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ESMO GASTROINTESTINAL ONCOLOGY

ORIGINAL ARTICLE

Baseline neutrophil-to-eosinophil ratio and neutrophil-to-lymphocyte ratio as prognostic markers in patients with unresectable biliary tract cancer treated with gemcitabine-cisplatin-durvalumab: an international, multicentre, retrospective cohort study

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Background: A combination of gemcitabine, cisplatin and durvalumab (anti-programmed death-ligand 1) is the current standard-of-care first-line therapy for advanced biliary tract cancer (BTC). To date, there is only limited evidence regarding prognostic biomarkers. Studies in other tumour types treated with immune checkpoint inhibitors have highlighted the pretreatment neutrophil-to-eosinophil ratio (NER) and neutrophil-to-lymphocyte ratio (NLR) as potential prognostic biomarkers for response to immunochemotherapy.
Our aim is to investigate whether NER and NLR can serve as potential prognostic biomarkers in advanced BTC.
Materials and methods: Patient data were retrospectively collected from 39 sites in 11 countries. The study population comprised patients with unresectable, locally advanced, or metastatic BTCs. The primary outcome was overall survival (OS). The secondary outcomes were progression-free survival (PFS) and objective response rate (ORR).
Results: A total of 509 patients were included in the analysis. In a multivariate Cox regression model, OS and PFS were significantly shorter for patients with NLR above the median value (OS: hazard ratio (HR) 2.08, 95% confidence interval (CI) 1.44-3.00, $P < 0.001$ and PFS: HR 1.57, 95% CI 1.17-2.10, $P = 0.002$). These associations were not found based on NER. There was no significant difference in ORR between patients stratified based on NER or NLR values.
Conclusion: A higher baseline NLR was significantly associated with worse PFS and OS. No significant association was found between NER and OS or PFS. These findings suggest that NLR can serve as a prognostic biomarker for patients with advanced BTC treated with gemcitabine-cisplatin-durvalumab.
Key words: advanced biliary cancer, durvalumab-gemcitabine-cisplatin, neutrophil-to-eosinophil ratio, neutrophil-to-lymphocyte ratio

INTRODUCTION

Biliary tract cancer (BTC) is a highly lethal malignancy of the epithelial cells of the liver and biliary tract. Its incidence has been steadily increasing worldwide, now accounting for ∼15% of all primary liver cancers and ∼3% of gastrointestinal malignancies.

Despite recent advances in diagnosis and treatment, BTC continues to have a high mortality rate, with 5-year overall survival (OS) rates ranging from just 7% to 20%.[1-3] This low survival rate contributes to BTC being responsible for ∼2% of cancer-related deaths globally each year.[4-6] Until today, the only curative option for BTC is surgery followed by adjuvant chemotherapy. However, due to the insidious onset and aggressive behaviour of BTC, almost 75% of patients have unresectable or metastatic disease at diagnosis.[4] In patients with advanced-stage disease, several systemic therapies are available, but efficacy in the unselected population is limited, likely due to the high inter- and intratumoral heterogeneity of BTCs at the genomic, epigenetic and molecular level.[7-9] Despite numerous clinical trials investigating a range of targeted therapies, the combination of gemcitabine and cisplatin chemotherapy continued to be the cornerstone of first-line treatment until 2022, yielding a median OS of 11.7 months. However, in 2022, the TOPAZ-1 phase 3 trial marked a significant advancement in the treatment of advanced BTC by demonstrating the benefit of combining immune checkpoint inhibitors (ICIs) with chemotherapy.[5,10] This landmark trial demonstrated a significant improvement in OS, with a hazard ratio of 0.8, establishing the combination of gemcitabine, cisplatin, and durvalumab (anti-programmed death-ligand 1) as the new standard-of-care first-line treatment for advanced BTC. In addition, the KEYNOTE-966 study also showed a significant effect on prognosis with combination therapy of gemcitabine-cisplatin-pembrolizumab (anti-programmed cell death protein 1) in advanced BTC, supporting the favourable effect of ICI.[11] However, response rates still hover around 25%-30%, and there is no effective prognostic biomarker to date that can effectively predict the effect of this treatment regimen.

Immune checkpoint inhibitors function by T cell-mediated tumour cell destruction through inhibition of immune checkpoint proteins that normally dampen immune responses.[5,10,12] By blocking these inhibitory pathways, ICI reinvigorates the immune system's ability to target and eliminate cancer cells. Therefore, it is plausible that a higher absolute lymphocyte count and a higher prevalence of tumour-infiltrating lymphocytes are associated with improved responses to ICI. Conversely, specific neutrophil phenotypes are known to suppress T cell proliferation and promote T cell apoptosis, which has led to findings that increased neutrophil counts are correlated with worse ICI treatment response and outcomes. This understanding has prompted investigations into the pretreatment neutrophil-to-lymphocyte ratio (NLR) as a potential biomarker for predicting ICI treatment outcomes in several solid tumours, such as advanced melanoma or renal cell carcinoma, with promising results.[13,14]

A recent meta-analysis investigated the value of the pretreatment NLR in prognosticating the outcome of patients with advanced cancer receiving immunotherapy. They found that an elevated NLR before immunotherapy was significantly associated with poor clinical outcomes in patients with advanced cancer.[15]

Eosinophils are known to have multiple antitumour functions, including their ability to recruit T cells into the tumour microenvironment, enhance antitumour immunity by modifying the tumour vasculature and reshape the immune landscape to support an effective immune response. Furthermore, eosinophils also have the potential for direct tumour cell cytotoxicity. Studies have reported an

association between greater eosinophil levels and improved survival outcomes in ICI-treated melanoma.[16,17] This suggests a potential role for baseline eosinophil count as a prognostic biomarker. Furthermore, in studies investigating other types of cancer, there was an association between improved OS with lower pretreatment neutrophil-to-eosinophil ratio (NER) and NLR.[13,15,18–24]

Although there is some limited evidence on the prognostic utility of NLR in BTC,[25-27] this is the first study to assess this in a homogenous cohort of patients receiving immunochemotherapy. To bridge this knowledge gap, an international, multicentre retrospective study was conducted to assess the potential of the NLR and NER as prognostic biomarkers for treatment outcomes in patients with BTC receiving immunochemotherapy.

