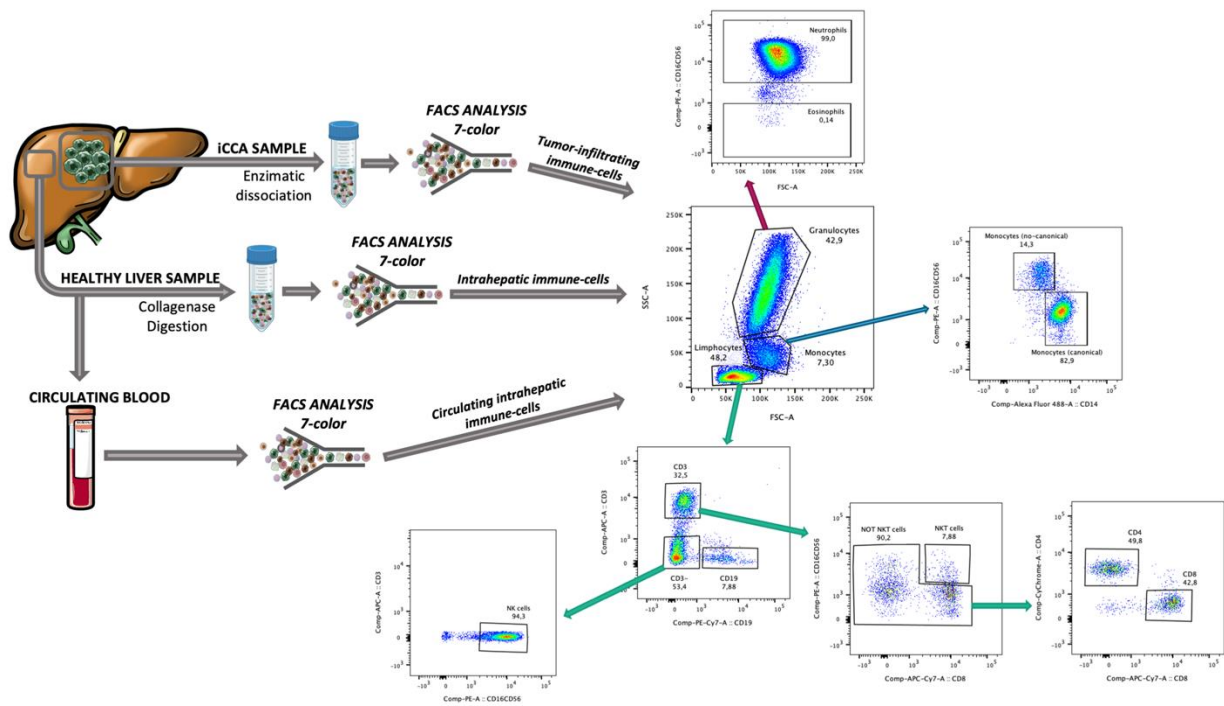**Unveiling the prognostic role of blood inflammatory indexes in a retrospective cohort of patients undergoing liver resection for intrahepatic cholangiocarcinoma**
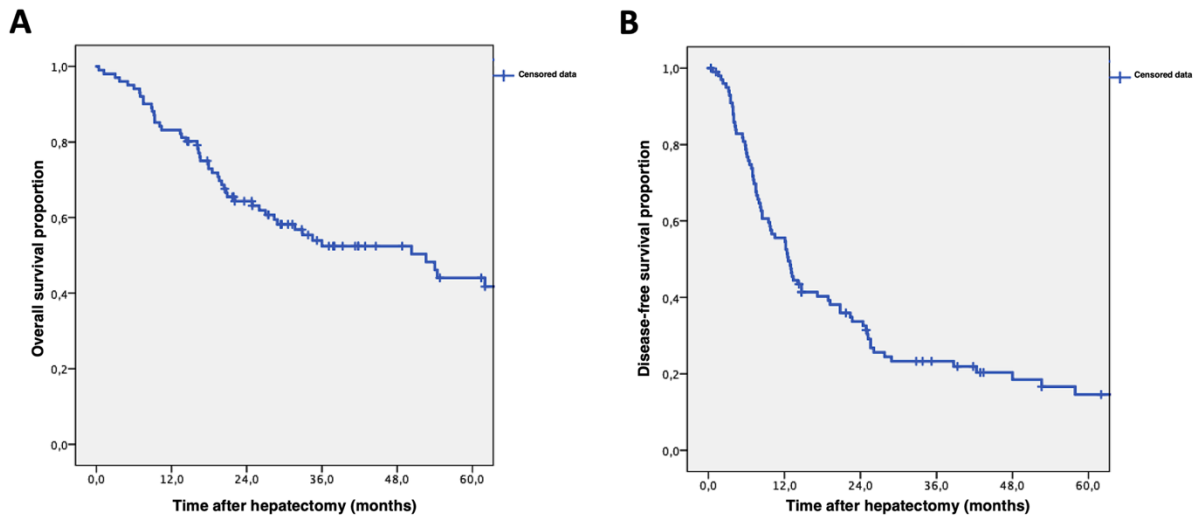
Milana F, et al

