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RESEARCH ARTICLE

Unveiling the mechanisms of immune evasion in pancreatic cancer: may it be a systemic inflammation responsible for dismal survival?

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Abstract

Purpose Pancreatic cancer (PC) is one of the most aggressive malignancies with no effective treatment if diagnosed in advanced stage. Systemic inflammation is a recognized characteristic of cancer progression, and we believe that the understanding of the influence of inflammatory parameters may contribute to therapeutic improvement in PC. Here, we validated the Eosinophil/Lymphocyte Ratio (ELR) together with the Neutrophil/Lymphocyte Ratio (NLR) and their components, as prognostic factors in PC patients treated with chemoradiation.

Methods A total of 66 consecutive patients (p) diagnosed with PC stage I–III and treated with External Beam Radiotherapy + chemotherapy ± surgery (28p) in our institution from 2007 to 2018 were retrospectively evaluated. The impact of pretreatment ELR ≥ 0.04, NLR ≥ 1.9, neutrophilia ($\geq 7.0 \times 10^{(9)}$/l), eosinophilia ($\geq 0.5 \times 10^{(9)}$/l) and lymphopenia ($< 1.0 \times 10^{(9)}$/l) on Overall Survival (OS) and Time-to-Progression (TTP) was evaluated both in the entire cohort and separately according to surgical status.

Results Higher ELR was associated with longer OS and TTP, both in surgically treated and not operable patients. On univariate analysis, elevated ELR was associated with better OS (HR = 0.3, 95% IC 0.13–0.65, $p = 0.003$), contrarily to neutrophilia (HR = 2.7, 95% IC 1.2–6.5, $p = 0.026$) and age > 50 years (HR = 2.6, 95% IC 1.03–6.6, $p = 0.044$), while NLR, lymphopenia and Ca-19.9 were not significant. On multivariate regression, independent prognosticators for OS were: ELR, age and neutrophilia; while for TTP: ELR, neutrophilia, eosinophilia and lymphopenia.

Conclusions The host's immune response influences survival outcomes of PC patients and may be of interest for future research.

Keywords Pancreatic cancer · Systemic inflammation · Neutrophil/lymphocyte ratio · Eosinophil/lymphocytes ratio · Eosinophils · Neutrophils

Introduction

Pancreatic cancer (PC) is an incurable disease with a 5-year survival rate of 8.5% that has not improved over the past two decades [1]. Although PC is the 11th in frequency of tumors incidence, it is currently the 4th leading cause of death by cancer, with a global median overall survival of 4.6 months [2]. Approximately, 55,440 new cases were diagnosed and 44,330 patients died of this cancer along 2018 in the USA [1]. The vast majority of patients are diagnosed with regional (29%) or distant dissemination (52%) that determines 88.5% and 97.3% 5-year mortality rate, respectively. Even among patients undergoing surgery (10% of all cases), only 34.3% of them are alive after 5 years [2].

This dismal prognosis is supposedly a consequence of a simultaneous alteration in the expression of oncogenes

Some preliminary results of this study were presented at the 20th World Congress on Gastrointestinal Cancer of the European Society for Medical Oncology (ESMO) held in Barcelona, Spain, in June 2018.

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Table 1 Characteristics of study population and inflammatory parameters ($n=66$)

