Combination of lymphocyte count and albumin concentration as a novel prognostic index in cholangiocarcinoma

Keywords
Prognosis, Immunity, Nutrition, Cholangiocarcinoma

Abstract
Introduction
Cholangiocarcinoma (CCA) is a cancer arising from the intra- or extrahepatic bile ducts Here, we evaluated the prognostic significance of a new inflammation-based scoring system derived from preoperative lymphocyte count x albumin (LA) in cholangiocarcinoma patients.

Material and methods
The study included 36 patients who underwent surgical treatment for cholangiocarcinoma between 2010 and 2021. We defined the LA as lymphocyte count (/L)×albumin (g/L) The cut-off point was determined by ROC curves. The patients were divided into two groups according to the cut-off point: Group1 (Low LA) and Group2 (High LA). These groups were compared for clinical characteristics, recurrence, and overall survival.

Results
The patients were divided into two groups as Group1 (Low LA) and Group2 (High LA) according to the cut-off point of 5400. The male sex was dominant in the groups (76.2% vs. 86.7%; p:0.434). Hgb was lower (11.7 vs. 13.7; p:0.002) and CA19.9 was higher (310 vs. 71; p:0.013) in Group1. The length of hospital stay, reoperation, and 90-day readmission rate, were similar in the groups. The tumor size (2.8 cm vs. 3.13 cm; p:0.683) was similar in the groups. At follow-up, 33% of the patients in Group1 and 6.7% of the patients in Group2 developed recurrence (p:0.104). The survival was shorter in Group 1 (18 vs. 41.5 months; p:0.003).

Conclusions
Our study established that an LA score below the cut-off point of 5400 was a prognostic factor associated with reduced survival. LA scores can be used to predict prognosis and make more individualized decisions in cholangiocarcinoma.

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Explanation letter
Dear Editor,
Thank you very much for your kind interest in our manuscript entitled “” I am enclosing the marked revision of the manuscript, the revised Combination of lymphocyte count and albumin concentration as a novel prognostic index in cholangiocarcinoma manuscript, and the responses to the reviewers with the corrections as you suggested. Corrections and amendments are marked in red in the revised manuscript and explained in the revision notes for each reviewer. Thank you for your consideration and for the suggested corrections, which have improved my article. The revision notes are as follows:With my best regards

Dear Reviewer 1
We would like to thank you for your nice comments and present our respect.

Dear Reviewer 2

We would like to thank you for your nice comments and present our respect. Unfortunately, Number of patients is not to big but on other hand this is rare tumor. We tried to present our limited number of patients in the discussion as a limitation of the study.

Dear Reviewer 3
We would like to thank you for your nice comments and present our respect.
1-The materials and methods section in the abstract We added the number of patients examined.
2- We have added the corresponding references to each scoring system in the introduction in the main text.

Dear Reviewer 4
We would like to thank you for your nice comments and present our respect. Actually, our number of patients is higher, but we did not include some patients in the study. Patients with chronic inflammatory diseases (tuberculosis, sarcoidosis), autoimmune diseases, hematological diseases, who were using steroids, who were diagnosed with inoperable cholangiocarcinoma and were considered unresectable at laparotomy and with inaccessible records were excluded from the study. This reduced our numbers. We think that the low AUC value is due to the small number of our patient group.
1- Added a sentence about CCA to the first line of the Summary as you suggested.
2- The number of patients examined has been added to the Summary, Methods section.
3- In summary, the wrong formula in the methods section has been corrected.
4- In line 6-7 it says the correction you suggested has been made
5- Fixed "p=0.257" at line 107.

Dear Reviewer 5
Dear referee, you have evaluated the study from a very correct point of view. We agree with your forward-looking comment. Thank you for giving us insight from a different point of view. Yes, we think that these prognostic indices can be used to predict prognosis and should not prevent us from performing radical surgeries. To give us an idea about the individualized treatment choices we expect from these prognostic indices. As we stated in our study and as you suggested, the results of different prospectively planned centers are needed.

