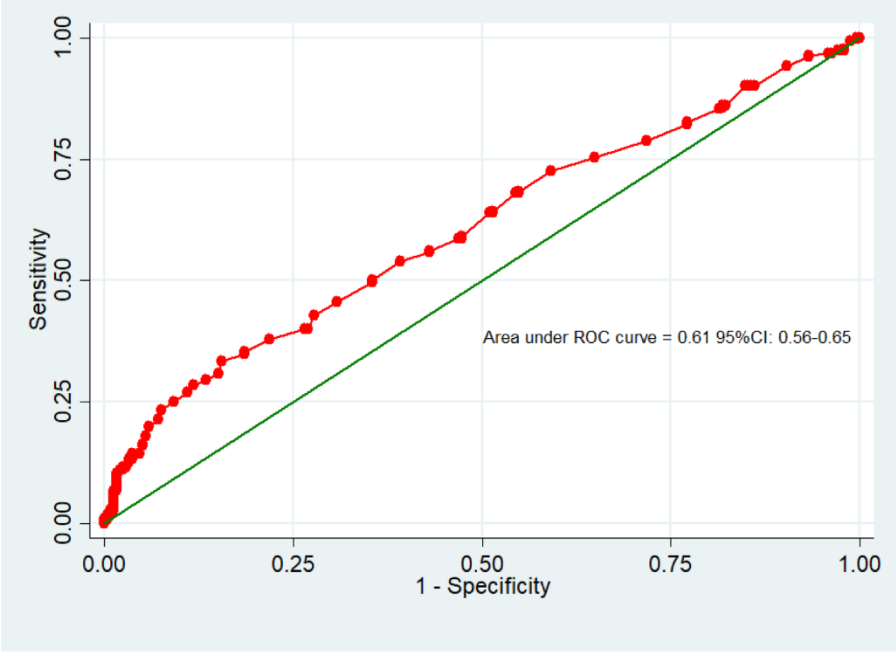

Fig. 2. ROC analyses for the neutrophil to lymphocyte ratio.

4. Discussion

4.1. Neutrophil-to-Lymphocyte ratio

The first report that described patients with intraabdominal CMM metastasis concluded that a high NLR value suggested an unfavorable progression[27]. Ferrucci et al. analyzed a cohort of 187 patients with metastasis, identifying that high NLR before ipilimumab is an independent marker of aggressive disease, associated with poor survival [28]. Cassidy et al. reported that NLR might be a unique predictive value in patients treated with immunotherapy[29]. To identify surgical candidates in Stage IV melanoma patients, they should have a low preoperative NLR to achieve favorable prognosis[30]. Most studies suggest that NLR is probably a significant biomarker for melanoma in clinical evolution as shown by the study of Lino-Silva et al., that was carried out mainly in patients from the clinical stages I to III[31].

Our study focused on comprehensive patient data and long-term follow-up in a well-established cohort describing demographics, tumor characteristics, and clinical management variables that impact overall and favorable-unfavorable evolution. In this study, we demonstrate that a basal NLR $\geq$ 3 was associated with unfavorable evolution. According to our analysis, we obtained NLR $\geq$ 3 in 24.2% of cases; however, the patients with the initial diagnosis in stage III and IV represented 60.2% of the cases, indicating that a high NLR is more associated with these

Table A.3
Multivariate Analysis of the Leukocyte Lymphocyte relationship in patients diagnosed with cutaneous Malign Melanoma of the INEN.