Patients' characteristics	All patients $n=66$ (100%)	ELR < 0.04 $n=9$ (13.6%)	ELR ≥ 0.04 $n=57$ (86.4%)	p value
Age at diagnosis (years)				
Mean (SD) median (range)	63.7 (10.8) 65.5 (42–84.0)	63.3 (10.9) 65.0 (44.0–80.0)	63.7 (10.9) 66.0 (42.0–84.0)	–
≤ 50 years old	9 (13.6%)	1 (11.1%)	8 (88.9%)	0.81
> 50 years old	57 (86.4%)	8 (14.0%)	49 (86.0%)	
TNM stage				
IA	5 (7.6%)	0	5 (100%)	0.38
IB	5 (7.6%)	1 (20%)	4 (80%)	
IIA	10 (15.2%)	2 (20%)	8 (80%)	
IIB	32 (48.5%)	6 (18.8%)	26 (81.2%)	
III	14 (21.2%)	0	14 (100%)	
Stage III				
No	52 (78.8%)	9 (17.3%)	43 (82.7%)	0.09
Yes	14 (21.2%)	0	14 (100%)	
Gender				
Men	41 (62.1%)	4 (9.8%)	37 (90.2%)	0.24
Women	25 (37.9%)	5 (16.0%)	20 (84.0%)	
Histology				
Adenocarcinoma	63 (95.6%)	8 (16.7%)	55 (83.3%)	0.31
Other histology	3 (4.5%)	1 (33.3%)	2 (66.6%)	
Tumor localization				
Head and neck	52 (78.8%)	7 (13.5%)	45 (86.5%)	0.94
Body and tail	14 (21.2%)	2 (14.2%)	12 (85.7%)	
Ca-19.9 level ($n=50$)				
Mean (SD) median (range)	570.2 (1476) 175.5 (0–10000)	793.4 (646.9) 921.0 (108–2005)	533.9 (1573.4) 149.0 (0–10000)	–
< 120	21 (42%)	1 (4.8%)	20 (95.2%)	0.11
≥ 120	29 (58%)	6 (20.7%)	23 (79.3%)	
Surgery				
No	39 (59%)	5 (12.8%)	34 (87.2%)	0.82
Yes	27 (41%)	4 (14.8%)	23 (85.2%)	
Status at the end of data collection				
Alive	11 (16.7%)	1 (9.1%)	10 (90.9%)	0.63
Dead (cancer-related deaths)	55 (83.3%)	8 (14.5%)	47 (85.5%)	
Patients with disease progression				
Non-progression	4 (6.1%)	0	4 (100%)	0.41
Progression	62 (93.9%)	9 (14.5%)	53 (85.5%)	
–Local progression	27 (40.9%)	3 (11.1%)	24 (88.9%)	0.69
–Distant progression	20 (30.3%)	4 (20.0%)	16 (80.0%)	
–Both	15 (22.7%)	2 (13.3%)	13 (86.7%)	
Eosinophils level (x $10^{(9)}$/l)				
Media (SD) median (range)	0.189 (0.22) 0.10 (0.0–1.7)	0.44 (0.05) 0.00 (0.0–0.1)	0.21 (0.23) 0.20 (0.1–1.7)	–
≥ 0.5	3 (4.5%)	0	3 (100%)	0.48
< 0.5	63 (95.5%)	9 (14.3%)	54 (85.7%)	
Lymphocytes level (x $10^{(9)}$/l)				
Media (SD) median (range)	1.79 (0.79) 1.7 (0.6–4.9)	2.3 (1.5) 2.6 (0.6–4.9)	1.7 (0.6) 1.7 (0.7–3.7)	–
< 1.0	9 (13.6%)	3 (33.3%)	6 (66.7%)	0.10
≥ 1.0	57 (86.4%)	6 (10.5%)	51 (89.5%)	
Neutrophils level (x $10^{(9)}$/l)				
Media (SD) median (range)	4.8 (1.58) 4.75 (1.7–9.4)	5.1 (1.96) 4.8 (3.0–9.4)	4.8 (1.5) 4.7 (1.7–8.3)	–
≥ 7.0	6 (9.1%)	1 (16.7%)	5 (83.3%)	0.82
< 7.0	60 (90.9%)	8 (13.3%)	52 (86.7%)	

Table 1 (continued)

Patients' characteristics	All patients $n=66$ (100%)	ELR < 0.04 $n=9$ (13.6%)	ELR ≥ 0.04 $n=57$ (86.4%)	p value
NLR				
Media (SD) median (range)	3.13 (1.65) 2.78 (1.0–10.4)	3.2 (2.22) 2.17 (1.0–7.5)	3.1 (1.6) 2.8 (1.05–10.4)	–
≥ 1.9	14 (21.2%)	2 (14.3%)	12 (85.7%)	0.94
< 1.9	52 (78.8%)	7 (13.5%)	45 (86.5%)	

and suppressor genes, but the underlying mechanisms may also include a complex tumor microenvironment that likely stimulates the immunologic evasion, thus conditioning therapy resistance [3–5]. For these reasons, an active search of mechanisms responsible of this pro-tumor status is especially needed to develop more effective treatment options.

In the last decade, the paradigm linking cancer progression with the host's systemic inflammatory response has increasingly been accepted, and multiple studies have evaluated the impact of circuiting blood cells on the survival outcomes in different cancer settings [6, 7]. The most frequently validated inflammatory ratio is the Neutrophil–Lymphocytes Ratio (NLR), and various attempts have been made to validate its ability to predict the response to different chemotherapeutics in PC [8–16].

Although NLR proved to be an independent prognosticator both in inoperable and surgically treated PC patients [8, 10, 12], the evidence about the role of other subtypes of white blood cells (WBC) in PC progression is scarce [12]. This issue is especially germane, since historically, the only WBC related with PC were eosinophils, responsible for eosinophilic pancreatitis mimicking cancer and reported occasionally in advanced disease [17, 18]. Interestingly, according to some publications from the late 90s, circulating eosinophils were capable to stimulate lymphocytes, which may have a special interest in PC, where the T cells do not penetrate to the stromal microenvironment [19–22]. Recently, the significance of eosinophils in oncology has caught more attention, although mostly focused on preclinical models and/or tumor-infiltrating eosinophils [20–23].

Our study aimed to validate the Eosinophil/Lymphocytes Ratio (ELR), previously described as an independent prognostic factor in uterine tumors [24, 25], in a cohort of PC patients treated with chemoradiation, as well as to explore a prognostic value of pre-treatment neutrophilia, eosinophilia, lymphopenia and NLR.

Materials and methods

After our Institutional Review Board approval, the clinical data of 85 consecutive patients (p) diagnosed with histologically proven PC between September 2007 and March 2018, were retrieved from our departmental database. All participants gave their informed consent for treatment and data processing. The study was conducted in accordance with ethical standards following the rules of the Declaration of Helsinki, (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013. Nineteen patients were excluded for the following reasons: neuroendocrine histology (2p), External Beam Radiotherapy (EBRT) not completed (6p), palliative RT for PC metastasis (8p), blood test data not available (1p), pancytopenia at cancer diagnosis (1p) and an extremely long survival of one patient (129.7 months), excluded to maintain the sample more homogeneous.