Response letter.docx

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Combination of lymphocyte count and albumin concentration as a novel prognostic index in cholangiocarcinoma

Introduction

Cholangiocarcinoma (CCA) is a primary liver cancer featuring differentiation of cholangiocytes, the epithelial cells lining the intra- and extrahepatic sections of the biliary tree. CCA is the most common biliary malignancy and 2nd most common primary hepatic malignancy after HCC. CCAs are heterogeneous biliary epithelial tumors divided into subtypes according to the anatomical location in the biliary tree as intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) CCA [1]

Surgical resection remains the best treatment approach to CCA, but unfortunately, most patients are diagnosed at an unresectable stage of the disease. Although the accuracy of available diagnostic methods has improved greatly, the 5-year overall survival (OS) remains low [2-4].

A growing number of studies have shown that cancer-related inflammation is responsible for poor prognosis. Inflammation also plays a strong role in tumor development, progression, and metastasis [5,6]. Albumin is a stable molecule synthesized by hepatocytes that maintain intravascular oncotic pressure and transport various substances. In clinical practice, hypoalbuminemia is a common indicator of malnutrition and hepatic dysfunction. Previous studies have shown that albumin alone or albumin-based markers are independent predictors of poor survival in some cancers [7-9].

Various systemic inflammatory response (SIR) markers, such as the platelet-to-lymphocyte ratio (PLR) [10], neutrophil-to-lymphocyte ratio (NLR)[11], lymphocyte-to-monocyte ratio (LMR)[12], and albumin-to-globulin ratio (AGR)[13], have been investigated and accepted as prognostic factors in various cancers. Chronic inflammation is an important predisposing factor for the development of biliary tract cancers (BTCs) [14] .

Based on the evidence on inflammation and nutrition in the literature, a recent study reported that LA, a new composite index calculated by the formula: (LA) = lymphocyte count (/L) × albumin (g/L), was associated with poor survival in rectal cancer [15].

Understanding the factors that determine prognosis is important to improve outcomes and allow clinicians to classify patients for treatment. Therefore, it is necessary to derive indices with prognostic significance. To the best of our knowledge, the present study is the

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first to analyze the significance of LA in cholangiocarcinoma. Here, we tried to establish the prognostic significance of the LA combination and its association with postoperative complications in patients with cholangiocarcinoma.

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Methods

The study included 36 patients who underwent surgical treatment for cholangiocarcinoma between 2010 and 2021 in the General Surgery Clinic of Çukurova University Faculty of Medicine. The study was approved by the Ethics Committee of Çukurova University Faculty of Medicine (IRB no :15.10.2021-14/3). Patients with chronic inflammatory diseases (tuberculosis, sarcoidosis), autoimmune diseases, hematological diseases, who were using steroids, who were diagnosed with inoperable cholangiocarcinoma and were considered unresectable at laparotomy and with inaccessible records were excluded from the study.

According to previous literature reports, we defined the LA as lymphocyte count (/L) × albumin (g/L). LA was calculated on admission for operation.

The cut-off point was determined by ROC curves. The patients were divided into two groups according to the cut-off point: Group 1 (Low LA) and Group 2 (High LA). These two groups were compared for demographic and clinical characteristics, laboratory parameters, tumor marker levels, history of preoperative Endoscopic Retrograde Cholangiopancreatography (ERCP) and biliary drainage, surgical methods, the presence of intraoperative complications, duration of operation, tumor localization, tumor size, the number of lymph nodes dissected, postoperative complications according to the Clavien-Dindo scoring, the length of postoperative hospital stay, the reoperation and unplanned readmission rates and reasons as postoperative quality indicators, the presence of recurrence, and overall survival characteristics using follow-up data.

Most of the patients treated in our clinic were referred from other centers after initial examinations such as Computed Tomography (CT) and Endoscopic Retrograde Cholangiopancreatography (ERCP). The stage and resectability of the tumor were determined by preoperative assessment with one or more of the following tests: Doppler Ultrasonography (USG), high-resolution spiral CT, Magnetic Resonance Cholangiopancreatography (MRCP), Percutaneous Transhepatic Cholangiography (PTC), and Endoscopic Ultrasonography (EUS). Positron Emission Tomography (PET) scanning was performed to evaluate the presence of extrahepatic metastases in selected cases. Patients with cholangitis or pancreatitis after previous biliary intervention were treated preoperatively.

Patients with imaging findings of peritoneal invasion, metastatic involvement of the hepatic parenchyma outside the resection site, and/or macroscopic paraaortic lymph node

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metastasis were considered unresectable. The American Joint Committee on Cancer staging manuals 2010 and 2016 were used for tumor staging.