Variables		Crude model			Adjusted model		
		HR	IC 95%	P	HR	IC 95%	P
Age (years)							
	≤ 60	Reference			Reference		
	> 60	1.26	(1.02–1.54)	0.029	1.51	(1.11–2.05)	0.008
Sex							
	Male	Reference			Reference		
	Female	1.62	(1.32–1.98)	<0.001	1.37	(1.02–1.84)	0.037
Breslow thickness (mm)							
	≤ 1	Reference			Reference		
	1.01 – 2	1.22	(0.71–2.11)	0.468	1.84	(0.65–5.23)	0.250
	2.01 – 4	1.40	(0.85–2.29)	0.184	1.05	(0.31–3.54)	0.943
	> 4	3.03	(1.93–4.75)	<0.001	1.68	(0.49–5.74)	0.407
Invasion level							
	V	Reference			Reference		
	IV	0.67	(0.52–0.86)	0.002	0.78	(0.56–1.11)	0.176
	III	0.36	(0.25–0.52)	<0.001	0.55	(0.29–1.03)	0.063
	II	0.37	(0.22–0.61)	<0.001	0.87	(0.29–2.66)	0.814
	I	0.56	(0.29–1.08)	0.085	0.39	(0.05–3.08)	0.373
Ulceration							
	Absent	Reference			Reference		
	Present	1.78	(1.41–2.26)	<0.001	1.03	(0.70–1.52)	0.872
Mitotic index (mitosis/mm2)							
	0	Reference			Reference		
	1 – 4	0.97	(0.72–1.30)	0.82	0.78	(0.50–1.21)	0.268
	5 – 10	1.46	(1.05–2.04)	0.025	1.04	(0.62–1.75)	0.881
	> 10	2.22	(1.49–3.31)	<0.001	1.15	(0.64–2.05)	0.642
TIL							
	Absent	Reference			Reference		
	Light / moderate	0.93	(0.65–1.33)	0.685	0.95	(0.62–1.45)	0.813
	Abundant	0.54	(0.21–1.38)	0.196	0.26	(0.06–1.14)	0.073
Histological Type							
	SSM	Reference			Reference		
	NM	1.69	(0.98–2.93)	0.061	0.62	(0.23–1.72)	0.361
	MLM	1.80	(0.90–3.61)	0.096	1.82	(0.53–6.23)	0.342
	ALM	1.93	(1.14–3.26)	0.014	0.88	(0.31–2.50)	0.805
Location							
	Head /neck	Reference			Reference		
	Trunk	0.84	(0.54–1.29)	0.420	1.37	(0.56–3.32)	0.487
	Upper limb	0.63	(0.40–1.01)	0.055	0.71	(0.28–1.76)	0.458
	Lower limb	0.98	(0.71–1.37)	0.926	1.15	(0.55–2.40)	0.709
Clinical stage							
	I	Reference			Reference		
	II	2.36	(1.48–3.75)	<0.001	1.49	(0.62–3.60)	0.370
	III	5.08	(3.23–8.01)	<0.001	3.20	(1.30–7.85)	0.011
	IV	22.1	(12.6–38.8)	<0.001	24.0	(7.01–82.4)	0.001
Neutrophil /lymphocyte ratio							
	< 3	Reference			Reference		
	≥ 3	2.52	(2.03–3.14)	<0.001	1.61	(1.16–2.24)	0.005

NRL: Neutrophil-Lymphocyte Ratio; H.R.: Hazard ratio; aHR: Adjusted Hazard Ratio. * = $P<0.05$; ** = $P<0.01$; *** = <0.001.

clinical stages and as such a better prognostic tool for these stages as well. In a previous study, the stage-for-stage analysis found that NLR was only significant for stage II [31]. Nowadays, the NLR cutoff value varies from 2 to 5 in the literature[32]. The results of our study confirm the results in multiple studies in solid tumors where a high proportion of neutrophils / NLR lymphocytes in the initial diagnosis or when metastases occur, derives in a poor survival rate. [26,31,33–36]

4.2. Female gender

Our study considered the female gender as a predictor of mortality in CMM patients. In contrast, a different author described the male gender as an independent unfavorable prognosis for stage I-II[37]. And a worse disease-specific survival in stage III-IV compared with the female gender [38,39]. However, another study showed no difference between men's and women's survival outcomes[40]. There is not enough literature to define female or male gender as a prognostic factor. For example, pregnancy-associated melanoma (PAM) represents 31% of all cancers identified during gestation[41]. It is interesting how the gender effect suggests that biological factors rather than behavioral differences alone account for, at least in part, the variable gender outcomes. We encourage the basic-science researchers to study the biological factors outcomes by gender.

4.3. Mixed-race skin / non-white skin

The incidence of CMM varies based on race. The lifetime risk of Caucasians is 2.6%, in African Americans 0.1%, and in Hispanics 0.58% [42]. Caucasians have a 10-fold increased risk of developing CMM compared with dark skin patients[43]. However, mixed-race skin patients have a worse overall outcome than white-skin patients[44]. This information is compatible with our results. In Latin America, mixed-race skin is common and are strongly associated with unfavorable CMM progression compared to the white-skin population. More research is necessary to analyze if the race is associate with social, clinical factors, or both. The novelty of the present study is that it was conducted with an entirely mixed-race population, for which there were no previous similar study that associated the Neutrophil-to-Lymphocyte Ratio NLR relationship and its prognosis. Therefore, focusing on the impact this relationship has on the prognosis of the Hispanic population with CMM.

Table A.4
Multivariate Analysis of the Neutrophil to Lymphocyte ratio for survival at five and ten years.