Of the remaining 66 patients included in the analysis, no patient used immunosuppressive drugs (including anti-inflammatory and steroids) or had evidence of active infections (including human immunodeficiency virus and hepatitis) or history of any other active malignant tumor in the 12 months previous to PC diagnosis.

The level of circulating WBC (eosinophils, neutrophils and lymphocytes) in pre-treatment blood tests was analysed, and two groups were created according to the standard laboratory cut-off for neutrophilia ($\geq 7.0 \times 10^{(9)}$/l), eosinophilia ($\geq 0.5 \times 10^{(9)}$/l) and lymphopenia ($< 1.0 \times 10^{(9)}$/l). Subsequently, the inflammatory ratios were calculated as follows: Neutrophil/lymphocyte ratio (NLR: neutrophils count divided by the lymphocyte count) and Eosinophil/lymphocyte ratio (ELR: eosinophils count divided by the lymphocyte count). To convert NLR and ELR into binary variables, the optimal cut-off values of the receiver-operating characteristic (ROC) curves were applied, generated separately for each parameter, that was 0.04 for ELR [an area under curve, AUC, of 0.602, a sensitivity (Se) of 0.8 and a specificity (Sp) of 0.9] and 1.9 for NLR (AUC 0.593, Se 0.8, Sp 0.6) [Fig. S1]. Furthermore, the subgroup of patients with histology of adenocarcinoma ($n=63$) was evaluated.

Statistics

All statistical tests were two-sided and the p value < 0.05 was considered as statistically significant. Only the variables with p value < 0.05 in Cox univariate analysis were analysed in multivariate regression.

Table 2 Analysis of overall survival: Kaplan–Meier analysis, Cox univariate and multivariate regression (ELR model) based on the data of the entire study population (n = 66)

n = 66 Variables	Kaplan–Meier survival analysis			Univariate cox regression		Multivariate cox regression—ELR model	
	n	*p* value (X2)	OS (range)	HR (IC 95%)	*p* value	HR (IC 95%)	*p* value
Age > 50 years (vs. ≤ 50 years)	57 vs. 9	**0.036** (4.4)	17.2 (14.7–19.7) vs. 31.6 (26.2–37.1)	**2.6 (1.03–6.6)**	**0.044**	2.3 (0.9–5.8)	0.087
Surgery (vs. no surgery)	27 vs. 39	0.209 (1.6)	23.1 (14.0–32.1) vs. 17.2 (15.8–18.6)	0.7 (0.4–1.2)	0.211	–	–
Ca-19.9 ≥ 120 (vs. < 120) *$n = 50$	29 vs. 21	**0.049** (3.9)	22.7 (12.4–23.5) vs. 26.2 (17.2–35.2)	1.9 (1.0–3.7)	0.052	–	–
ELR ≥ 0.04 (vs. < 0.04)	57 vs. 9	**0.001** (10.2)	19.5 (16.5–22.5) vs. 10.6 (8.2–13.0)	**0.3 (0.1–0.7)**	**0.003**	**0.3 (0.14–0.69)**	**0.004**
NLR ≥ 1.9 (vs. < 1.9)	52 vs. 14	0.093	17.9 (14.6-21.2) vs. 26.2 (18.1–34.3)	1.8 (0.9–3.4)	0.098	–	–
Eosinophils ≥ 0.5 (vs. < 0.5)	3 vs. 63	0.269 (1.2)	6.2 (2.2–10.2) vs. 18.7 (16.4–21.1)	1.9 (0.6–6.3)	0.300	–	–
Neutrophils ≥ 7.0 (vs. < 7.0)	6 vs. 60	**0.021** (5.4)	13.1 (7.8–18.4) vs. 18.9 (16.1–21.8)	**2.7 (1.2–6.5)**	**0.026**	**2.5 (1.1–6.2)**	**0.039**
Lymphocytes < 1.0 (vs. ≥ 1.0)	9 vs. 57	0.078	14.3 (13.8–14.8) vs. 19.5 (16.4–22.6)	1.9 (0.9–4.0)	0.083	–	–

p value <0.05 are in bold

All parameters analysed through Kaplan–Meier (KM) analysis and Cox regression were defined as binary variables by finding the cut-off value from a ROC curve. Additionally, a cut-off finder program software was applied. Correlations between variables were assessed via χ^2 or Fisher's exact tests where appropriate. As all deaths were a consequence of PC progression, the primary endpoint was OS, which in this study was equal to cancer-specific survival (CSS) and defined as the time from cancer diagnosis to cancer-related death or last visit. The secondary endpoint was Time-to-Progression (TTP), defined as the time from cancer diagnosis to the first disease progression. Patients who did not present any event at the time of data collection were censored at the time they were last known to be event free. Additionally, a predefined subgroup analysis of patients surgically treated or not operable was conducted. The survival outcomes were analysed using log rank test. All statistical analyses were performed using SPSS v. 23 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of patients and treatments

The median age at PC diagnosis was 65.5 years, mean 63.7, standard deviation (SD) 10.8, range 42–84. The most frequent histology was adenocarcinoma—63p (95.5%). Other histological types were: acinar carcinoma, adenosquamous carcinoma and mixed histology (ductal + clear cells carcinoma), each of these histologies represented by only one patient (1.5%). In 52 patients (78.8%) the primary tumor was localized in the pancreatic head (47 p, 71.2%) or neck (5p, 7.6%); whereas in 14 p (21.2%), in the body and/or tail. All patients were staged using radiological imaging as stage: IA in 5p, IB—5p, IIA—10p, IIB—32p and III—14p. Ten patients (15.2%) were classified as borderline, 39p (59.1%) as unresectable and 17p (25.8%) as resectable. The baseline patients' characteristics are detailed in Table 1.

