



Original Research

The A.L.A.N. score identifies prognostic classes in advanced biliary cancer patients receiving first-line chemotherapy



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Abstract Background: Chemotherapy is the mainstay treatment for advanced biliary cancer (ABC). Best supportive care and clinical trials are currently alternative options. The identification of a prognostic score that can be widely applied to daily practice has the potential to better inform clinical management of ABC patients.

Methods: A cohort of 123 ABC patients undergoing first-line chemotherapy was used as an exploratory cohort to define the prognostic value of laboratory tests routinely performed in clinical practice. Kaplan–Meier analysis was used to investigate the association between the variables and overall survival (OS). Those variables that were statistically significant at the multivariate analysis were combined in a multiplex score. Performance of the novel prognostic score was confirmed in a validation cohort of 60 ABC patients.

Results: Baseline actual neutrophil count, lymphocytes-monocytes ratio, neutrophil-lymphocytes ratio and albumin (A.L.A.N.) correlated with OS at the multivariate analysis in the

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exploratory cohort. When combined in the multiplex, A.L.A.N. score was able to identify three classes of ABC patients with significantly different OS (high-risk: median OS, 5 months; intermediate-risk: median OS, 12 months and low-risk: median OS, 22 months; $p < 0.001$). The score performed well in the different subtypes of ABC and was independent of stage, performance status and chemotherapy regimen. The performance of the A.L.A.N. score was confirmed in a validation cohort of cholangiocarcinoma patients (high-risk: median OS, 4.3 months; intermediate-risk: median OS 9.3 months, low-risk: median OS 13 months; $p: 0.005$). **Conclusions:** The A.L.A.N. score can be derived by variables routinely recorded in clinical practice and can provide prognostic assessment of ABC patients considered for first-line treatment.

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1. Introduction

Biliary tract cancers are heterogeneous tumour entities arising from the biliary tree that encompass intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), distal cholangiocarcinoma (dCCA) and gallbladder carcinoma (GBC) [1]. Although relatively uncommon in Western countries with roughly 9000 new cases in the United States [2], 2000 new cases in UK and 4900 new cases in Italy [3] annually, their incidence is increasing worldwide [4]. Curative-intent surgical resection can be pursued only in 10–20% of cases, and recurrence rates remain as high as 40–60% [5]. The vast majority of patients presents with advanced disease at diagnosis. Chemotherapy is the mainstay of treatment for advanced biliary cancers (ABCs) with median overall survival (OS) hardly exceeding 12 months [6]. It is of paramount importance to properly select patients to treat those more likely to benefit, while sparing others from unacceptable toxicities. Hence, different research efforts have attempted to develop clinically useful tools aiding patients' stratification. To date, several factors have shown to be correlated with survival of ABC: Eastern Cooperative Oncology Group (ECOG) performance status (PS), primary tumour location, disease status and number of metastatic sites [7–9]. However, in ABC patients fit to receive first-line chemotherapy, the capability of these models to accurately predict prognosis is limited, making the development of biomarkers for patients' selection still an unmet need both in daily practice and within clinical trials. Growing evidence is supporting the involvement of the immune system in the modulation of response to chemotherapy [10–12]. ABC is known to arise in the background of chronic inflammation (e.g. cholecystitis, hepatobiliary fluke infestation and primary sclerosing cholangitis) and to be characterised by an enrichment of inflammatory mediators [13,14], raising the interest around host immune system and inflammation determinants as predictors of outcome. Interestingly, several reports suggested that the derived neutrophil/lymphocyte ratio (NLR) can more precisely predict prognosis than ECOG PS, showing the latter as

insufficient to reflect the complex biological impact of this disease [15]. In this view, neutrophil, lymphocyte and platelet count, their ratios and the dynamic change of these ratios during chemotherapy, are known to reflect both systemic inflammation and immune system fitness and are thus regarded as promising prognostic factors in ABC [16,17]. More recently, monocytes have emerged as an important determinant of prognosis in ABC [18,19], a finding that is also supported by biological evidence of the role of myeloid-derived suppressive cells in the pathogenesis of cancers [20].

In the present study, we investigated the prognostic value of clinical factors together with an extensive panel of immune-inflammatory markers in ABC patients treated with first-line chemotherapy with the aim of developing a prognostic model to improve patients' risk-stratification in the daily practice.