MATERIALS AND METHODS

Study population

The study population comprised patients with unresectable, locally advanced or metastatic BTCs, including intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA) and gallbladder carcinoma (GBC) that were treated with gemcitabine-cisplatin-durvalumab.

Patient data was retrospectively collected from 39 sites in 11 countries (Italy, Germany, Austria, Spain, the UK, the United States, the Republic of Korea, China, Hong Kong Special Administrative Region of China, Japan and Belgium).

Patients were treated with durvalumab combined with gemcitabine and cisplatin administered intravenously on a 21-day cycle for up to eight cycles. Durvalumab (1500 mg) was administered on day 1 of each cycle, in combination with gemcitabine (1000 mg/m^2) and cisplatin (25 mg/m^2), which were administered on days 1 and 8 of each cycle. After completion of gemcitabine and cisplatin, durvalumab monotherapy (1500 mg) was administered every 4 weeks until clinical or imaging disease progression or unacceptable toxicity.

The present study was approved by the local ethics committee at each centre, complied with the provisions of the Good Clinical Practice guidelines and the Declaration of Helsinki and local laws, and fulfilled the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons regarding the processing of personal data.

Statistical analysis

Leukocyte values, including total white blood cell count, eosinophil count, neutrophil count and lymphocyte count, were measured at treatment initiation. NER and NLR were calculated by dividing the absolute neutrophil count by the absolute eosinophil count (AEC) and absolute lymphocyte count (ALC), respectively. To prevent undefined or infinite results, an AEC or ALC of zero was adjusted to 0.01×10^9/l for the purpose of NER or NLR calculations.

The primary outcome was OS, defined as the time from initiation of gemcitabine-cisplatin-durvalumab to death from any cause. Progression-free survival (PFS) was calculated from treatment initiation to radiographic progression based on RECIST criteria v1.1 or death, whichever occurred first. The secondary endpoint, objective response rate (ORR), was calculated as the sum of the rate of complete responses and partial responses as best responses based on RECIST v1.1 criteria.

Patients were stratified into subgroups based on the median values of NLR and NER measured before ICI initiation. Differences in baseline patient characteristics between these subgroups were assessed using either the chi-squared test or Fisher's exact test. Univariate and multivariate Cox proportional hazards models were then employed to evaluate the association between predictors and OS or PFS. Correlated predictors were excluded from a multivariate Cox proportional hazards model.

For the multivariate Cox model, NLR was selected over NER to avoid bias and statistical errors due to their correlation. Kaplan-Meier curves of OS and PFS for NER and NLR were constructed, and a log-rank test was used to compare the differences between groups.

To account for multiple comparisons, the Bonferroni correction was applied within each family of related hypotheses by dividing the nominal significance level ($\alpha = 0.05$) by the number of comparisons performed.

RESULTS

Descriptive statistics

From February 2022 to January 2024, a total of 666 patients with unresectable, locally advanced or metastatic BTC were included in the study. Patients without a pretreatment eosinophil count were excluded, leaving 509 patients. Subsequently, patients with missing neutrophil or lymphocyte counts were excluded, resulting in a final cohort of 454 patients with calculable NLR and NER for statistical analysis. Missing cell counts were primarily due to center-specific differences in data availability. No association with clinical outcomes was observed. At data cutoff (1 April 2024), the median duration of follow-up was 8.5 months [95% confidence interval (CI) 7.7-9.7]. The median age at the start of the therapy was 67 years (interquartile range 15.0). Among the cohort, 267 patients (52.5%) were male and 242 (47.5%) were female. Regarding the primary tumour site, 287 patients (56.4%) had iCCA, 121 (23.8%) had eCCA and 101 (19.8%) had GBC. Most patients (412, 80.9%) had metastatic disease at diagnosis. Additional patient demographics and disease characteristics are detailed in Table 1. We further stratified the cohort into subgroups based on the median NLR and median NER. Comparison of demographics and disease characteristics between these subgroups identified a significant difference in primary tumour site and prior surgery prevalence between patients with high and low NLR.

Univariate Cox regression model for PFS and OS

After Bonferroni correction for multiple testing across 13 comparisons ($P < 0.0038$ as significant), univariate Cox regression analysis showed that metastatic disease [hazard

Table 1. Patient demographics and disease characteristics.