Primary treatment was performed in accordance with the European guidelines conforming to the cancer stage at diagnosis [4]. All patients were treated with chemoradiation, based on weekly chemotherapy and EBRT with a total dose initially planned of 45 Gray, although in 7 cases it was not achieved because of a clinical worsening of patients. In 39 cases (59.1%), chemo-radiation was an exclusive treatment. Surgical resection was applied in 27 patients (40.9%) with neo-adjuvant (13p, 19.7%) or adjuvant (14p, 21.2%) chemo-radiation (including one patient who was operated before and after chemoradiation).

The most frequently used surgical technique was pancreaticoduodenectomy (Whipple procedure) in 21 p (21.2%), followed by total pancreatectomy (2p), distal pancreatectomy (2p), Roux-Y-hepaticojejunostomy (2p). In 22 patients (33.3%) the first treatment consisted of 3 cycles of gemcitabine [1000 mg/m^2 on days 1, 8, 15 of a 28-day cycle] followed by chemoradiation. In 27 patients (40.9%), the first treatment was chemoradiation based on EBRT in a total dose of 45 Gy in 25 fractions (1.8 Gy/fraction) concomitant with gemcitabine, fluorouracil or capecitabine.

Fig. 1 Impact of pre-treatment Eosinophil–Lymphocytes Ratio (ELR, ▶ cut-off 0.04) on overall survival (OS). **a** Entire study population of PC patients: $n=66$ (57 vs. 9). **b** Patients not treated with surgery: $n=39$ (34 vs. 5). **c** Patients treated with surgery: $n=27$ (23 vs. 4)

In 3 patients (4.5%), RT and chemotherapy were administrated sequentially. Other chemotherapy schemes were the following: gemcitabine–capecitabine, 5-fluorouracil, GEMOX (6p), FOLFIRINOX (7p), carbotaxol (2p), gemcitabine–abraxane (17p) and gemcitabine–everolimus (6p).

Characteristics of inflammatory parameters detected at cancer diagnosis

A high pre-treatment ELR ≥ 0.04 was observed in 9 patients (13.6%), NLR ≥ 1.9 in 52 patients (78.8%), neutrophilia in 6 patients (9.1%), eosinophilia in 3 patients (4.5%), and lymphopenia in 9p (13.5%). After the analysis of correlations, no statistically significant association was found between a high level of eosinophils, a low level of lymphocytes and a high ELR. All detailed data of the levels of studied parameters are exposed in Table 1.

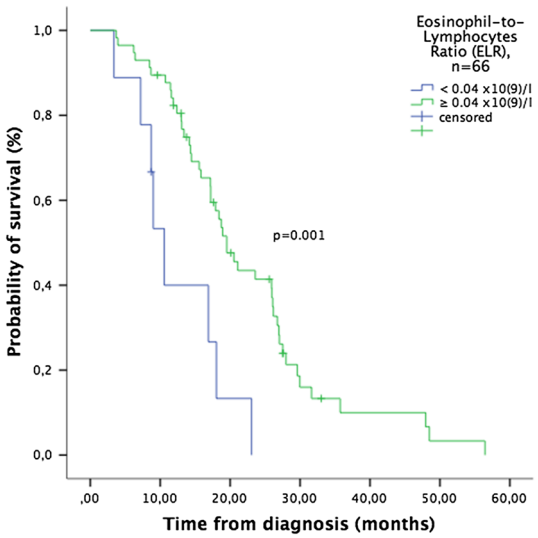
A Sialyl-Lewis carbohydrate antigen Ca-19.9, the more sensitive and specific serum marker for pancreatic cancer used in clinical practice, was retrieved from a blood test at cancer diagnosis, but was only available for 50 patients [3]. As 13 patients (26%) presented values < 37 U/ml, including 6 p (9%) with antigen lower than the sensibility of the laboratory test, we decided to establish a cut-off according to the ROC curve for our cohort in Ca-19.9 ≥ 120 (AUC 0.505, Se 0.6, Sp 0.5), observed in 29 p (43.3%).