2. Materials and methods

2.1. Patient selection

Patients with cytohistologically proven unresectable biliary tract cancer treated with first-line chemotherapy were retrospectively identified from the Modena Cancer Centre (exploratory cohort) and the Royal Marsden Hospital (validation cohort) Biliary Tract Cancer Databases, after review from the appropriate health research authorities (HRA). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Data from the Modena cohort were collected under the protocol 183/2019 that was reviewed by the Area Vasta Emilia Nord Ethics committee, while data from the Royal Marsden cohort were collected under the research protocol CCR4415 that has received approval from the HRA Yorkshire & The Humber South Yorkshire Research Ethics Committee.

Patients with mixed hepatocellular-cholangiocellular carcinoma as well as ampullary carcinoma were excluded. Patients with signs and/or symptoms suggestive for infectious disease within 2 weeks of starting treatment were not included in the analysis. Radiotherapy treatment was not allowed. None of the patients

underwent local interventional procedures (such as ablation) before first-line chemotherapy nor received ablation and/or surgery of the metastatic sites. Various chemotherapy regimens were administered as indicated in the result section. Clinical and laboratory data were retrieved through electronic medical records review. The following baseline variables were collected and analysed before the commencement of first-line chemotherapy: age, gender, ECOG PS, primary tumour site, disease status, first-line regimen, hematological and biochemical parameters including white blood cell count (cell/ μ l), haemoglobin (gr/dl), platelet count (cell/ μ l), bilirubin (mg/dL), alkaline phosphatase (ALP; IU/L), lactate dehydrogenase (LDH U/L), alanine aminotransferase (IU/L), aspartate aminotransferase (AST; IU/L), albumin (g/dL), carbohydrate antigen 19-9 (CA 19-9) (U/mL) and carcinoembryonic antigen (CEA) (ng/ml).

2.2. Statistical analysis

The primary end-point was OS calculated from the date of the first cycle of front-line chemotherapy to the date of death for any cause or last follow-up visit. Continuous variables were reported as the median and 25–95 percentile, while categorical variables were reported as absolute and percentage frequencies. Laboratory variables initially recorded as continuous parameters were later dichotomised according to usual clinical thresholds reported in the literature or according to their upper 75 percentile, chosen as worst status. The OS was calculated using Kaplan-Meier estimators [21]. Statistical comparisons between curves were performed with the log-rank test, and the effects were estimated using the Cox proportional hazard (PH) regression analysis, with a confidence interval at 95% (95% CI) [22]. The proportionality of hazard was checked graphically with scaled Schoenfeld residuals [23]. The prognostic performance of each variable on OS was first evaluated by means of Cox PH univariate model, selecting covariates with p value <0.20 . The final model was developed step by step in multiple Cox PH regression using the likelihood ratio test. Furthermore, the over-optimism and calibration of the model was computed over 250 bootstrap replications by means of Harrell's methods [24]. For all tests, a two-sided p -value <0.05 was considered to demonstrate a moderate strength of evidence against the null hypothesis. This level of probability is helpful for providing clinically useful advice.

3. Results

3.1. Exploratory cohort

Overall, 218 patients with biliary tract cancer were identified through the Modena Cancer Centre Biliary Tract Cancer Database search from 1st January 2010 to

31st July 2017. One hundred and twenty-three patients (56%) fulfilled all the above-mentioned criteria and were therefore included in the analysis. The median age of the patients was 67 years (range 29–85 years), and 65 (53%) of them were women. Primary tumour sites of disease were iCCA (50%), GBC (31%) and eCCA (19%; 12% pCCA and 7% dCCA). Amongst patients with iCCA, the prevalence of liver cirrhosis was 8%. One hundred eight patients (88%) had metastatic disease, while the remaining 15 (12%) had unresectable locally advanced disease. One hundred ten (89%) patients received doublet chemotherapy as first-line treatment and 104 (85%) platinum/gemcitabine combination. Overall, 58% received cisplatin-gemcitabine, while 42% received other regimens (which included 15% single-agent chemotherapy and 85% other combinations). Disease control was achieved in 75% of cases with an objective response rate of 23%. Thirty-six patients (29%) received second-line chemotherapy. Other baseline clinical and laboratory characteristics are summarised in Table 1. As of data cut-off, 111 patients had died, median OS in the whole patients' population was 12 months (95% CI, 7–14 months) and 1-year OS was 52% (Supplementary Fig. 1A).