All surgical resections were performed to achieve microscopically negative surgical margins. Frozen section examination was performed if the involvement of the surgical margin was suspected. During laparotomy, a comprehensive examination was performed to exclude metastatic disease, and then in patients who would not receive pancreaticoduodenectomy, the bile duct resection was advanced from the level where the bile duct enters the pancreas to the location of the tumor-free bile duct in the hilum of the liver. The surgical margins of the proximal and distal bile ducts were intraoperatively assessed by frozen section analysis. Partial hepatectomy (hemi-hepatectomy, extended hepatectomy with or without caudate lobe resection), and all extended right hepatectomy and left hepatectomy procedures included caudate lobectomy. Biliary continuity was restored with a Roux-en-Y biliary-enteric anastomosis. Pancreaticoduodenectomy (the Whipple procedure) was performed for the tumors located inferior to the cystic duct. The Whipple procedure group was included in the biliary resection group. The lymph node dissection was advanced from the lymphatic tissue in the hepatoduodenal ligament to the level of the celiac trunk.

Statistical assessment

Statistical analysis of the data was performed using SPSS v23.0. Categorical measurements were summarized using number and percentage, and continuous measurements using mean, standard deviation, and minimum-maximum. The normality of the data was analyzed by the Shapiro-Wilk test. Categorical variables were compared using the Chi-square and Fisher’s tests. Independent Samples (Student's) t-test was used for the normally distributed groups and Mann-Whitney U test for non-normally distributed groups. The sensitivity and specificity of the LA score (Lymphocyte count x Albumin) were calculated based on the mortality of the study patients and the cut-off point was established by examining the area under the ROC curve. The statistical significance level was set at 0.05 for all tests.

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Results

A ROC analysis was conducted and a ROC curve was created to determine a cut-off point for the LA score. The patients were divided into two groups according to survival and the ROC analysis was performed according to these groups. The ROC analysis yielded an area under the ROC curve of 70.8%. In other words, the cut-off point gives a correct answer rate of 70.8%. According to the cut-off point, LA scores of < 5400 suggested the mortality of patients at follow-up, with a sensitivity of 75% and a specificity of 75% *(Graph 1 and Table 1)*.

The male sex was dominant in all groups (76.2% vs. 86.7% p= 0.434). The mean age, ASA scores, and presenting symptoms were similar in the groups (p > 0.05). Preoperative Percutaneous Biliary Drainage and ERCP stent rates were similar in the groups (52.4% vs. 33.3%; p=0.257 and p= 0.320) (*Table 2*).

As we expected, the lymphocyte count (1.400 vs. 2560 p < 0.0001) and the albumin level (2.67 vs. 3.45; p < 0.001) were lower in Group 1. The Hgb level (11.7 vs. 13.7; p= 0.002) was lower and the CA19.9 level (310 vs. 71; p =0.013) was higher in Group 1 *(Table 3)*.

The most common surgery was biliary resection in the groups (61.9% vs. 66.7%; p: 0.441). The most common tumor localization was the perihilar region (42.9%) in Group 1 and the distal region (46.7%) in Group 2. Intraoperative small bowel injury developed in one patient in Group 2. The length of hospital stay, postoperative mortality rate, reoperation, and 90-day readmission rate, which are among the postoperative quality indicators, were similar in the groups. The results are presented in *Table 4*.

The tumor size (2.8 cm vs. 3.13 cm; p= 0.683) and the number of lymph nodes dissected (7 vs. 8; p = 0.392) were similar in the groups. Stage II disease was the most common in both groups (52.4% vs. 60% p= 0.210). The results are presented in *Table 5*.

At follow-up, 33% of the Group 1 patients and 6.7% of the Group 2 patients developed recurrence (p= 0.104), while the mortality rate was 54.7% in Group 1 and 40% in Group 2 (p: 0.004) (*Table 6*). The survival was shorter in Group 1 (18 vs. 41.5 months; p= 0.003) (*Table 7, Graph 2*).

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Discussion

Cholangiocarcinoma is an aggressive malignancy with an overall poor prognosis. Prognostic markers are needed for these patients. Previous studies on prognostic factors have identified preoperative albumin, tumor grade/size, size of resection, lymph node/vascular involvement, and elevated CEA/CA19-9 tumor markers as significant prognostic factors [7, 9, 16, 17].