Patient characteristics ($N = 509$)	All ($N = 509$)	NER ($n = 454$)			NLR ($n = 454$)		
		<mNER ($n = 227$)	≥mNER ($n = 227$)	*P* value	<mNLR ($n = 225$)	≥mNLR ($n = 229$)	*P* value
Gender				0.110			0.918
Male (%)	267 (52.46%)	128 (56.39%)	110 (48.46%)		119 (52.89%)	119 (51.97%)	
Female (%)	242 (47.54%)	99 (43.61%)	117 (51.54%)		106 (47.11%)	110 (48.03%)	
Age at start Gem-Cis-Durva Years, median (IQR)	67 (15)	66 (15.5)	69 (13)	0.045	68 (14.75)	67 (16)	0.679
Primary tumor site				0.037			<0.0001*
Intrahepatic (%)	287 (56.39%)	126 (55.51%)	141 (62.11%)		110 (48.89%)	157 (68.56%)	
Extrahepatic (%)	121 (23.77%)	65 (28.63%)	42 (18.50%)		68 (30.22%)	39 (17.03%)	
Gallbladder (%)	101 (19.84%)	36 (15.86%)	44 (19.38%)		47 (20.88%)	33 (14.41%)	
Previous surgery				0.461			<0.0001*
Yes	135 (26.52%)	66 (29.07%)	58 (25.55%)		85 (37.78%)	39 (17.03%)	
No	374 (73.48%)	161 (70.93%)	169 (74.45%)		140 (62.22%)	190 (82.97%)	
Previous adjuvant therapy after surgery				0.138			0.520
Yes	84 (62.22%)	46 (69.70%)	32 (55.17%)		54 (63.53%)	24 (61.54%)	
No	51 (37.78%)	20 (30.30%)	26 (44.83%)		31 (36.47%)	15 (38.46%)	
Disease stage				0.116			0.032
Locally advanced	97 (19.06%)	49 (21.59%)	35 (15.42%)		51 (22.67%)	33 (14.41%)	
Metastatic	412 (80.94%)	178 (78.41%)	192 (84.58%)		174 (77.33%)	196 (85.59%)	
Eastern Cooperative Oncology Group performance status				0.075			0.301
0	242 (47.54%)	125 (55.07%)	105 (46.26%)		120 (53.33%)	110 (48.03%)	
0	267 (52.46%)	102 (44.93%)	122 (53.74%)		105 (46.67%)	119 (51.97%)	
Carbohydrate antigen 19-9 UI/ml				0.583			0.014
Median (IQR)	107.5 (847.02)	106.5 (721.48)	114 (1198.5)		81.65 (433.7)	138.09 (1938)	
Carcinoembryonic antigen ng/ml				0.390			0.700
Median (IQR)	3 (7.4)	2.8 (6.5)	3.1 (7.69)		2.8 (7.4)	3.1 (6.94)	
NLR				<0.0001*			
Median (IQR)	3.33 (3.21)	2.99 (2.45)	4.06 (3.55)				
Not reported	55 (10.81%)	/	/				
Eosinophils baseline/mcL				<0.0001*			0.007
Median (IQR)	130 (200)	200 (220)	80 (70)		130 (120)	100 (150)	
NER							<0.0001*
Median (IQR)	35.92 (68.88)				25.87 (36.94)	52.91 (96.78)	
Not reported	55 (10.81%)				/	/	

IQR, interquartile range; mNER, median neutrophil-to-eosinophil ratio; mNLR, median neutrophil-to-lymphocyte ratio; NER, neutrophil-to-eosinophil ratio; NLR, neutrophil-to-lymphocyte ratio.
*Statistical significance of $P < 0.0045$ (Bonferroni correction, $P = 0.05/1$).

ratio (HR) 2.09, 95% confidence interval (CI) 1.45-3.01, $P < 0.001$], Eastern Cooperative Oncology Group (ECOG) performance status (PS) >0 (HR 1.63, 95% CI 1.28-2.09, $P < 0.001$) and NLR above the median (HR 1.57, 95% CI 1.22-2.03, $P < 0.001$) were significantly associated with shorter PFS.

For OS, metastatic disease (HR 3.11, 95% CI 1.76-5.50, $P < 0.001$), ECOG PS >0 (HR 2.36, 95% CI 1.69-3.31, $P < 0.001$), NLR above the median (HR 2.03, 95% CI 1.46-2.84, $P < 0.001$) and NER above the median (HR 1.66, 95% CI 1.19-2.31, $P = 0.003$) remained significantly associated with shorter OS. Additional results are presented in Table 2.

Sensitivity analyses confirmed the robustness of the association between NLR and outcomes. When modelled as a continuous (log-transformed) variable, higher NLR remained significantly associated with shorter PFS (HR 1.34, 95% CI 1.14-1.59, $P < 0.001$) and OS (HR 1.54, 95% CI 1.27-1.86, $P < 0.001$). Similarly, using an established cutoff (NLR ≥ 3), higher NLR was associated with worse PFS (HR 1.54, 95% CI 1.18-2.00, $P = 0.001$) and OS (HR 2.18, 95% CI 1.53-3.11, $P < 0.001$) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmogo.2026.100335).

Kaplan-Meier analysis for PFS and OS

Since baseline NLR was significantly associated with OS and PFS and baseline NER with OS and a nonsignificant association with PFS in the univariate Cox regression, Kaplan-Meier curves were generated for both variables. The log-rank test confirmed a significant difference in OS and PFS between patients with a baseline NLR above versus below the median (Figure 1), with similar findings for NER (Figure 2).

Multivariate Cox model

In a multivariate Cox regression model, PFS was significantly shorter for patients with metastatic disease (HR 2.19, 95% CI 1.40-3.43, $P < 0.001$), ECOG PS >0 (HR 1.80, 95% CI 1.35-2.40, $P < 0.001$), higher carbohydrate antigen 19-9 levels (HR 1.00, 95% CI 1.00-1.01, $P = 0.038$), and NLR

Table 2. Univariate Cox regression model for PFS and OS of patients with unresectable biliary tract cancer.