SDC, Figure 1. **Schematic representation of the experimental approach used to analyze the immune cells in iCCA samples.**

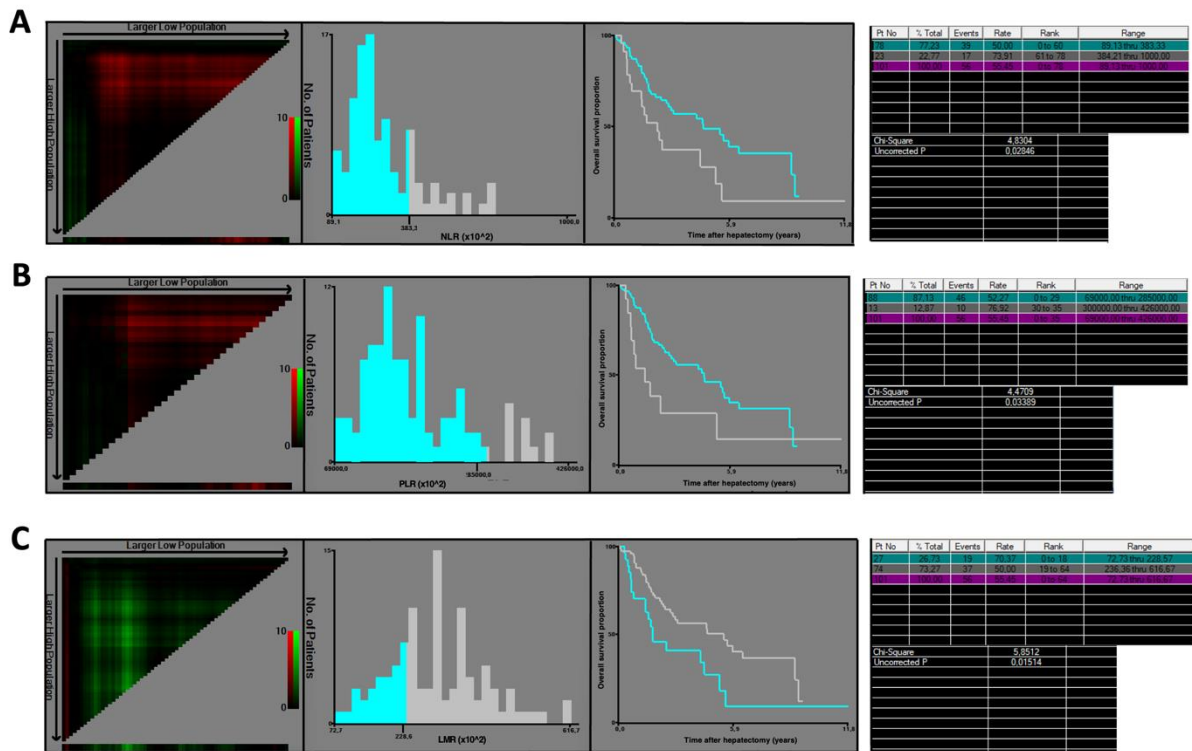*At left, the digestion methods used for iCCA samples.*
At right, the gating strategy applied to analyze the immune-cells subpopulations.

*SDC, Figure 2.* **Survival of the entire cohort.**

A

B

A) Overall Survival, B) Disease-Free Survival.

SDC, Figure 3. X-tile plots.

A) Neutrophil-to-lymphocyte ratio (NLR) X-tile plots, **B) Platelet-to-lymphocyte ratio (PLR)** X-tile plots, **C) Lymphocyte-to-monocyte ratio (LMR)** X-tile plots. *X-tile plots are created by dividing marker data into two populations: low and high. All possible divisions of the marker data are assessed. Associations can be calculated at each division by the log-rank test for overall survival. The data are represented graphically in a right-triangular grid where each point (pixel) represents the data from a given set of divisions. The vertical axis represents all possible "high" populations, with the size of the high population increasing from top to bottom. Similarly, the horizontal axis represents all possible "low" populations, with the size of the low population increasing from left to right. The coloration of the plot represents the strength of the association at each division, ranging from low (dark, black) to high (bright, green, or red). Inverse associations between marker expression and survival (e.g., high expression connotes poorer survival) are colored red, whereas direct associations are colored green.*