The present study, which examined the prognostic value of the LA score in cholangiocellular carcinoma patients who underwent surgical treatment, established that an LA score of < 5400 was a prognostic factor associated with reduced survival. A low LA score also predicted patient mortality at follow-up. On the other hand, a low LA score was not associated with demographic and clinical data, postoperative period, and tumor stage.

Hypoalbuminemia is associated with the progression of various diseases. It is generally accepted that a low ALB level is a poor prognostic indicator of survival in various types of cancer, such as colorectal cancer, pancreatic carcinoma, hepatocellular carcinoma, and lung cancer [1,18]. A recent study identified low serum levels of albumin as an independent predictor of survival in CCA [16]. The potential mechanism for the development of this condition may be multifactorial. Hypoalbuminemia is common in patients with malnutrition and cachexia and increases the risk of chemotherapy-induced toxicity. In addition, hypoalbuminemia is associated with the failure of several immune system components and promotes tumor growth [19]. On the other hand, lymphocytes are vital in immune defense against tumor cells. Infiltration of CD4+ T cells triggers immune activation of CD8+ T cells and activated CD8+ T cells release cytotoxic factors, resulting in apoptosis of cancer cells [20]. In our study, a low LA score did not affect postoperative short-term outcomes and was not associated with disease stage but associated with reduced long-term survival and poor oncologic outcomes.

Many inflammation-based scores have emerged in the literature over the past decade as prognostic indicators for various malignancies. Estimation of survival and prognosis has been tried using various composite indices derived from parameters evaluating immune and nutritional status [8, 15,21,22].

In their study of 206 patients with CCA, Jing et al. identified the albumin-to-gamma-glutamyl transferase ratio (AGR) as an independent predictor of overall survival (OS; $p = 0.003$) and recurrence-free survival (RFS; $p = 0.046$) [6]. The meta-analysis by Hu et al.

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showed that the pre-treatment platelet-to-lymphocyte ratio could serve as a useful prognostic biomarker in cholangiocarcinoma [2]. Similarly, the meta-analysis by Tan et al. established that a high preoperative neutrophil-to-lymphocyte ratio was associated with lower survival rates in cholangiocarcinoma patients [20]. Saito, et al., in turn, determined that indices such as prognostic nutritional index (PNI), the C-reactive protein-to-albumin ratio (CAR), and the platelet-to-albumin ratio (PAR) were associated with survival in CCA [22].

Yamamoto T et al. included 448 patients with stage II/III rectal cancer who had received curative resection in their study. The researchers found a low LA score to be associated with reduced overall survival and relapse-free survival, and concluded that it could be used to identify a high-risk subgroup for recurrence, and might also help decide on a postoperative treatment to prevent recurrence [15]. Similarly, reduced survival was associated with LA scores in our study.

Tumor markers were evaluated as prognostic factors in patients undergoing surgical resection for cholangiocarcinoma. The prognostic significance of CEA and CA 19-9, in particular, was demonstrated [23,24]. In our series, an increased level of CA19.9 was associated with a low LA score. We may think that high levels of CA19.9 are likely to be indirectly associated with reduced survival in the Low LA patient group. Similarly, anemia was found to be a poor prognostic factor for CCA [25,26]. This should also be considered an anecdote that directly or indirectly affects the outcomes in multiple ways.

The most important limitations of our study were its retrospective design and a limited number of patients. However, we believe that it will contribute to the literature as it is the first study showing that the LA score can be used as a new immunonutritional index in Cholangiocellular Carcinoma.

In conclusion, our study showed that the LA ratio is an independent predictor of prognosis in CCA. Based on this finding, LA-based nomograms can be developed to predict prognosis and make more individualized decisions in CCA. Prospective research with different cohorts is needed in the future.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