3.2. Univariate analysis, multivariate analysis and prognostic score development

When assessed by univariate analysis, ECOG PS ≥ 2 , GBC as primary tumour site, metastatic disease, monotherapy, LDH $>$ upper normal limit, AST > 40 IU/L, ALP ≥ 100 IU/L, albumin <3.5 gr/dl, absolute neutrophil count (ANC) $> 8000/\mu$ l, lymphocyte/monocyte ratio (LMR) < 2.1 , NLR >3 , platelet/lymphocyte ratio ≥ 160 , CEA > 9.5 ng/ml and CA19-9 >700 U/L were significantly associated with shorter OS (Table 2).

At multivariate analysis, the following variables retained statistical significance as poor prognostic factors: ANC $>8000/\mu$ l, LMR <2.1 , albumin <3.5 gr/dl, NLR >3 , ECOG PS ≥ 2 , metastatic status and CEA >9.5 ng/ml (Table 3). On this basis, we depicted a prognostic model by combining the four immune-inflammation variables within the A.L.A.N. score (Actual neutrophil count, lymphocytes-monocytes ratio, albumin and neutrophil-lymphocytes ratio) and by assigning weight = 1 to each of the following: ANC $> 8000/\mu$ l, LMR < 2.1 , albumin <3.5 gr/dl and NLR > 3 . Accordingly, patients were stratified into three different risk groups as follows: low-risk group (0 negative prognostic factors), intermediate-risk group (from 1 to 2 negative prognostic factors) and high-risk group (from 3 to 4 negative prognostic factors). Globally, 38 patients were categorised as low-risk, 55 patients as intermediate-risk and 30 as high-risk. Survival curves according to the prognostic model are shown in Fig. 1A. Median OS for low-, intermediate- and high-risk group was 22 months (95% CI, 14–32 months), 12 months

Table 1
Patients characteristics in the exploratory cohort (n = 123).

Variable	N (%)
Age, years (median, range)	67 (29–85)
Gender	
Female	65 (53%)
Male	58 (47%)
Performance status	
ECOG 0-1	101 (82%)
ECOG ≥ 2	22 (18%)
Primary tumour site,	
iCCA	61 (50%)
pCCA	15 (12%)
dCCA	9 (7%)
GBC	38 (31%)
Disease status	
Locally advanced	15 (12%)
Metastatic	108 (88%)
Number of metastatic sites	
0	15 (12%)
1	59 (48%)
2	28 (23%)
3	15 (12%)
Metastatic sites	
Liver	82 (76%)
Abdominal lymph node (M1)	27 (25%)
Peritoneum	21 (19%)
Lung	15 (14%)
Others	12 (11%)
First-line chemotherapy regimen	
Cisplatin/Gemcitabine	71 (58%)
GEMOX	33 (27%)
Others	11 (9%)
Gemcitabine	8 (6%)
Laboratory tests (median, range)	
ANC, cells/ μ l	5504 (1690–36230)
Haemoglobin, gr/dl	12.4 (9.0–16.2)
Platelets, cells/ μ l	255 (86–1160)
Albumin, gr/dl	37 (21–49)
ALP, IU/L	227 (11–1387)
AST, IU/L	34 (9–1088)
ALT, IU/L	36 (6–721)
Bilirubin, gr/dl	0.75 (0.03–9.6)
CEA, ng/ml	2.6 (0.2–2029)
CA19-9, U/ml	120 (0.6–49454)

iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; ANC, absolute neutrophil count; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; GBC, gallbladder carcinoma.

(95% CI, 8–15 months) and 5 months (95% CI, 2–8 months), respectively. The difference in survival was statistically significant between groups ($p < 0.001$). The score did not significantly associate with other relevant prognostic factors such as PS (Chi-square test p : 0.68) and disease status (Chi-square test p : 0.13). The prognostic performance of the model was maintained regardless of primary tumour site (Supplementary Fig. 1B) and chemotherapy regimen (Supplementary Fig. 1C).

Table 2
Univariate analysis in the exploratory cohort.