Survival outcomes of the entire cohort and according to the surgical status

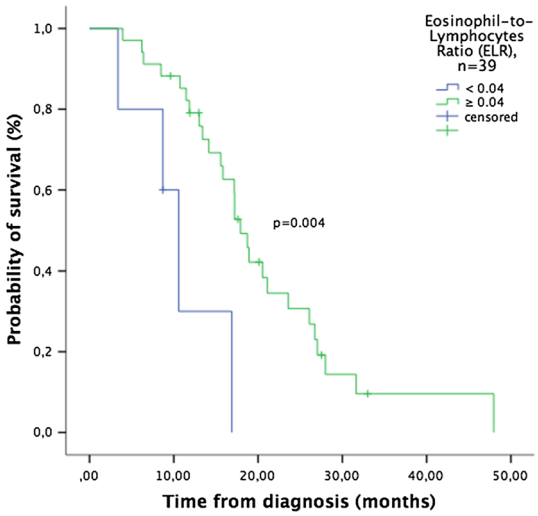
Overall survival

Median OS of the entire cohort was 17.4 months (range 3.4–56.4), with 21 months for surgically treated patients (range 3.7–56.4) and 19.5 months (range 3.4–48.0) for not operable patients. Fifty-five patients (83.3%) were dead at the time of data collection and all deaths were cancer related. In the entire study population ($n=66$), 1-, 2-, 3- and 4-year OS rates were of 77% (48p), 37%(20p), 10% (3p) and 7% (2p), respectively. In the surgically treated cohort ($n=39$), the survival rates in 1, 2, 3 and 4 years after diagnosis were as follows: 81% at 12 months (22p), 47% at 24 months (12p), 13% at 36 and 48 months (2p), while in patients with no possibility of surgery ($n=27$) were 73% at 12 months (26p), 28% at 24 months (8p), 8% at 36 months (1p) with no survivals at 48 months.

A Entire study population of PC patients: n=66 (57 vs. 9).



B Patients not treated with surgery: n=39 (34 vs. 5).



C Patients treated with surgery: n=27 (23 vs. 4).

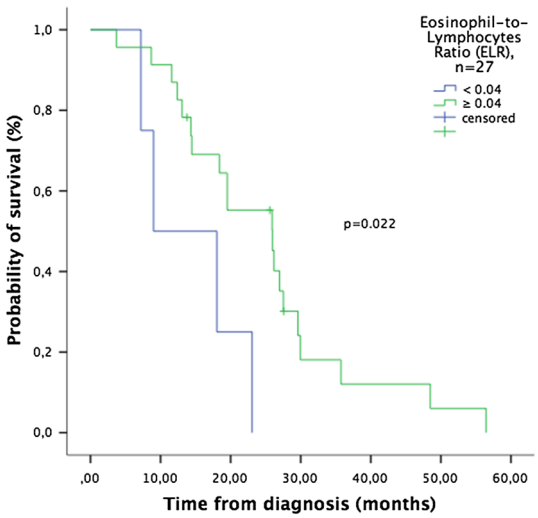


Table 3 Time-to-Progression: Kaplan–Meier, Cox univariate and multivariate regression (ELR model) based of the data of the entire study population ($n=61$)

$n=61$ Variables	Kaplan–Meier survival analysis			Univariate Cox regression		Multivariate cox regression—ELR model	
	n	*p* value	OS (range)	HR (IC 95%)	*p* value	HR (IC 95%)	*p* value
Age > 50 years (vs. ≤ 50 years)	53 vs. 8	0.121 (2.4)	10.0 (8.7–11.3) vs. 14.8 (4.5–25.1)	1.8 (0.8–3.8)	0.128	–	–
Surgery (vs. no surgery)	23 vs. 38	0.680 (0.17)	10.2 (7.6–12.7) vs. 10.0 (8.2–11.8)	0.9 (0.5–1.5)	0.681	–	–
Ca-19.9 ≥ 120 (vs. < 120) *$n=48$	27 vs. 21	0.317 (1.0)	9.7 (7.9–11.5) vs. 11.4 (7.4–15.4)	1.9 (1.0–3.7)	0.319	–	–
ELR ≥ 0.04 (vs. < 0.04)	52 vs. 9	**0.000 (21.2)**	**10.9 (9.9–11.9) vs. 5.7 (2.2–9.3)**	**0.2 (0.07–0.39)**	**0.000**	**0.13 (0.05–0.33)**	**0.000**
NLR ≥ 1.9 (vs. < 1.9)	48 vs. 13	0.402 (0.7)	9.7 (8.3–11.1) vs. 10.8 (9.9–11.8)	1.3 (0.7–2.4)	0.404	–	–
Eosinophils ≥ 0.5 (vs. < 0.5)	3 vs. 58	**0.014 (6.2)**	**2.3 (0.4–4.2) vs. 10.2 (8.8–11.6)**	**3.9 (1.2–13.2)**	**0.024**	**7.1 (2.03–24.6)**	**0.002**
Neutrophils ≥ 0.19 (vs. < 0.19)	6 vs. 55	**0.000 (14.5)**	**2.9 (1.7–4.0) vs. 10.8 (9.8–11.9)**	**5.0 (2.0–12.4)**	**0.001**	**6.2 (2.4–16.1)**	**0.000**
Lymphocytes < 1.0 (vs. ≥ 1.0)	9 vs. 52	**0.016 (5.8)**	**5.2 (3.2–7.1) vs. 10.6 (9.5–11.7)**	**2.4 (1.2–5.0)**	**0.020**	**2.6 (1.2–5.4)**	**0.014**