None

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Tables

Graph 1 Receiver operating characteristic (ROC) curve analyses for mortality

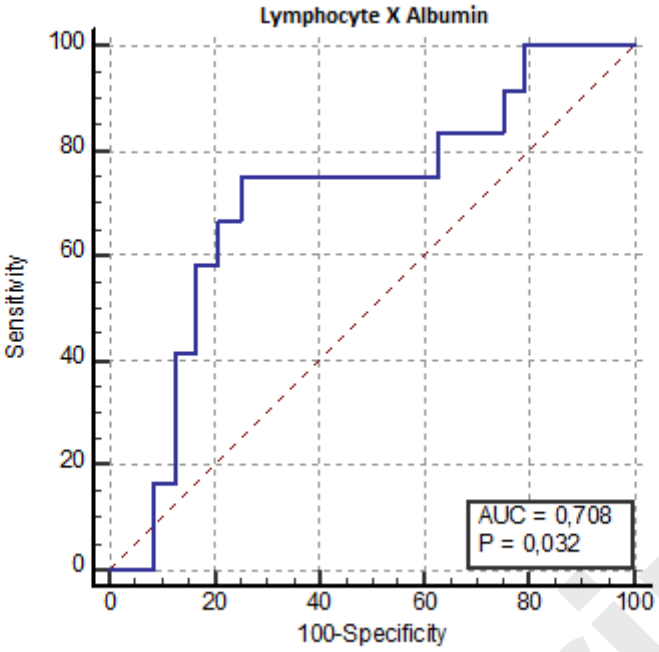

Table 1 **Proposed cut-off values for significant parameters in overall survival**

	Cut-off	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**AUC (95% CI)**	**p**
Lymphocytes x albumin	<5.400	75 (42.8–94.5)	75 (53.3–90.2)	60 (41.1–76.3)	85.7 (68.7–94.3)	0.708 (0.533–0.847)	**0.032**

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Table 2. Demographic and clinical data

	Low (n=21)	High (n=15)	p
	n (%)	n (%)	
Sex			
Male	16 (76.2)	13 (86.7)	0.434
Female	5 (23.8)	2 (13.3)	
Age[b]	65 (45–85)	64 (28–74)	0.872
ASA			
1	3 (14.3)	4 (26.7)	0.336
2	11 (52.4)	9 (60)	
3	7 (33.3)	2 (13.3)	
Abdominal pain	13 (61.9)	8 (53.3)	0.607
Jaundice	10 (47.6)	8 (53.3)	0.735
Preoperative biopsy	1 (4.8)	2 (13.3)	0.359
Preoperative percutaneous biliary drainage	11 (52.4)	5 (33.3)	0.257
Preoperative ERCP stent	11 (52.4)	5 (33.3)	0.320
Neoadjuvant therapy	1 (4.8)	-	NA

a Independent Student's t-test, Mann-Whitney U test

Table 3 Laboratory parameters

	Low (n=21)	High (n=15)	p
Wbc mm3 /L[b]	10.5 (5.3–30)	10.6 (6.1–31)	0.700
Neutrophil count mm3 /L[b]	8.8 (3.3–27.2)	6.8 (3.4–27.8)	0.553
Lymphocyte count mm3 /L[b]	1.4 (0.8–2.5)	2.56 (1.3–8.8)	**<0.001****
Platelet count mm3 /L[a]	304.5 ± 22.1	272.4 ± 20.6	0.314
CRP[b]	10.3 (0.3–170)	5.52 (0.2–61)	0.748
Preop Hgb gr/dl[a]	11.7 ± 0.4	13.7 ± 0.4	**0.001****
Preop albumin gr/dl[a]	2.67 ± 0.1	3.45 ± 0.1	**0.002****
Total bilirubin mg/dL[b]	7 (0.4–25.4)	2.29 (0.4–18.8)	0.078
Direct bilirubin mg/dL[b]	3.84 (0.1–12.1)	0.82 (0.1–15.9)	0.095
Ast[b]	71 (18–465)	115 (24–537)	0.178
Alt[b]	80 (13–648)	137 (14–623)	0.248
Alp[b]	284 (58–1769)	319 (47–670)	0.700
Ggt[b]	194 (30–1155)	327 (42–1739)	0.312
Cea[b]	3.5 (0.9–137)	2.1 (0.6–6)	0.095
Ca19.9[b]	310 (0.8–20270)	71 (1.6–1631.5)	**0.013***

a Independent Student's t-test, Mann-Whitney U test

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Table 4. Operational details