Covariate	HR	95% CI	p value
Gender , female versus male	0.69	0.47–1.01	0.058
Age , ≥ 70 versus < 70 years	1.98	1.29–3.04	0.058
Performance status ECOG, ≥ 2 versus 0-1	2.15	1.46–3.15	< 0.001
Site , GBC versus CCA	1.68	1.13–2.50	0.011
Disease status , metastatic versus LA	1.71	1.13–2.60	0.011
First-line , doublet versus monochemotherapy	0.65	0.44–0.96	0.028
Haemoglobin , < 12 versus ≥ 12 gr/dl	1.30	0.88–1.90	0.187
WBC , $> 10,000$ versus $\leq 10,000$	1.20	0.18–1.90	0.377
ANC , > 8000 versus ≤ 8000	2.40	1.57–3.67	< 0.001
NLR , > 3 versus ≤ 3	2.76	1.81–4.20	< 0.001
LMR , < 2.1 versus ≥ 2.1	2.23	1.44–3.47	< 0.001
PLR , > 160 versus ≤ 160	1.52	1.03–2.23	0.034
LDH , $> \text{ULN}$ versus $\leq \text{ULN}$	1.99	1.25–3.17	0.004
Albumin , < 3.5 versus ≥ 3.5	1.69	1.11–2.50	0.013
Bilirubin , > 1.3 versus ≤ 1.3	1.12	0.73–1.72	0.601
ALP , > 100 versus ≤ 100 IU/l	1.64	1.13–2.40	0.010
AST , > 40 versus ≤ 40 IU/l	1.60	1.09–2.35	0.017
ALT , > 45 versus ≤ 45 IU/l	1.10	0.75–1.61	0.625
CEA , > 9.5 versus ≤ 9.5 ng/dl	2.28	1.46–3.55	< 0.001
CA19-9 , > 700 versus ≤ 700 IU/l	2.25	1.48–3.43	< 0.001

CCA, cholangiocarcinoma; ANC, absolute neutrophil count; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ECOG, Eastern Cooperative Oncology Group; GBC, gallbladder carcinoma; UNL: upper normal limit; NLR, neutrophil/lymphocyte ratio; LMR, lymphocyte/monocyte ratio; PLR, platelet/lymphocyte ratio; WBC, white blood cells; LA, locally advanced.

Table 3
Multivariate analysis in the exploratory cohort.

Covariate	HR	95% CI	p value
LMR			
< 2.1	1.60	1.02–3.08	0.045
Albumin g/dl			
< 3.5	1.62	1.04–2.50	0.031
NLR			
> 3	1.74	1.03–2.97	0.042
ANC			
> 8000	2.12	1.27–3.54	0.004
Performance status			
ECOG ≥ 2 versus 0-1	2.16	1.28–3.64	0.004
Disease status			
Metastatic versus LA	2.22	1.30–3.78	0.003
CEA ng/ml			
> 9.5	2.59	1.55–4.32	< 0.001

ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil/lymphocyte ratio; LMR, lymphocyte/monocyte ratio; ANC, absolute neutrophil count; CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazards ratio; LA, locally advanced

Variables that resulted statistically significant in the multivariate analysis are reported. Shrinkage (overfitting) 0.099. c-Harrell Train 0.702 Test 0.692.

3.3. External validation data set

Patients diagnosed with cholangiocarcinoma within the CCR4415 protocol were identified at the Royal