Variable	PFS		OS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender (male versus female)	1.016 (0.797-1.295)	0.898	1.0542 (0.765-1.454)	0.747
Age at start Gem-Cis-Durva	0.998 (0.986-1.009)	0.694	1.003 (0.987-1.018)	0.740
Primary tumour site				
Extrahepatic	Reference	Reference	Reference	Reference
Intrahepatic	1.439 (1.061-1.952)	0.019	1.736 (1.123-2.682)	0.013
Gallbladder	1.607 (1.106-2.334)	0.013	1.690 (0.999-2.861)	0.051
Disease stage (metastatic versus locally advanced)	2.087 (1.448-3.007)	<0.001*	3.113 (1.761-5.503)	<0.001*
Eastern Cooperative Oncology Group performance status (>0 versus 0)	1.634 (1.279-2.087)	<0.001*	2.363 (1.687-3.312)	<0.001*
Carbohydrate antigen 19-9 UI/ml	1.003 (1.001-1.005)	0.005	0.999 (0.995-1.003)	0.712
Carcinoembryonic antigen ng/ml	1.056 (0.998-1.118)	0.060	1.073 (1.013-1.136)	0.016
Presence of an immune-related adverse event (yes versus no)	0.676 (0.498-0.917)	0.012	0.632 (0.413-0.967)	0.034
Neutrophil-to-lymphocyte ratio (≥mNLR versus <mNLR)	1.573 (1.220-2.029)	<0.001*	2.033 (1.455-2.840)	<0.001*
Eosinophils baseline	1.036 (0.901-1.192)	0.618	0.725 (0.509-1.034)	0.076
Change in eosinophils (increase versus decrease)	1.324 (0.928-1.889)	0.121	1.161 (0.699-1.930)	0.564
Neutrophil-to-eosinophil ratio (≥mNER versus <mNER)	1.448 (1.121-1.869)	0.0045	1.655 (1.187-2.309)	0.003*

HR, hazard ratio; mNER, median neutrophil-to-eosinophil ratio; mNLR, median neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival.
*Statistical significance of $P < 0.0038$ (Bonferroni correction: $P = 0.05/13$).

above the median (HR 1.57, 95% CI 1.17-2.10, $P = 0.002$). OS was significantly shorter for patients with metastatic disease (HR 2.14, 95% CI 1.17-3.93, $P = 0.014$), ECOG PS >0 (HR 2.51, 95% CI 1.72-3.69, $P < 0.001$), and NLR above the median value (HR 2.08, 95% CI 1.45-3.00, $P < 0.001$). The results are reported in Table 3.

Response rates and adverse events

There was no significant difference in ORR between patients stratified based on NER or NLR values. Regarding adverse events (AEs) and immune-related adverse events (irAEs), no significant differences were observed in either the incidence or severity of these events between patients stratified by NER or NLR (Table 4).

Univariate Cox regression model on treatment

A univariate Cox regression model showed no statistically significant relationship between absolute on-treatment eosinophil count and OS or PFS. Similarly, changes in eosinophil levels on treatment (increase versus decrease) showed no significant association with these outcomes

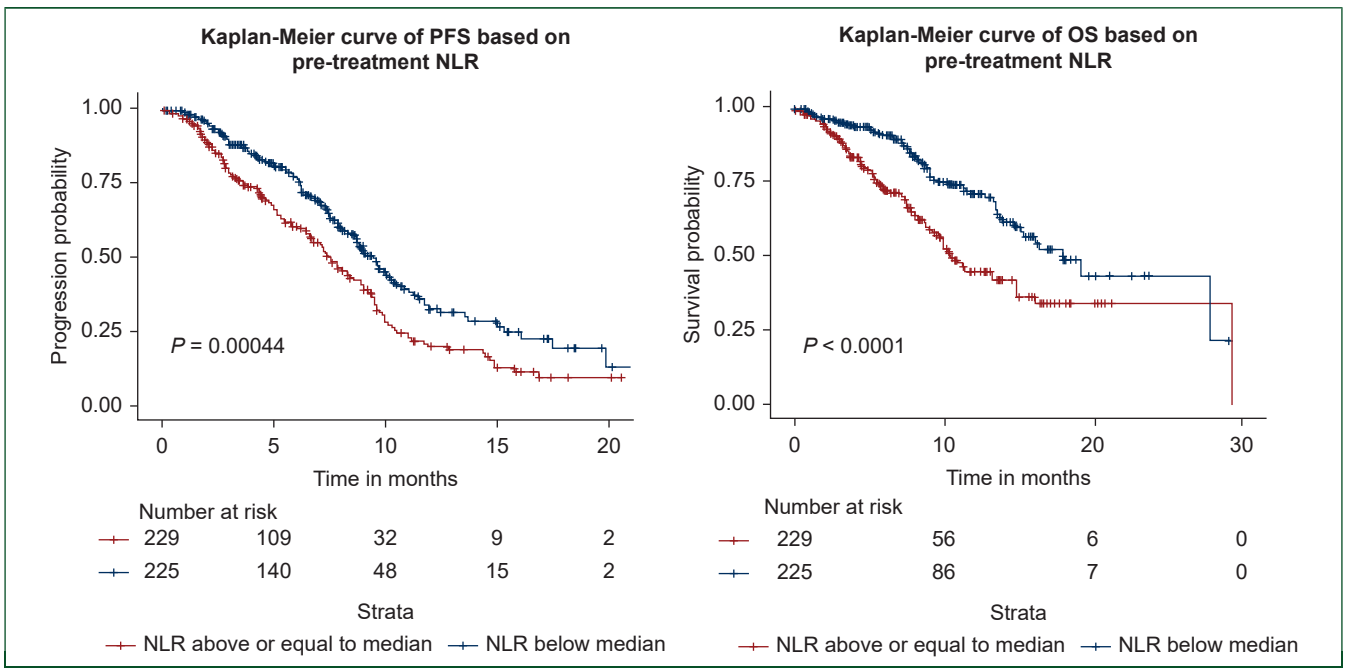

Figure 1. Kaplan-Meier curves for PFS and OS in patients with unresectable BTC stratified according to baseline NLR.
BTC, biliary tract cancer; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival.