	Low (n=21)	High (n=15)	p
	n (%)	n (%)	
Surgery			
Biliary	13 (61.9)	10 (66.7)	0.441
Hepatic	2 (9.5)	3 (20)	
Hepatic + biliary	6 (28.6)	2 (13.3)	
Tumor localization			
Intrahepatic (iCCA)	4 (19)	6 (40)	0.133
Perihilar (pCCA)	9 (42.9)	2 (13.3)	
Distal (dCCA)	8 (38.1)	7 (46.7)	
Intraoperative complications	-	1 (6.7)	0.230
Vascular invasion	1 (4.8)	4 (26.7)	0.061
Duration of operation[a]	280.2 ± 31.5	268.2 ± 21.1	0.773
Length of postoperative hospital stay[a]	16.5 ± 1.4	14.9 ± 1.7	0.497
Postoperative complications according to Clavien-Dindo			
1	19 (90.5)	13 (86.7)	0.472
2	2 (9.5)	1 (6.7)	
3B	-	1 (6.7)	
Postoperative mortality	-	2 (13.3)	0.085
Reoperation	-	1 (6.7)	0.230
90-day unplanned readmission	9 (42.9)	2 (13.3)	0.058

a Independent Student's t-test, Mann-Whitney U test

Table 5. Pathological characteristics

	Low (n=21)	High (n=15)	p
	n (%)	n (%)	
Tumor size[b]	2.8 (0.7–10)	3.13 (0.5–6.2)	0.683
Number of lymph nodes[b]	7 (1–18)	8 (3–47)	0.392
Number of metastatic lymph nodes[b]	4.5 (2–10)	4.5 (4–5)	NA
Pathological grade			
1	-	1 (6.7)	0.210
2	11 (52.4)	9 (60)	
3A	3 (14.3)	3 (20)	
3C	7 (33.3)	1 (6.7)	
4	-	1 (6.7)	

a Independent Student's t-test, Mann-Whitney U test

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Table 6. Oncological follow-up results

	Low (n=21)	High (n=15)	p
Recurrence	7 (33.3)	1 (6.7)	0.104
Survival			
Non-survived	18 (85.7)	6 (40)	**0.004****
Length of follow-up (months)[b]	17 (1–72)	13 (0–48)	0.618

Graph 2 **Overall survival by LA groups**

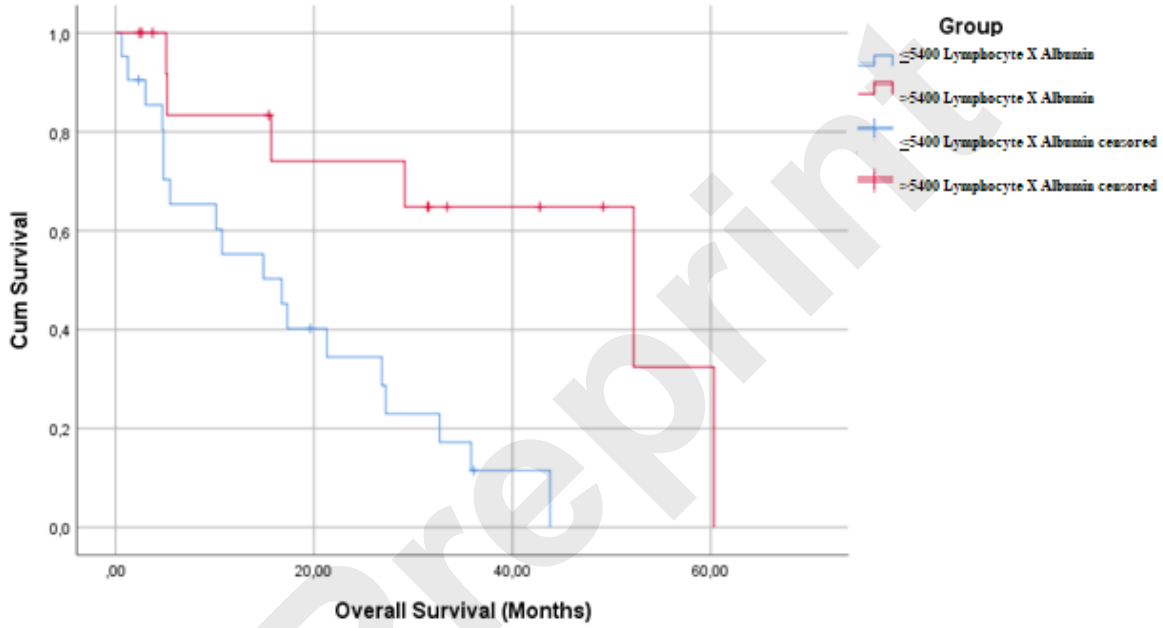

Table 7. **Overall survival by LA groups**

	Mean ± SD	95% CI		p
		Lower	Upper	
≤ 5.400	18.1 ± 3.3	11.7	24.4	**0.003****
> 5.400	41.5 ± 7.1	27.7	55.3	

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