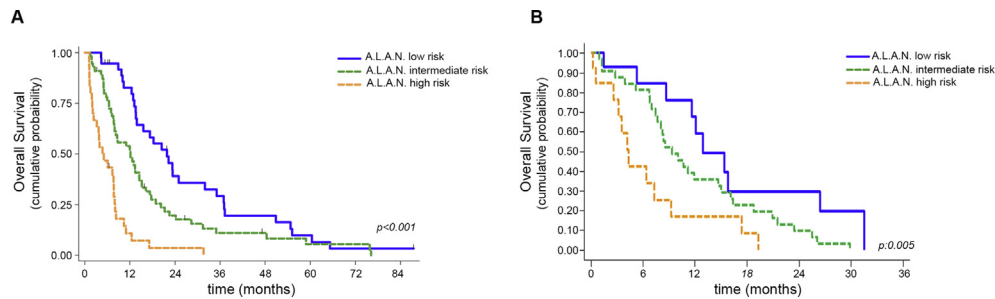


Fig. 1. **A. Overall survival according to the A.L.A.N. score in the exploratory cohort.** Patients were classified according to the A.L.A.N. score in low-risk (0), intermediate-risk (1–2) and high-risk (3–4) groups. Median OS was 22 months in the low-risk, 12 months in the intermediate-risk and 5 months in the high-risk group. Log-rank $p < 0.001$. **B. Overall survival by the A.L.A.N. score in the validation cohort.** Patients were classified according to the A.L.A.N. score in low-risk (0), intermediate-risk (1–2) and high-risk (3–4) groups. Median OS was 12.9 (95% CI: 8.7–26.4) months in the low-risk, 9.3 (95%CI: 7.4–14.7) months in the intermediate-risk and 4.3 (95%CI: 2.6–9.2) months in the high-risk group. Log-rank $p = 0.005$. OS, overall survival; CI, confidence interval; A.L.A.N., actual neutrophil count, lymphocytes-monocytes ratio, neutrophil-lymphocytes ratio and albumin.

Marsden Hospital, London, UK, from 1st January 2010 to 1st July 2015. Out of 96 patients, 60 with ABC treated with first-line chemotherapy were eligible for this study (Table 4). Median age was 64 years, 31 were male and 45 (75%) had metastatic disease. Thirty-three (53%) patients received combination chemotherapy with cisplatin and gemcitabine, and 24 (25%) received second-line treatment. The median OS of the validation cohort was 9.24 months (Supplementary Fig. 1D). At univariate analysis, the variables included in the A.L.A.N. immunoscore were significantly associated to OS when considered independently (Supplementary Table 1). When combined in the A.L.A.N. score, the population was classified in three separate groups with significantly different OS: low-risk group (12.9 median OS, 95% CI 8.7–26.4; $N = 14$), intermediate-risk group (9.3 median

OS, 95% CI 7.4–14.7; $N = 33$) and high-risk group (4.3 median OS, 95% CI 2.6–9.2; $N = 13$) (Fig. 1B). The score maintained statistical significance at the multivariate analysis when stage of disease and PS were assessed (Table 5).

4. Discussion

Herein, we provide initial evidence of a prognostic score which takes into account markers of inflammation and immunity, demonstrating a good performance in risk-stratifying ABC patients treated with first-line chemotherapy into three statistically significant different groups. Of note, the discriminant power of the score was independent of primary tumour site.

The value of immune-inflammatory markers in cancer patients stands on the potential of mirroring the complex network of cancer-related inflammation within the tumour microenvironment, an established hallmark of cancer. Chronic biliary tract inflammation is well-known to promote cholangiocarcinogenesis as well as

Table 4
Patients characteristics in the validation cohort ($n = 60$).

Variable	N (%)
Age, years (median, range)	64 (54–70)
Gender	
Female	31 (52%)
Male	29 (48%)
Performance status	
ECOG 0–1	50 (83%)
ECOG ≥ 2	10 (17%)
Primary tumour site	
iCCA	17 (28%)
pCCA	18 (30%)
dCCA	19 (32%)
Unknown	13 (20%)
Disease status	
Locally advanced	15 (25%)
Metastatic	45 (75%)
First-line chemotherapy regimen	
Cisplatin/gemcitabine	33 (55%)
Gemcitabine	13 (22%)
Others	14 (23%)

ECOG, Eastern Cooperative Oncology Group; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma.

Table 5
Multivariate analysis for the validation cohort.

Covariate	HR	95% CI	p value
A.L.A.N. score,			
Low-risk	Reference category		
Intermediate-risk	2.46	(0.92–6.58)	0.07
High-risk	6.79	(2.22–20.82)	0.001
Age	1.00	0.96–1.03	0.80
Gender			
Female versus male	1.08	0.56–2.11	0.82
Performance status			
ECOG 2 versus 0–1	1.66	0.64–4.28	0.63
Disease status			
Metastatic versus LA	0.81	0.35–1.89	0.63

OS, overall survival; CI, confidence interval; A.L.A.N., actual neutrophil count, lymphocytes-monocytes ratio, neutrophil-lymphocytes ratio and albumin; ECOG, Eastern Cooperative Oncology Group. LA, locally advanced.