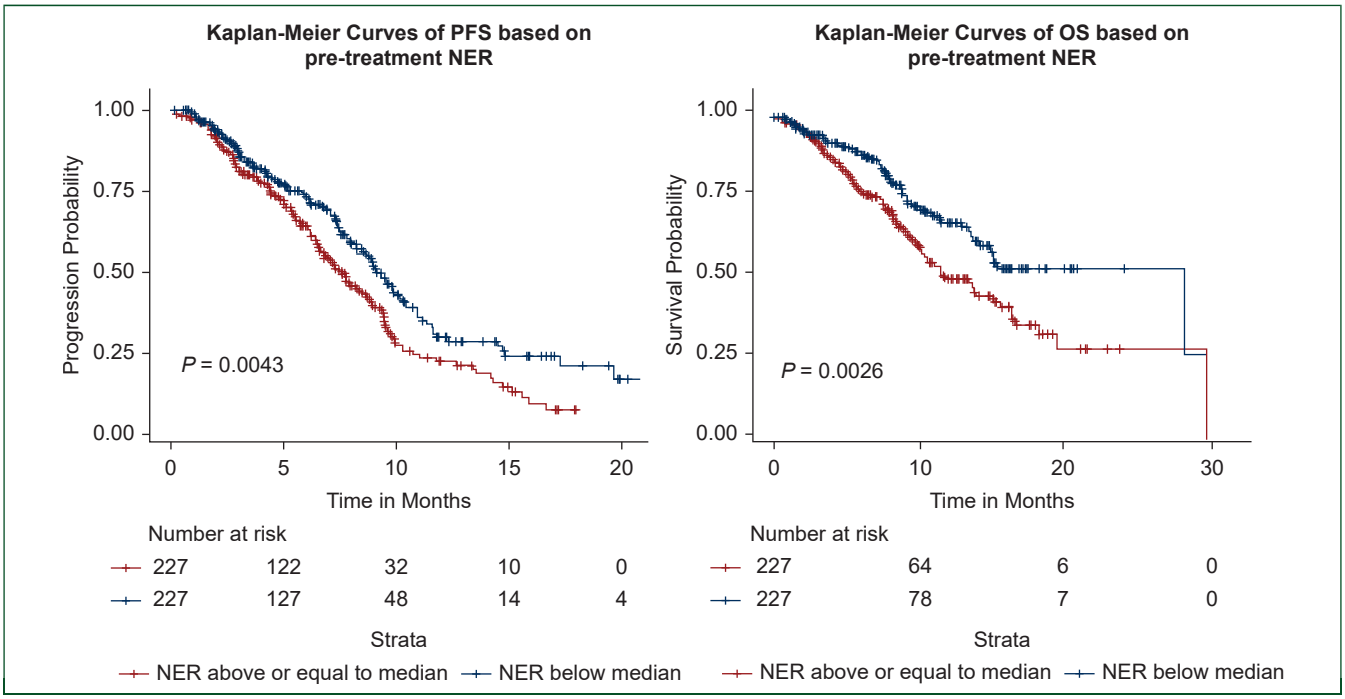

Figure 2. Kaplan-Meier curves for PFS and OS in patients with unresectable BTC stratified according to baseline NER.
BTC, biliary tract cancer; NER, neutrophil-to-eosinophil ratio; OS, overall survival; PFS, progression-free survival.

(Supplementary Table S2, available at https://doi.org/10.1016/j.esmogo.2026.100335).

DISCUSSION

BTC is a highly lethal malignancy with an increasing incidence and mortality worldwide.[4] Nevertheless, the recent introduction of ICI has resulted in an important change in the treatment landscape of patients diagnosed with unresectable disease. The TOPAZ-1 trial demonstrated that ∼25% of patients treated with gemcitabine, cisplatin, and durvalumab showed a response to this regimen, with 2.1% even achieving a complete response.[5] Given its promising results, this treatment regimen is now the standard first-line treatment for patients with unresectable BTC. However, not all patients derive benefit, and toxicity remains a significant concern. Therefore, identifying patients most likely to respond to treatment is crucial.

Blood-based inflammatory biomarkers are increasingly explored as predictors of survival and response to treatment across cancer types.[28–30] Lymphocytes are key mediators of antitumour immunity. High levels of tumour-infiltrating lymphocytes have been linked to favourable outcomes in various cancers, and elevated peripheral lymphocyte counts have similarly been associated with better responses and prolonged survival in patients treated with ICI, highlighting their potential as prognostic markers for immunotherapy efficacy.[15,19,20,23,24,28,30-32]

Table 3. Multivariate Cox regression analysis for PFS and OS of patients with unresectable biliary tract cancer.