SDC, Figure 4. Survival according to **inflammatory ratio cut-offs.**
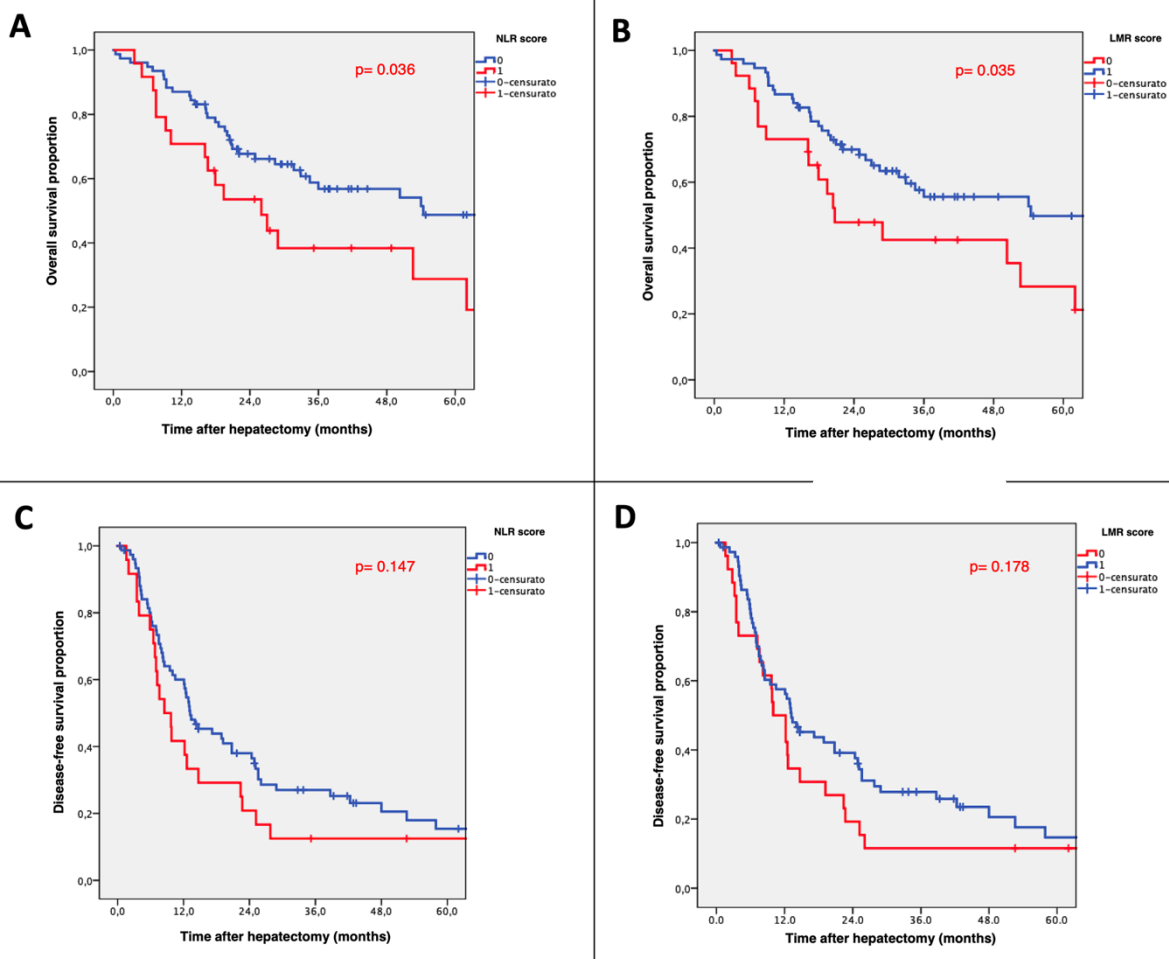
a) Overall survival (OS) according to the neutrophil-to-lymphocyte ratio (NLR) **b)** OS according to the lymphocyte-to-monocyte ratio (LMR) **c)** Disease-free survival (DFS) according to NLR **d)** DFS according to LMR.

SDC, Table 1. Clinical and Pathological characteristics of the analyzed population.