Variables		Survival percentage 5 years	IC 95%	10 years	IC 95%
Age (years)					
	≤ 60	51.2	45.3–56.8	38.8	32.1–45.3
	> 60	40.0	34.5–45.3	31.3	25.6–37.1
Sex					
	Male	54.1	48.3–59.5	42.6	36.0–49.1
	Female	36.3	30.9–41.8	26.9	21.5–32.6
Breslow thickness (mm)					
	≤ 1	66.2	52.2–76.9	62.3	48.1–73.6
	1.01 – 2	68.8	56.9–78.0	50.3	36.2–62.9
	2.01 – 4	59.9	50.7–67.9	43.0	31.4–54.0
	> 4	31.0	25.6–36.5	23.6	18.2–29.3
Invasion level					
	V	28.8	21.3–36.6	23.1	16.0–30.9
	IV	45.3	40.0–51.4	31.7	24.0–39.7
	III	68.2	57.5–76.7	53.8	42.3–63.9
	III	68.1	52.2–79.7	53.2	33.0–69.8
	I	47.40	24.4–67.3	47.40	24.4–67.3
Ulceration					
	Absent	59.4	52.3–65.8	49.4	41.1–57.2
	Present	39.3	34.1–44.5	28.7	23.3–34.3
Mitotic index (mitosis/mm^2)					
	0	51.8	42.2–60.6	44.0	34.6–53.1
	1 – 4	54.8	48.5–60.7	39.5	31.1–47.7
	5 – 10	35.8	27.1–44.6	30.1	21.8–38.7
	> 10	23.5	12.8–36.0	–	–
TIL					
	Absent	47.2	33.9–59.5	26.5	12.5–42.8
	Light/ moderate	46.2	40.5–51.7	38.3	32.4–44.2
	Abundant	81.8	44.7–95.1	–	–
Histological type					
	SSM	68.6	50.5–81.2	55.3	36.8–70.4
	NM	45.9	37.3–54.0	37.3	28.4–46.2
	MLM	46.2	26.6–63.6	33.7	16.4–51.9
	ALM	40.60	35.4–45.8	30.1	23.9–36.5
Location					
	Head/neck	47.6	34.9–59.3	34.2	21.9–46.8
	Trunk	53.0	40.4–64.2	39.2	26.9–51.3
	Upper limb	63.0	50.1–73.5	47.1	32.7–60.2
	Lower limb	41.0	36.1–45.8	32.1	26.8–37.6
Clinical stage					
	I	79.6	68.8–89.9	72.4	60.7–81.1
	II	58.4	51.6–64.6	40.5	32.3–48.5
	III	27.2	21.6–33.2	21.9	16.4–27.8
	IV	3.0	0.23–13.4	3.0	0.23–13.4
Neutrophil/Lymphocyte ratio					
	< 3	52.7	48.0–57.2	41.1	35.7–46.3
	≥ 3	22.1	15.8–29.2	14.8	9.23–21.6

NRL: Neutrophil-Lymphocyte Ratio; Survival at five and ten years.

4.4. Stage of melanoma

Our study reports higher mortality risks for stages III and IV, which is congruent with previous studies. The mortality of stages I-II was low, and stages III-IV was high; the staging is essential to identify the appropriate treatment[45]. Zhang et al. found that clinical stage and treatment were independent prognostic factors in patients with CMM [46].

4.5. Breslow thickness

Breslow depth is measured from the surface to the most in-depth base of the tumor[47]. Maurichi et al. found that increasing depth was a significant independent prognostic factor for melanomas, and a Breslow thickness >0.75 mm had a positive sentinel lymph node biopsy[48] compared with tumors <0.75 mm[49]. About 5-year survival, a more remarkable survival was associated in stages I-II (90%) compared to stages III/IV (73% and 53%)[50]. In our study, Breslow thickness is associated with unfavorable prognosis in stage III-IV.

4.6. Limitations

The present study has several limitations. First, the retrospective nature of the study may have led to selection and information bias. Second, the patients were treated by multiple oncologists, which probably, influenced the outcome. Third, we did not determine the optimal time for NLR measurement (before or after the treatment) and did not differentiate by stages. Fourth, it is not a multicenter study but a retrospective review of cases from a single center, the Peruvian Institute of Neoplastic Diseases (INEN) (Lima, Peru). Fifth, data on the type of adjuvant treatment received especially by stage III and IV patients were not available and should be included in future research.

Additionally, in the period where the data of the study was being collected, immunotherapy was not part of the standard treatment, so the implications of NLR in the immunotherapy for melanoma were not considered.

4.7. Areas of future research

One could investigate the relationship of NLR with other tests that also assess the inflammatory reaction, such as c-reactive protein and platelet - lymphocyte ratio, including an assessment of this relationship for each clinical stage of CMM. The use of NLR as a biomarker in the new immunological therapies that are being used in melanoma currently could also be investigated.

5. Conclusion

In this study, we observed a strong association between pretreatment high NLR and higher mortality risk, as shown in our statistical analysis. We also reported that female gender, non-white skin color, higher stage at diagnosis, and Breslow thickness correlated with mortality. Our findings are consistent with previous findings demonstrating that a high neutrophil-lymphocyte ratio was associated with unfavorable CMM progression.

The NLR should be measured in every patient who is diagnosed with malignant melanoma during their first blood count. It should then be carried out in follow-up controls for patients of clinical stage III and IV only, or in patients who present a relapse.

Further studies are required to evaluate whether clinical management of patients with high NLR should be more aggressive.

CRediT author statement

Mirian Elizabeth Pinto-Paz: Conceptualization, Methodology, Data curation, Software. **Jose Manuel Cotrina-Concha:** Visualization, Supervision. **Vicente A. Benites-Zapata:** Software, Validation, Writing - review & editing

Appendix

Table A.1, Table A.2, Table A.3, Table A.4

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