Variable	PFS		OS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender (male versus female)	1.237 (0.929-1.648)	0.145	1.271 (0.889-1.817)	0.189
Age at start Gem-Cis-Durva	0.999 (0.986-1.014)	0.967	0.992 (0.975-1.009)	0.360
Primary tumour site				
Extrahepatic	Reference	Reference	Reference	Reference
Intrahepatic	1.046 (0.727-1.506)	0.807	1.192 (0.731-1.943)	0.482
Gallbladder	1.204 (0.747-1.941)	0.446	1.596 (0.866-2.941)	0.134
Disease stage (metastatic versus locally advanced)	2.189 (1.396-3.433)	<0.001*	2.144 (1.169 -3.930)	0.014*
Eastern Cooperative Oncology Group performance status (>0 versus 0)	1.797 (1.348-2.397)	<0.001*	2.514 (1.716-3.685)	<0.001*
Carbohydrate antigen 19-9 UI/ml	1.003 (1.000-1.006)	0.038*	0.999 (0.994-1.004)	0.625
Carcinoembryonic antigen ng/ml	1.029 (0.970-1.091)	0.341	1.036 (0.977-1.098)	0.241
Presence of an immune-related adverse event (yes versus no)	0.664 (0.474-0.931)	0.018*	0.650 (0.415-1.017)	0.059
Neutrophil-to-lymphocyte (≥mNLR versus <mNLR)	1.570 (1.174-2.100)	0.002*	2.082 (1.444-3.002)	<0.001*
Eosinophils baseline	0.813 (0.542-1.221)	0.319	0.913 (0.574-1.453)	0.702

HR, hazard ratio; mNLR, median neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival.
*Statistical significance of $P < 0.05$.

Table 4. Objective response rate, presence of AEs and presence of irAEs, stratified according to NER and NLR.

Response rates and adverse events							
All ($N = 509$)		NER			NLR		
		<mNER ($n = 227$)	≥mNER ($n = 227$)	*P* value	<mNLR ($n = 225$)	≥mNLR ($n = 229$)	*P* value
Objective response rate (per RECIST v1.1)				0.838			0.305
Yes (complete response + partial response)	159 (31.24%)	72 (31.72%)	78 (34.36%)		81 (36.00%)	69 (30.13%)	
No (stable disease + progressive disease)	299 (58.74%)	127 (55.95%)	131 (57.71%)		125 (55.56%)	133 (58.08%)	
Unknown or NA	51 (10.02%)	28 (12.33%)	18 (7.93%)		19 (8.44%)	27 (11.79%)	
AE				0.906			0.254
No AE	37 (7.27%)	18 (7.93%)	17 (7.49%)		22 (9.78%)	13 (5.68%)	
Grades 1-2	246 (48.33%)	106 (46.70%)	112 (49.34%)		109 (48.44%)	109 (47.60%)	
Grades 3-4	225 (44.20%)	102 (44.93%)	98 (43.17%)		93 (41.33%)	107 (46.72%)	
NA	1 (0.20%)	1 (0.44%)	0		1 (0.44%)	0	
irAE				0.160			1
No irAE	384 (75.44%)	157 (69.16%)	177 (77.97%)		168 (74.67%)	166 (72.49%)	
Grades 1-2	84 (16.50%)	45 (19.82%)	34 (14.98%)		42 (18.67%)	37 (16.16%)	
Grades 3-4	12 (2.36%)	4 (1.76%)	8 (3.52%)		6 (2.67%)	6 (2.62%)	
NA	29 (5.70%)	21 (9.25%)	8 (3.52%)		9 (4.00%)	20 (8.73%)	

AE, adverse event; irAE, immune-related adverse event; NA, nonapplicable; NER, neutrophil-to-eosinophil ratio; NLR, neutrophil-to-lymphocyte ratio; mNER, median neutrophil-to-eosinophil ratio; mNLR, median neutrophil-to-lymphocyte ratio.

In addition to lymphocytes, eosinophils have also emerged as promising immunological biomarkers due to their modulatory role in both innate and adaptive immunity. Preclinical studies show that activated eosinophils enhance antitumour responses. Given their interaction with the innate immune system, eosinophils may also synergise with ICI to boost immunotherapy efficacy. Clinically, tumour-associated eosinophilia and elevated peripheral eosinophil counts have been linked to a favourable prognosis in several cancer types.[33-41]

In contrast to the favourable role of lymphocytes and eosinophils, elevated neutrophil levels have been associated with tumour progression and poor clinical outcomes.[42] Neutrophils contribute to cancer development by promoting angiogenesis and remodelling of the extracellular matrix, thereby facilitating tumour invasion and metastasis. Additionally, tumour-associated neutrophils exert immunosuppressive effects, further enabling immune evasion within the tumour microenvironment.[10,17,18,34]

Therefore, composite blood-based inflammatory markers like NLR and NER have emerged as prognostic indicators in oncology.[7,24]

A recent meta-analysis by Su et al,[15] including 120 studies and 17 969 patients with advanced cancer, demonstrated that elevated pretreatment NLR was significantly associated with poorer OS, PFS and ORR in patients receiving immunotherapy.[15] These findings have been consistently confirmed across multiple independent studies.[43-48] Composite blood-based markers offer several practical advantages, including low cost, ease of implementation and suitability for longitudinal monitoring.[10]

The aim of our study was to evaluate whether NLR and NER could serve as prognostic biomarkers for treatment outcomes in patients with BTC receiving immunochemotherapy. Although there is some recent data on the prognostic utility of NLR in BTC, this is the first study to assess both NER and NLR in a large and homogenous cohort of patients with BTC receiving immunochemotherapy.

Our findings are in line with previous studies that demonstrated the prognostic value of blood-based inflammatory markers in cancer patients treated with immunochemotherapy. In our large cohort of 509 patients with advanced BTC, we found that a pretreatment NLR above the median was significantly associated with a shorter PFS and OS. Similarly, a higher NER at baseline was also associated with inferior OS. These results support the growing body of evidence suggesting that NLR and NER may serve as prognostic biomarkers for patients with BTC receiving immunochemotherapy, reflecting the pretreatment immunological state of the patient.