Variables	Patients (n=101)
Age Years, median (IQR)	70.6 (62.9 – 75.3)
Sex Male, n (%)	49 (48.5)
G stage, n (%)	
G1	8 (7.9)
G2	62 (61.4)
G3	30 (29.7)
missing	1 (1)
T stage, n (%)	
T1a	30 (29.7)
T1b	16 (15.8)
T2	34 (33.7)
T3	16 (15.8)
T4	3 (3)
missing	2 (2)
N stage, n (%)	
No lymphadenectomy	57 (56.4)
N0	29 (28.7)
N1	13 (12.9)
missing	1 (1)
MicroVascularInvasion, yes, n (%)	35 (34.7)
Surgical Margins, positive, n (%)	35 (34.7)
Tumor nodule, median (IQR)	1 (1-2)
>1 nodule, n (%)	28 (27.7)
Tumor size, median cm (IQR)	5.7 (3.9-8.7)
>5cm, n (%)	54 (53.5)
CEA (n.v. 0-5 ng/mL), median (IQR)	2.00 (1.05-3.55)
>5ng/mL, n (%)	10 (9.9)
CA19.9 (n.v. 2-40 IU/mL), median (IQR)	29.50 (9.00-98.20)

***>40IU/ml**, n (%)*		42 (41.6)
***Liver pathological substrate,** n (%)*		
Normal		44 (43.6)
Steatosis/Steatohepatitis		52 (51.5)
Cirrhosis		5 (5)
***Viral infection,** n (%)*		
No		83 (83.2)
HBV		8 (7.9)
HCV		10 (9.9)

Abbreviations: n, number**; IQR,** interquartile range; **G,** grade; **T,** tumor; **N,** lymph nodal;

CEA, carcinoembryonic antigen; **CA 19.9**, carbohydrate antigen 19.9.

SDC, Table 2. Inflammatory score distribution in the examined population and High-Risk score distribution obtained after the combination of neutrophil-to-lymphocyte (NLR) and lymphocyte-to-monocyte (LMR) values.

Inflammatory Scores	Score Value	Patients (n=101)
NLR, median (IQR)		2.42 (1.96-3.66)
NLR< 3.83, n (%)	0	77 (76.2)
NLR≥ 3.83, n (%)	1	24 (23.8)
LMR, median (IQR)		3.00 (2.25-3.73)
LMR< 2.28, n (%)	0	26 (25.7)
LMR≥ 2.28, n (%)	1	75 (74.3)
PLR, median (IQR)		130.76 x10^3 (92.47 – 169.70) x10^3
PLR<285.00 x10^3, n (%)	0	95 (94.1)
PLR≥285.00 x10^3, n (%)	1	6 (5.9)
Risk Score*		
HRS, n (%)	**1**	**34 (33.7)**
LRS, n (%)	**0**	**67 (66.3)**

SDC, Table 3. Univariate and Multivariate Cox regression analysis for Disease-Free Survival (DFS).

VARIABLE	UNIVARIATE (DFS)		MULTIVARIATE (DFS)	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Age>70yo	0.840 (0.540-1.308)	0.441		
Sex (male)	1.391 (0.893-2.167)	0.144		
G stage				
G1	Ref		Ref	
G2	1.664 (0.660-4.194)	0.280	1.337 (0.473-3.784)	0.584
G3	**2.333 (0.884-6.156)**	**0.087**	1.667 (0.532-5.227)	0.380
T stage				
T1a	Ref		Ref	
T1b	**2.497 (1.272-4.903)**	**0.008**	0.990 (0.379-2.589)	0.984
T2	**2.300 (1.277-4.143)**	**0.006**	0.949 (0.408-2.207)	0.903
T3	**2.090 (1.012-4.315)**	**0.046**	0.853 (0.313-2.319)	0.755
T4	**3.203 (0.930-11.037)**	**0.065**	1.074 (0.229-5.046)	0.928
*N stage**				
N0	Ref			
N1	1.761 (0.832-3.725)	0.139		

MVI	1.255 (0.789-1.997)	0.337		
Surgical margins (R1)	**1.671 (1.052-2.655)**	**0.030**	1.198 (0.697-2.059)	0.513
N nodule (>1)	**2.538 (1.542-4.179)**	**<0.001**	**1.979 (1.109-3.532)**	**0.021**
Size (>5cm)	**2.459 (1.545-3.914)**	**<0.001**	1.988 (0.997-3.965)	0.051
RiskScore (High)	**1.532 (0.971-2.417)**	**0.067**	1.102 (0.633-1.917)	0.732
CEA elevated	1.522 (0.751-3.082)	0.244		
CA19-9 elevated	**1.859 (1.173-2.947)**	**0.008**	1.442 (0.845-2.460)	0.179
Liver path substrate§	0.811 (0.520-1.265)	0.356		
Viral infection (yes)	0.783 (0.423-1.450)	0.436		

*N stage is based on patients undergone lymphadenectomy

§Liver path substrate is intended for patients having normal liver background Vs those with hepatopathy (from steatosis to cirrhosis)

Viral infections are considered both HBV/HCV

Abbreviations: DFS, disease-free survival; **G,** grade; **T**, tumor; **N**, lymph nodal; **MVI**, microvascular invasion; **R1**, microscopic residual tumor, **n,** number**; CEA**, carcinoembryonic antigen; **CA 19.9**, carbohydrate antigen 19.9.