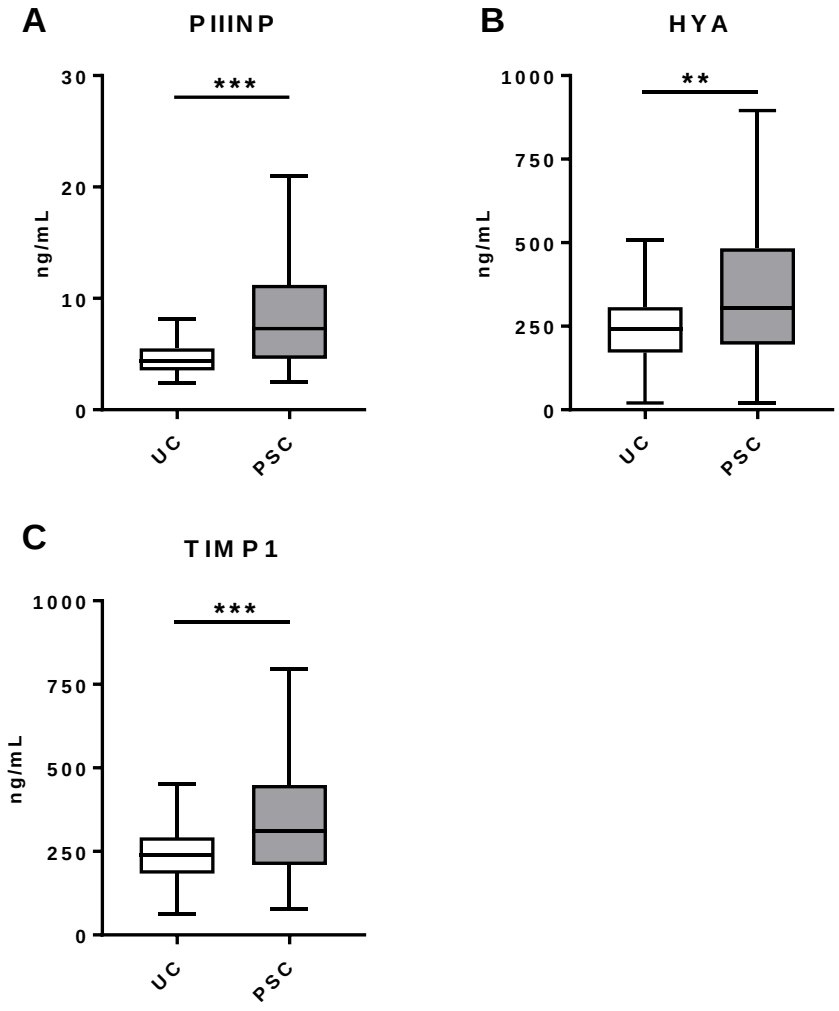
Supplementary material

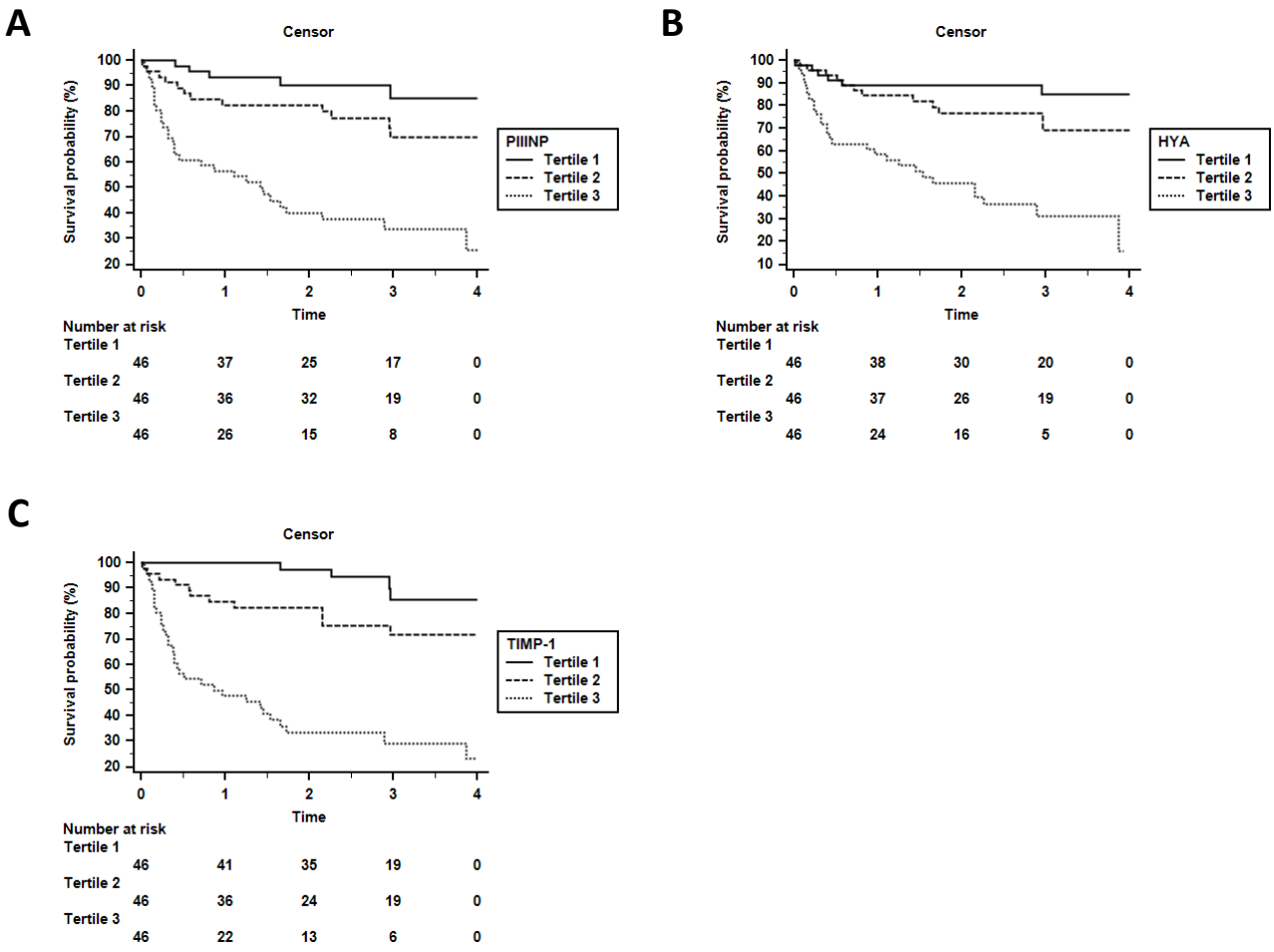
Serological markers of extracellular matrix remodeling predict transplant-free survival in Primary Sclerosing Cholangitis patients