In contrast to their prognostic value, neither NLR nor NER correlated with ORR in our cohort. Although previous studies in other tumour types, such as non—small cell lung cancer, have reported associations between higher NLR and lower ORR with ICIs, these findings cannot be directly extrapolated to our cohort.[49]

Furthermore, we observed that neither NLR nor NER was associated with the occurrence of immune-related or other AEs. This aligns with the conflicting nature of the available evidence on the role of NLR and NER in predicting AEs. Although some studies have found a lower pretreatment NLR linked to a higher incidence of irAEs, others report no significant association.[50,51]

A key strength of this study is its large, real-world cohort of 509 patients with advanced BTC, from geographically diverse centres, which strengthens the robustness and enhances the generalizability of our findings. However, several limitations must be acknowledged. First, the retrospective design of the study. Second, peripheral blood counts, used to compose NLR and NER, are nonspecific and

can be influenced by various confounding factors, including infections, smoking, autoimmune diseases and stress. These markers are also dynamic and may fluctuate throughout the treatment course. Another challenge is the lack of standardised cutoff values for NLR and NER. We applied the cohort median to dichotomise patients, but cutoff values in the literature vary widely, particularly for NLR, where thresholds between 2 and 5 have been proposed. Establishing unified, evidence-based cutoffs will be essential for broader clinical application.

In conclusion, our findings support the prognostic relevance of NLR in patients with advanced BTC receiving immunochemotherapy. This simple, low-cost biomarker may help identify patients with a worse baseline prognosis, thereby aiding in risk stratification.

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REFERENCES

1. Banales JM, Rodrigues PM, Affò S, et al. Cholangiocarcinoma 2026: status quo, unmet needs and priorities. *Nat Rev Gastroenterol Hepatol*. 2026;23:65-96.
2. Sarcognato S, Sacchi D, Fassan M, et al. Cholangiocarcinoma. *Pathologica*. 2021;113:158-169.
3. Vithayathil M, Khan SA. Current epidemiology of cholangiocarcinoma in Western countries. *J Hepatol*. 2022;77:1690-1698.
4. Banales JM, Marin JJ, Lamarca A, et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol*. 2020;17:557-588.
5. Oh DY, Ruth He A, Qin S, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. *NEJM Evidence*. 2022;1(8):EVIDoa2200015.
6. Brindley PJ, Bachini M, Ilyas SI, et al. Cholangiocarcinoma. *Nat Rev Dis Primers*. 2021;7(1):65.
7. Vogel A, Bridgewater J, Edeline J, et al. Biliary tract cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34:127-140.
8. Ilyas SI, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology*. 2013;145:1215-1229.
9. Banales JM, Cardinale V, Carpino G, et al. Expert consensus document: cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol*. 2016;13:261-280.
10. Rimini M, Fornaro L, Rizzato MD, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer: a large real-life worldwide population. *Eur J Cancer*. 2024;208.
11. Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet*. 2023;401(10391):1853-1865.
12. Gooden MJ, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer*. 2011;105(1):93-103.
13. Pozorski V, Park Y, Mohamoud Y, et al. Neutrophil-to-eosinophil ratio as a biomarker for clinical outcomes in advanced-stage melanoma