p value <0.05 are in bold

In the KM analysis of the entire cohort ($n=66$), age, Ca-19.9, ELR and neutrophilia influenced OS were significantly associated with OS; whereas tumor localization (in the pancreatic head–neck vs. body–tail) and stage III (vs. stage I–II) were not [Table 1]. Univariate analyses showed an increased risk of death with a higher age and neutrophilia, while a high ELR was associated with better OS (HR 0.3, $p=0.003$) [Table 2]. Beneficial impact of a higher ELR was confirmed separately for surgically treated patients (HR 0.214, IC 95%; 0.07–0.7, $p=0.022$) and not operable (HR 0.032, IC 95% 0.086–0.9, $p=0.004$), although these results should be evaluated with caution due to a very small sample size in low ELR cohorts [Fig. 1]. Of note, a high NLR, Ca-19.9 and lymphopenia did not show a significant impact on OS in our cohort ($p=0.098$, 0.052 and 0.083, respectively).

On multivariate Cox regression, only ELR (HR 0.31, $p=0.004$) and neutrophilia (HR 2.54, $p=0.039$) proved to be independent prognosticators for OS [Table 2].

In the entire study population ($n=66$), the survival rates at 1-, 2-, 3- and 4-year after diagnosis for patients with ELR ≥ 0.04 (57p) were as follows: 82% (45p), 42% (20p), 12% (3p) and 8% (2p), while in patients with ELR < 0.04 (9p) the 1-year OS was of 41% (3p) with no survivals at 24 months.

Time-to-progression

Sixty-one patients (92.4%) presented disease progression during the study. The first progression occurred in a median time of 10.2 months (range 0.7–37.5) in the entire cohort, being 12.4 months (range 1.1–36.4) after surgical resection and 10.1 months (range 0.7–37.5) in not operable patients. A total of 27 patients (40.9%) presented exclusively local progression, 20 patients (30.3%) had only distant recurrence while 15 patients (22.7%) presented both local and distant failure. The most frequent localization of metastasis was liver (25p, 71.4%), lymphatic nodes (5p), peritoneal implants (4p) and lung (3p).

In the Kaplan–Meier survival analysis, a shorter TTP was associated with lower ELR ($p=0.00$), both in surgically treated and not operable cohort ($p=0.001$ and $p=0.003$, respectively), eosinophilia ($p=0.014$), neutrophilia ($p=0.00$) and lymphopenia ($p=0.016$) [Table 3, Figs. 2 and 3]. The unadjusted HR for progression was statistically significant for higher ELR (HR 0.2, $p=0.00$), eosinophilia (HR = 3.9, $p=0.02$), neutrophilia (HR = 5.0, $p=0.00$) and lymphopenia (HR = 2.4, $p=0.02$) [Table 3]. On multivariate analysis ELR (HR = 0.13, $p=0.00$), neutrophilia (HR = 6.2, $p=0.00$), lymphopenia (HR 2.6, $p=0.014$) and eosinophilia (HR 7.1, $p=0.002$) proved to be independent prognosticators for TTP [Table 3].

Higher Ca-19.9 at cancer diagnosis did not influence TTP of the entire study population ($p=0.032$) but was statistically significant ($p=0.025$) in patients treated with surgery (5.7 months vs. 11.4 months in patients with Ca-19.9 < 120).

Fig. 2 Impact of pre-treatment Eosinophil–Lymphocytes Ratio (ELR, ▶ cut-off 0.04) on Time-to-Progression (TTP). **a** Entire study population of PC patients staged I–III: $n=61$ (52 vs. 9). **b** Patients not treated with surgery: $n=38$ (33 vs. 5). **c** Patients treated with surgery: $n=23$ (19 vs. 4)

Separately, the patients with adenocarcinoma histology were analysed ($n=63$), and the 1-, 2-, 3- and 4-year OS rates were 77% (46p), 35% (18p), 9% (2p) and 4% (1p), respectively. In the surgically treated cohort ($n=25$), the survival rates in 1 , 2 , 3 and 4 years after diagnosis were as follows: 84% at 12 months (21p), 47% at 24 months (11p), 9% at 36 and 48 months (1p), while in patients with no possibility of surgery ($n=38$) were 73% at 12 months (25p), 26% at 24 months (7p), 9% at 36 months (1p) with no survivals at 48 months. The independent prognosticators were ELR ≥ 0.04 (HR 0.4, $p=0.017$) and neutrophilia (HR 2.5, $p=0.047$) for OS, while ELR (HR = 0.17, $p=0.00$), eosinophilia (HR = 3.6, $p=0.035$) and neutrophilia (HR = 4.8, $p=0.00$) were so for TTP [Table S1, S2, S3].