Supplementary Figure 1



Serum levels of the constituents of the Enhanced Liver Fibrosis Score and the derived ELF Score are elevated in PSC patients compared to UC patients. A) Serum levels of N-terminal propeptide of type III collagen, PIIINP; B) Serum levels of hyaluronic acid, HYA; and C) Serum levels of tissue inhibitor of metalloproteinase-1, TIMP1. Data are shown as Tukey's boxplot. Asterisks indicate significant differences as indicated by bars: **p<0.01; ***p<0.001.

Supplementary Figure 2



Prediction of transplant free survival by the individual components of ELF Test. The figure shows Kaplan-Meier curves of time of transplantation or death for PSC patients stratified into tertiles of A) PIIINP, B) HYA, and C) TIMP1.

Supplementary Table 1: AUROC analyses in 105 patients with low-risk (Mayo risk score <2) PSC showing the discriminatory ability of the ECM remodeling markers for detecting patients reaching the primary end-point (all-cause death or liver transplantation due to hepatic decompensation)

	Prev	Marker	AUC	Cut-off	Sens	Spec	PPV	NPV	+LR	LR-	p-value
	25%	ELF	0.72	10.2	62	77	47	86	2.7	0.5	***<0.001***
Patients with vs.		PRO-C3	0.74	35.1	62	86	60	87	4.4	0.5	***<0.001***
without end-point		C3M	0.77	36.7	65	80	52	87	3.2	0.4	***<0.001***
		C4M	0.78	110.3	81	70	47	92	2.7	0.3	***<0.001***
		PRO-C5	0.76	626.9	81	62	42	91	2.1	0.3	***<0.001***
		APRI	0.65	0.51	62	65	37	84	1.8	0.6	***0.01***

Prev, prevalence i.e. patients with end-point; AUC, Area under curve, Sens, sensitivity, spec, specificity, PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; APRI, aspartate aminotransferase to platelet ratio index. Cut-off is given in units for the ELF ng/mL

Supplementary Table 2: Univariate and multivariate Cox regression analyses to identify variables associated with transplant-free survival in a low-risk (Mayo risk score <2) subset of PSC patients. Cox proportional-hazards regression calculated for tertiles of each novel marker of extracellular matrix remodeling using forward elimination

Univariate	**HR**	**95% CI**	***P* value**	**Wald**	**N**
ELF Test	2.03	1.21, 3.41	*0.007*	7.3	105
PRO-C3	2.53	1.49, 4.31	*0.001*	11.8	105
PRO-C5	2.89	1.71, 4.88	*<0.001*	15.7	105
C3M	2.13	1.26, 3.58	*0.005*	8.1	105
C4M	2.89	1.66, 5.05	*<0.001*	14.0	105
APRI	1.76	1.07, 2.90	*0.03*	4.9	104
Multivariate Cox*	**HR**	**95% CI**	***P value***	**Wald**	**N**
Age at diagnosis	-	-	-	-	105
Sex	-	-	-	-	
Ln ALP	-	-	-	-	
Mayo risk score	-	-	-	-	
ELF Test	-	-	-	11.3	
PRO-C5	2.53	1.47, 4.36	*0.001*	6.9	
PRO-C3	2.00	1.19, 3.35	*0.009*	-	
C3M	-	-	-	-	
C4M	-	-	-		

ALP, alkaline phosphatase; HR, hazard ratio; CI, confidence interval.