tumour progression through chronic exposure to and stimulation by mediators acting as potent biliary mitogens. Interleukin-6, whose serum levels are elevated in cholangiocarcinoma, has been shown to contribute via autocrine and/or paracrine mechanism to growth stimulation of malignant cholangiocytes [25]. The monocyte-derived cytokine tumour necrosis factor can promote lymphangiogenesis in ABC via multiple pathways [26]. In addition to tumoural mediators, cellular components such as neutrophils, monocytes-macrophages and lymphocytes are pivotal orchestrators of cancer-promoting inflammation via extracellular matrix remodelling, enhancement of cancer cell invasion and metastasis, angiogenesis, cancer cell proliferation, lymphangiogenesis and inhibition of the antitumoural immune surveillance [27]. High tumour-associated neutrophils, low CD8+T cells and high T regulatory cells have been reported to be significantly associated with worse OS in a series of resected ABC [28]. Their value in peripheral blood is thought to reflect and inform on the balance between systemic inflammation and immune system in cancer patients in a reliable and easily accessible way.

Our findings are in keeping with published literature suggesting NLR as an independent prognostic factor in patients with both early and advanced biliary cancers [7,15]. The prognostic significance of NLR relies on the rise of absolute neutrophil count (a poor prognostic factor per se) and the decrease in lymphocyte count that likely reflects an insufficient antitumour immunological reaction. To our knowledge, this is the first study showing a prognostic role also for LMR in biliary tract cancer. A previous meta-analysis showed that pretreatment low LMR was associated with unfavourable OS in patients with both early-stage and advanced-stage solid cancers [29] but included gastrointestinal tumours arising outside the biliary tract. It is difficult to speculate if the effect on the outcome is related more to the increase in monocyte counts or the depletion of lymphocytes. Both events occur in ABC, where the circulating CD14⁺/CD16⁺ monocytes are thought to be the precursors of resident macrophages that contribute to tumourigenesis via paracrine stimuli cancers [30,31]. Nonetheless evidence points to a reduced activation of lymphocytes with antitumour activity in ABC that characterises an immunosuppressive milieu [10]. Consistently with previous reports, we confirmed that low albumin levels (<3.5 gr/dl) were predictive of shorter OS in patients receiving first-line chemotherapy in ABC [32]. Hypoalbuminaemia is linked to cancer-related inflammation and cachexia and has been associated with higher mortality rates in several cancer types [33].

The combination of all these single parameters in a multiplexed score, such as the one we propose, has the potential to reflect the various inflammatory/immunity reactions occurring in cancer patients. Indeed, conversely to single parameters (i.e. as LNR) the

A.L.A.N. score can differentiate three different groups with clearly separated OS, which allows discussion for better tailored treatment. The A.L.A.N. score can identify patients with an extremely poor OS (<5 months). On the contrary, patients in the low- and intermediate-risk groups are likely to receive more than one line of treatment, and therefore, discussion of clinical trials as first-line or second-line choice represent a feasible option that is unlikely to be limited by a rapid deterioration of the disease. We acknowledge that our data support a prognostic assessment of ABC patients, while more information is needed to understand a predictive value of the score that could inform chemotherapy decision.

The retrospective design of our study along with its relatively small sample size is a limitation to be acknowledged. Other inflammation markers (i.e. C-reactive protein) have not been included in the analysis, given our intent was to propose a score that would be pragmatically applicable in routine clinical practice; however, we acknowledge that the incorporation of other parameters may improve the performance of the score. In addition, full molecular characterisation of these tumours has not been performed, and therefore, we cannot weigh the prognostic value of indicated gene mutations. Nonetheless, we believe that the A.L.A.N. score can be widely accessible to oncologists across the world because it is not associated to additional costs and provides an advantage over PS alone to prognostically classify ABC patients undergoing first-line chemotherapy, by identifying a limited group of patients with particularly adverse prognosis.

5. Conclusions

In conclusion, while waiting for molecular biomarkers to enable better risk stratification, our prognostic model represents a useful tool to add to established clinical parameters to ameliorate the accuracy of patients' selection in daily practice. Notably, the better patients' stratification by inflammation status and immune cell profile can have interesting therapeutic implications, especially in the light of latest immunological approaches and antiinflammatory drugs available.

Conflict of interest statement

The authors declare no conflict of interest related to the reported data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2019.05.030>.

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