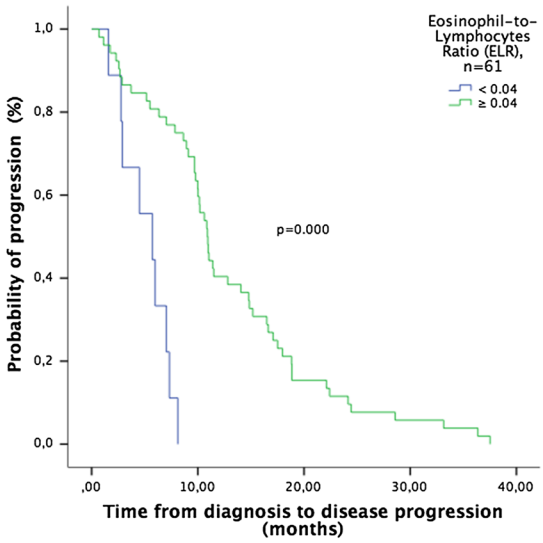
Discussion

Recently, we have witnessed a change in the paradigm of prognostic factors in cancer research: more and more studies provide results about the prognostic and even predictive role of systemic inflammation for survival outcomes in several malignancies [6, 7]. In PC, due to a lack of efficient treatment, the main interest focus on factors that may help to understand the complex microenvironment of this tumor, which generate the status of immune evasion [26–28]. Although the standards of care for metastatic patients have changed in favor to mFOLFIRINOX and Gemcitabine–Abraxane instead of gemcitabine monotherapy, a median OS of these patients is still poor in comparison to other aggressive malignancies such as melanoma or non-small cell lung cancer [3, 4]. At present, the only viable prognosticator of PC progression is a tumor stage at diagnosis, which determines the operability, [3, 4].

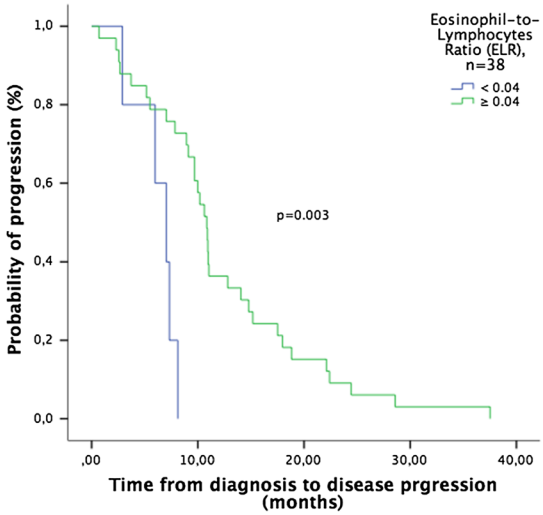
We demonstrated that a higher pre-treatment ELR portend better survival outcomes (HR = 0.3 for OS that indicates a 70% relative reduction in the risk of death for patients with ELR ≥ 0.04 at cancer diagnosis, and HR = 0.13 for TTP), while neutrophilia was inversely correlated with OS and TTP.

Even though the tumor-associated blood eosinophilia was described in some cases of advanced PC, we confirmed that it is really infrequent in clinical practice (in our cohort 3p, 4.5%), as well as lymphopenia (9p, 13.8%), and did not correlate with high ELR ($p=0.48$ and $p=0.1$, respectively). For this reason, our hypothesis relies on the relation between

A Entire study population of PC patients staged I-III: n=61 (52 vs. 9).



B Patients not treated with surgery: n=38 (33 vs. 5).



C Patients treated with surgery: n=23 (19 vs. 4).

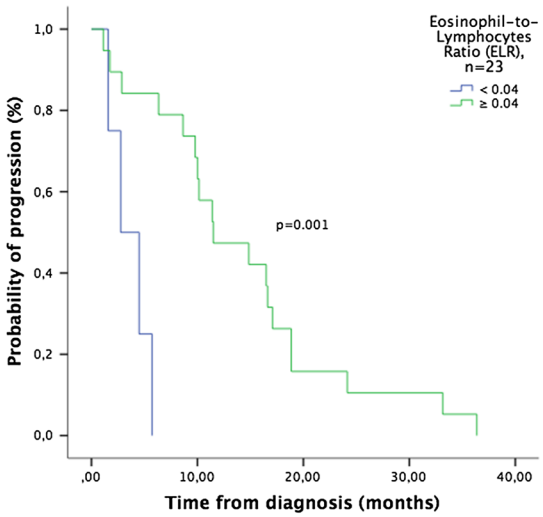
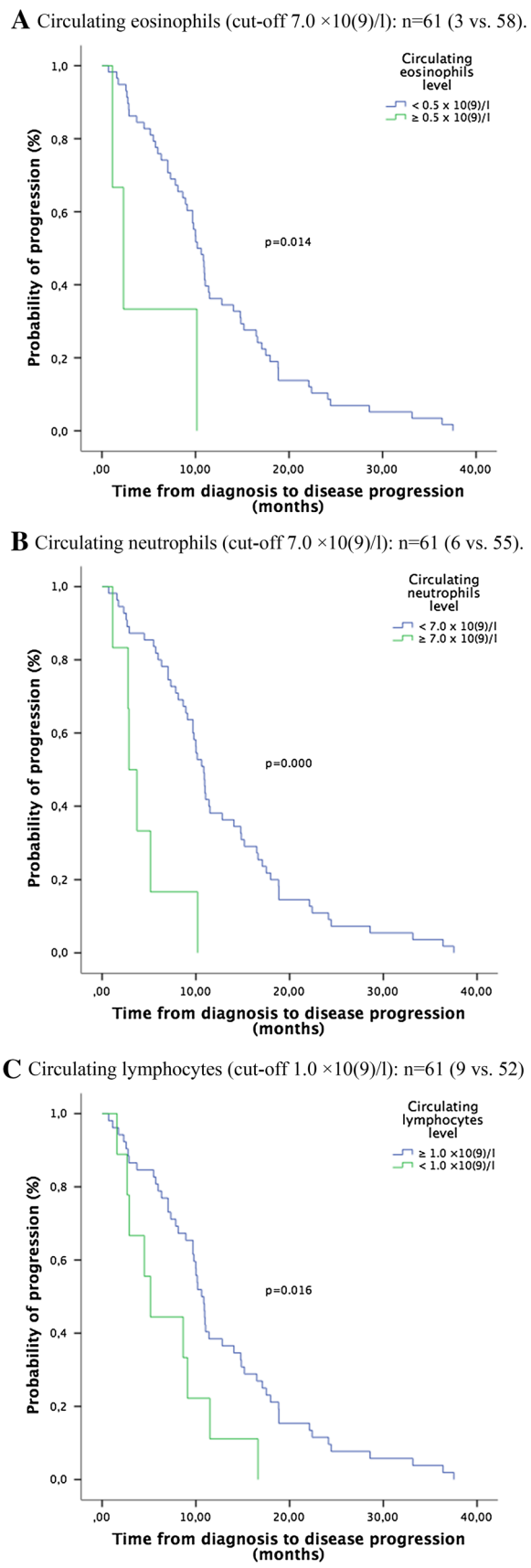