Supplementary Table 3: Multivariate Cox regression analyses to identify independent predictors of transplant-free survival in PSC patients. Cox proportional-hazards regression calculated for tertiles of each novel marker of extracellular matrix remodeling using forward elimination

Multivariate Cox*	**HR**	**95% CI**	**P value**	**Wald**	**N**
PRO-C3	2.39	1.52-3.75	*0.0002*	14.2	138
PRO-C5	1.80	1.19-2.72	*0.0053*	7.7	
C3M	-				
C4M	-				
PRO-C3	*1.80*	*1.19-2.72*	*0.0053*		138
PRO-C5	*2.39*	*1.52-3.75*	*0.002*		
ELF Test	-				
Age at diagnosis	1.03	1.00, 1.05	*0.036*	4.4	129
Sex	-	-	-	-	
Ln ALP	-	-	-	-	
Mayo risk score	1.46	1.08, 1.99	*0.016*	5.9	
ELF Test	1.45	1.13, 1.88	*0.004*	8.3	
Age at diagnosis	1.04	1.02, 1.06	*0.001*	11.3	129
Sex	-	-	-	-	
Ln ALP	-	-	-	-	
Mayo risk score	-	-	-	-	
ELF Test	1.69	1.39, 2.06	*<0.001*	26.9	
PRO-C5	1.92	1.28, 2.88	*0.002*	10.0	
Age at diagnosis	1.04	1.02, 1.06	*0.001*	10.9	129

Sex	-	-	-	-	
Ln ALP	-	-	-	-	
Mayo risk score	-	-	-	-	
ELF Test	1.70	1.39, 2.07	*<0.001*	26.9	
C4M	1.98	1.26, 3.09	*0.003*	8.9	
Age	-	-	-	-	128
Age at diagnosis	1.04	1.02, 1.07	*<0.001*	12.6	
Sex	0.42	0.21, 0.83	*0.012*	6.2	
Ln ALP	-	-	-	-	
Albumin	-	-	-	-	
PRO-C3	2.5	1.58, 4.0	*<0.001*	15.1	
C3M	-	-	-	-	
C4M	2.10	1.32, 3.35	*0.002*	9.7	

ALP, alkaline phosphatase; HR, hazard ratio; CI, confidence interval.

Supplementary Table 4: AUROC analyses in 138 patients with PSC showing the discriminatory ability of ELF Test and ECM remodeling markers for detecting patients reaching the secondary end-point (all-cause death or liver transplantation due to hepatic decompensation)

	Prev	Marker	AUC	Cut-off	Sens	Spec	PPV	NPV	+LR	LR-	p-value
	18%	ELF	0.84	>11.2	72	86	53	93	5.1	0.3	***<0.0001***
Patients with vs.		PRO-C3	0.78	>36.1	76	73	39	93	2.9	0.3	***<0.0001***
without end-point		C3M	0.71	>35.5	80	61	31	93	2.1	0.3	***<0.0001***
		C4M	0.72	>120.6	76	65	33	93	2.2	0.4	***<0.0001***
		PRO-C5	0.67	>632.9	84	50	27	93	1.7	0.3	***0.003***
		APRI	0.69	>0.7	68	66	31	90	2.0	0.5	***0.001***

Prev, prevalence i.e. patients with end-point; AUC, Area under curve, Sens, sensitivity, spec, specificity, PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; APRI, aspartate aminotransferase to platelet ratio index. Cut-off is given in units for the ELF ng/mL

Supplementary Table 5. Univariate Cox regression analyses of factors associated with transplant-free survival in PSC patients (n=138) using the secondary endpoint of all-cause death or hepatic decompensation (n=25, 18%). Cox proportional-hazards regression rates were calculated for tertiles of each of the novel markers and ELF Test. PRO-C3 (collagen III formation marker) performed best out of the novel markers.

	HR	**95% CI**	***P* value**	**Wald**	**N**
ELF Test	2.21	1.76, 2.79	*<0.001*	45.8	138
PRO-C3	3.59	1.89, 6.81	*<0.001*	15.2	138
PRO-C5	2.20	1.30, 3.73	*0.003*	8.7	138
C3M	1.75	1.04, 2.96	*0.04*	4.4	138
C4M	3.01	1.67, 5.44	*<0.001*	13.3	138
APRI	2.46	1.42, 4.29	*0.001*	10.1	128

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IBD, inflammatory bowel disease; INR, international normalized ratio; HR, hazard ratio; CI, confidence interval; APRI, AST to platelet ratio index.