patients treated with anti-PD-1 therapy. *Pigment Cell Melanoma Res*. 2023;36(6):501-511.
14. Beulque Y, Kinget L, Roussel E, et al. Baseline neutrophil-to-eosinophil-ratio and outcome in metastatic clear-cell renal cell carcinoma treated with nivolumab or ipilimumab/nivolumab. *Acta Oncol*. 2024;63:658-668.
15. Su J, Li Y, Tan S, Cheng T, Luo Y, Zhang L. Pretreatment neutrophil-to-lymphocyte ratio is associated with immunotherapy efficacy in patients with advanced cancer: a systematic review and meta-analysis. *Sci Rep*. 2025;15(1):446.
16. Ghaffari S, Rezaei N. Eosinophils in the tumor microenvironment: implications for cancer immunotherapy. *J Transl Med*. 2023;21(1):551.
17. Blomberg OS, Spagnuolo L, Garner H, et al. IL-5-producing CD4+ T cells and eosinophils cooperate to enhance response to immune checkpoint blockade in breast cancer. *Cancer Cell*. 2023;41:106-123.
18. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2014;106(6):dju124.
19. Bowen RC, Little NA, Harmer JR, et al. Neutrophil-to-lymphocyte ratio as prognostic indicator in gastrointestinal cancers: a systematic review and meta-analysis. *Oncotarget*. 2017;8:32171.
20. Pirozzolo G, Gisbertz SS, Castoro C, et al. Neutrophil-to-lymphocyte ratio as prognostic marker in esophageal cancer: a systematic review and meta-analysis. *J Thorac Dis*. 2019;11:3136.
21. Holub K, Biete A. New pre-treatment eosinophil-related ratios as prognostic biomarkers for survival outcomes in endometrial cancer. *BMC Cancer*. 2018;18(1):1280.
22. Sahin TK, Ayasun R, Rizzo A, Guven DC. Prognostic value of neutrophil-to-eosinophil ratio (NER) in cancer: a systematic review and meta-analysis. *Cancers*. 2024;16(21):3689.
23. Cho H, Hur HW, Kim SW, et al. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. *Cancer Immunol Immunother*. 2009;58(1):15-23.
24. Zhu J, Jiao D, Zhao Y, et al. Development of a predictive model utilizing the neutrophil to lymphocyte ratio to predict neoadjuvant chemotherapy efficacy in early breast cancer patients. *Sci Rep*. 2021;11:1-10.
25. McNamara MG, Templeton AJ, Maganti M, et al. Neutrophil/lymphocyte ratio (NLR) as a prognostic factor in biliary tract cancer (BTC). *J Clin Oncol*. 2013;31. 4130-4130.
26. Beal EW, Wei L, Ethun CG, et al. Elevated NLR in gallbladder cancer and cholangiocarcinoma—making bad cancers even worse: results from the US Extrahepatic Biliary Malignancy Consortium. *HPB*. 2016;18(11):950-957.
27. Tang H, Lu W, Li B, et al. Prognostic significance of neutrophil-to-lymphocyte ratio in biliary tract cancers: a systematic review and meta-analysis. *Oncotarget*. 2017;8. 36857-36868.
28. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140(6):883-899.
29. Ohkuma R, Kubota Y, Horiike A, et al. The prognostic impact of eosinophils and the eosinophil-to-lymphocyte ratio on survival outcomes in stage II resectable pancreatic cancer. *Pancreas*. 2021;50:167-175.
30. Tanrikulu AC, Abakay A, Komek H, Abakay O. Prognostic value of the lymphocyte-to-monocyte ratio and other inflammatory markers in malignant pleural mesothelioma. *Environ Health Prev Med*. 2016;21:304-311.
31. Dimitriou N, Felekouras E, Karavokyros I, Alexandrou A, Pikoulis E, Griniatsos J. Neutrophils to lymphocytes ratio as a useful prognosticator for stage II colorectal cancer patients. *BMC Cancer*. 2018;18(1):1202.
32. Jiang T, Qiao M, Zhao C, et al. Pretreatment neutrophil-to-lymphocyte ratio is associated with outcome of advanced-stage cancer patients treated with immunotherapy: a meta-analysis. *Cancer Immunol Immunother*. 2018;67(5):713-727.
33. Mingomataj EÇ. Eosinophil-induced prognosis improvement of solid tumors could be enabled by their vesicle-mediated barrier permeability induction. *Med Hypotheses*. 2008;70(3):582-584.
34. Furuta GT, Atkins FD, Lee NA, Lee JJ. Changing roles of eosinophils in health and disease. *Ann Allergy, Asthma and Immunology*. 2014;113(1):3-8.
35. Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. *Immunol Rev*. 2011;242(1):161-177.
36. Shamri R, Xenakis JJ, Spencer LA. Eosinophils in innate immunity: an evolving story. *Cell Tissue Res*. 2011;343(1):57-83.
37. Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol*. 1986;39:177-253.
38. Long H, Liao W, Wang L, Lu Q. A player and coordinator: the versatile roles of eosinophils in the immune system. *Transfus Med Hemother*. 2016;43:96.
39. Davis BP, Rothenberg ME. Eosinophils and cancer. *Cancer Immunol Res*. 2014;2(1):1-8.
40. Moreira A, Leisgang W, Schuler G, Heinzerling L. Eosinophilic count as a biomarker for prognosis of melanoma patients and its importance in the response to immunotherapy. *Immunotherapy*. 2017;9(2):115-121.
41. Wei Y, Zhang X, Wang G, et al. The impacts of pretreatment circulating eosinophils and basophils on prognosis of stage I-III colorectal cancer. *Asia Pac J Clin Oncol*. 2018;14(5):e243-e251.
42. Awasthi D, Sarode A. Neutrophils at the crossroads: unraveling the multifaceted role in the tumor microenvironment. *Int J Mol Sci*. 2024;25(5):2929.
43. Sacdalan DB, Lucero JA, Sacdalan DL. Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: a review and meta-analysis. *Onco Targets Ther*. 2018;11:955-965.
44. Valero C, Lee M, Hoen D, et al. Pretreatment neutrophil-to-lymphocyte ratio and mutational burden as biomarkers of tumor response to immune checkpoint inhibitors. *Nat Commun*. 2021;12(1):1-9 (12).
45. Booka E, Kikuchi H, Haneda R, et al. Neutrophil-to-lymphocyte ratio to predict the efficacy of immune checkpoint inhibitor in upper gastrointestinal cancer. *Anticancer Res*. 2022;42(6):2977-2987.
46. Chen X, Meng F, Jiang R. Neutrophil-to-lymphocyte ratio as a prognostic biomarker for patients with metastatic renal cell carcinoma treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Front Oncol*. 2011;11:746976.
47. Park W, Lopes G. Perspectives: neutrophil-to-lymphocyte ratio as a potential biomarker in immune checkpoint inhibitor for non—small-cell lung cancer. *Clin Lung Cancer*. 2019;20(3):143-147.
48. Ogihara K, Kikuchi E, Shigeta K, et al. The pretreatment neutrophil-to-lymphocyte ratio is a novel biomarker for predicting clinical responses to pembrolizumab in platinum-resistant metastatic urothelial carcinoma patients. *Urol Oncol*. 2020;38(6):602.e1. 602.e10.
49. Nguyen CT, Van TN, Huong PT. Predictability of neutrophile to lymphocyte ratio and platelet to lymphocyte ratio on the effectiveness of immune checkpoint inhibitors in non-small cell lung cancer patients: a meta-analysis. *Cancer Control*. 2024;31:1-11.
50. Fujimoto A, Toyokawa G, Koutake Y, et al. Association between pretreatment neutrophil-to-lymphocyte ratio and immune-related adverse events due to immune checkpoint inhibitors in patients with non-small cell lung cancer. *Thorac Cancer*. 2021;12(15):2198-2204.
51. Tasaki Y, Hamamoto S, Yamashita S, et al. Eosinophil is a predictor of severe immune-related adverse events induced by ipilimumab plus nivolumab therapy in patients with renal cell carcinoma: a retrospective multicenter cohort study. *Front Immunol*. 2025;15:1483956.