Fig. 3 Impact of pre-treatment circulating WBC on Time-to-Progression in PC patients ($n=61$). **a** Circulating eosinophils (cut-off $7.0\times10(9)/l$): $n=61$ (3 vs. 58). **b** Circulating neutrophils (cut-off $7.0\times10(9)/l$): $n=61$ (6 vs. 55). **c** Circulating lymphocytes (cut-off $1.0\times10(9)/l$): $n=61$ (9 vs. 52)



circulating eosinophils and lymphocytes, and not on a high level of its components. Nevertheless, we observed similar pattern for neutrophils and NLR in reference to patients' survival (opposite to tendency of ELR) [Fig. S2].

In our study, ELR ≥ 0.04 was observed in 86.4% of patients but this proportion is similar to the percentage of patients with lower values of NLR in studies published in PC [10–16]. The cut-off for NLR was similar to a lower value of this index in the healthy population [29]; nevertheless ELR is a new ratio, of which we do not have any reference data. However, if we compose ELR with the laboratory ranges for normal levels of eosinophils and lymphocytes, and assuming the lower values for both components, our cut-off corresponds to a lower extreme of ELR range in the healthy population. Additionally, the cut-off finder software confirmed the cut-off 0.04 for ELR as presented in Fig. S3 [30].

These findings of correlation between higher ELR with better survival are in accordance with our results, published previously, in a cohort of patients with cervical cancer, but opposite to the observations in patients with endometrial cancer [24, 25]. Nevertheless, other authors who studied the role of eosinophils in cancer patients arrived to the conclusion that the impact of eosinophils in tumor development is cancer specific [22, 23]. We may speculate that the characteristics of each malignancy, such as an intrinsic aggressiveness conditioned by tumor biology, may change the function of eosinophils [19–23]. Unveiling the potential role of WBC in cancer progression adjusted to specific cancer histology and localization makes this issue extremely captivating for further research.

We outline that the utility of inflammatory ratios changes substantially according to the tumor general prognostic and available therapies. In not so aggressive malignancies these ratios may be proposed to find patients in a higher risk of progression, therefore candidates for more intensive treatment [24]. As in PC up to now, there are no more efficient treatment options, the studies of systemic inflammation should be driven to understand the tumor biology more than to look for direct clinical application of the results of blood tests to identify patients with a higher risk of progression [5–7].

To make any conclusive remarks, our results should be confirmed in a larger, prospective and multicentre cohort. Moreover, due to a lack of effective treatment, multiple chemotherapy regimes were administrated in our hospital and some blood test results were not accessible. We recognize that our uni-center, retrospective study based on a

small cohort with heterogonous chemotherapy and surgery schedules may not give more than a hint conducing to an interesting field for future investigation on the PC biology [26–28].

Despite these limitations, we believe that our study provides some new evidence about the role of systemic inflammation in PC outcomes and may generate more questions about the mechanism of WBC interaction and its influence on tumor progression. Based on our findings, we conclude that a higher level of ELR may be a part of anti-tumor host's defence, and for this reason, it impacts on the OS and TTP of PC patients, independent of surgical status.

Conclusions

A high ELR (≥ 0.04) detected at PC diagnosis was associated with better OS and longer TTP in the entire cohort, regardless of the surgical treatment. Contrarily, a higher level of neutrophils and age > 50 years were correlated with poor survival outcomes. This new evidence about the role of circulating eosinophils, lymphocytes and neutrophils in PC progression merits further research to clarify their influence on the complex immune-evasive pancreatic microenvironment.

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Compliance with ethical standards

Data availability The datasets used and/or analysed during the current study are available on reasonable request.

Conflict of interest No potential conflict of interest to disclose.

Ethics approval Our study was approved by the Ethical Committee of the Hospital Clinic de Barcelona.

Informed consent All patients included in the study signed the informed consent for treatment, data processing and publication.

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