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ORIGINAL ARTICLE

Serological Risk Index Based on Alpha-Fetoprotein and C-Reactive Protein to Indicate Futile Liver Transplantation Among Patients with Advanced Hepatocellular Carcinoma

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Abstract

Background The aim of this study was to establish a preoperatively available serological risk index using alpha-fetoprotein (AFP) and C-reactive protein (CRP) for predicting oncologically futile liver transplantation (LT) in hepatocellular carcinoma (HCC) patients.

Methods A total of 119 liver transplant patients with HCC were retrospectively analyzed. The prognostic impact of clinical and histopathologic factors including pre-LT serum AFP and CRP values was determined.

Results Apart from microvascular tumor invasion (MVI; odds ratio [OR] 15.77), pretransplant serum levels of AFP > 100 ng/ml (OR 13.31) and CRP > 0.8 mg/dl (OR 13.97) were identified as independent predictors of HCC recurrence. The cumulative risk of HCC relapse at 5 years post-LT was 2.3% in low serological tumor activity (STA) index (AFP ≤ 100 ng/ml + CRP ≤ 0.8 mg/dl), 17.1% in intermediate STA (AFP ≤ 100 ng/ml or CRP ≤ 0.8 mg/dl), and 91.6% in high STA index (AFP > 100 ng/ml + CRP > 0.8 mg/dl; $p < 0.001$), respectively. High STA index was identified as most powerful pre-LT available predictor of MVI (OR 15.31) and posttransplant HCC recurrence (OR 54.44). Five-year recurrence-free survival rate in Milan Out patients with high STA was 0%, compared to 91.7% and 83.6% in those with low or intermediate STA index ($p < 0.001$), respectively.

Conclusion Our proposed serological risk index based on pretransplant serum AFP and CRP values is able to predict oncologically futile LT among advanced HCC patients.

Keywords Alpha-fetoprotein · C-reactive protein · Hepatocellular carcinoma · Liver transplantation · Tumor recurrence · Milan criteria

Abbreviations

AFP	Alpha-fetoprotein
AUC	Area under the curve
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
HCC	Hepatocellular carcinoma
IL	Interleukin
LT	Liver transplantation
LVI	Lymphovascular invasion
MC	Milan criteria
MELD	Model of end-stage liver disease
MRI	Magnetic resonance imaging
MVI	Microvascular invasion
OR	Odds ratio
OS	Overall survival
RFS	Recurrence-free survival
STA	Serological tumor activity
TACE	Transarterial chemotherapy

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Introduction

Despite significant advancements in surgical resection techniques, locoregional procedures, and medical therapies, liver transplantation (LT) remains the best curative treatment option for patients with early-stage hepatocellular carcinoma (HCC) in liver cirrhosis [1]. Only LT is able to remove the tumor and the pro-cancerogenic cirrhosis [2]. The introduction of the Milan criteria (MC) in 1996 (single tumor up to 5 cm, or up to 3 tumors with none of them exceeding 3 cm, absence of macrovascular invasion) for strict transplant patient selection represents a milestone, resulting in excellent disease-free survival rates above 70% [3, 4]. Several studies have since validated the MC, and consequently, they were implemented for prioritization in Model for End-Stage Liver Disease (MELD) score-based allocation systems [5, 6].

In the last two decades it became apparent that the MC are too rigid, excluding a large number of advanced HCC patients from potentially curative LT. Apart from that, discrepancies between radiographic and histopathologic tumor staging additionally limited their prognostic accuracy [7, 8].

Numerous expanded criteria sets have been proposed in recent years producing acceptable posttransplant survival rates above 50%, such as the University of California San Francisco criteria, the Valencia criteria, and the registry-based up-to-7 criteria [9–12]. However, none of them have implemented aspects of cancer biology and, besides, they were still based on quite unflexible tumor size limitations.

There is general agreement that biological tumor behavior rather than tumor load determines posttransplant outcome. In particular, microvascular invasion (MVI) and pathologic tumor differentiation were shown to indicate tumor virulence and poor prognosis [13–15]. Unfortunately, these cancer features may not reliably be assessed prior to LT, but have to be determined at explant liver pathology [16, 17].

Serum alpha-fetoprotein (AFP) has indisputably shown to correlate with tumor aggressiveness and posttransplant outcome [18–20]. Due to lack of a widely accepted threshold value along with presence of AFP negative tumors, AFP has not yet been implemented as standard in patient selection process [18–20, 22–25]. Apart from that, AFP is an oncoprotein describing cancer invasiveness, whereas other non-cancer factors are not captured. In particular, inflammation and immunologic activation were shown to promote tumor aggressiveness and risk of HCC recurrence [26–28]. Therefore, C-reactive protein (CRP), a widely used inflammatory parameter, may serve as useful biomarker in many tumor entities [29–31]. It was reported to correlate with outcome in conservative therapies and surgical resection of HCC [32–35]. Recently, some groups suggested perioperative CRP level to correlate with risk of tumor relapse in the LT setting [36–39].

We hypothesized that combining pretransplant serum levels of AFP and CRP might improve prognostic power of each by reflecting different aspects of tumor biology. The major aim of this study was to establish a pre-LT available serological tumor activity index (STA) for predicting oncologically futile LT, particularly in patients with beyond MC tumors.

Patients and Methods

The study protocol was approved by the ethical committee of the Technical University Munich (Nr. 217/15). Prior to transplant, all patients gave informed consent that follow-up data may be used for academic analysis. In a prospectively managed database, 119 consecutive liver transplant patients with HCC were identified and retrospectively analyzed.

HCC Staging and Listing

Diagnosis of HCC was based on radiographic staging by computed tomography (CT) or magnetic resonance imaging (MRI), visualizing arterial enhancement of a tumor nodule of > 1 cm and contrast washout during venous phase [40]. For safety reasons, we did not perform transhepatic tumor biopsy. Negative AFP levels did not exclude HCC diagnosis. The MC were the guiding framework for patient listing. Since December 2007, patients with tumors meeting the MC (Milan In) received standard exceptional upgrades according the MELD scoring allocation system. Based on an interdisciplinary individual decision-making process, patients with tumors exceeding the MC were put on the waiting list without assignment of MELD points. These patients were scheduled for rescue liver graft allocation. Tumor re-evaluation was performed every 6 weeks by liver ultrasound and laboratory analysis, including AFP and CRP values. In addition, CT/MRI scans were performed every 3 months or in the case of suspected tumor progression. At final pretransplant radiographic staging, all patients were classified as Milan In or Milan Out.

Bridging Therapies

Since waiting time for LT was expected to exceed 12 months in our transplant area, locoregional tumor therapies were discussed in all patients in a weekly multidisciplinary hepato-transplant board. If being feasible for functional (liver cirrhosis Child A or B) and topographical/morphological aspects, transarterial chemotherapy (TACE) was the

predominantly indicated procedure. Surgical resection was not used as bridging concept prior to LT.

Dropout Criteria

Macrovascular invasion, lymphonodular tumor infiltration, extrahepatic metastasis, and tumor-related symptoms, such as progressive weight loss, cachexia, or tumor-induced pain, were absolute contraindications for LT and resulted in dropout from the waiting list.

Cutoff Values of AFP and CRP

The upper values of AFP and CRP based on our central laboratory standard were 6 ng/ml and 0.5 mg/dl, respectively. The last pretransplant available AFP (within 6 weeks prior to LT) and CRP level (at LT) were used for further analysis. By means of receiver operating characteristics analyses, the optimal cutoff values for AFP and CRP to predict HCC recurrence were 100 ng/ml (area under the curve [AUC] = 0.830; $r = 0.042$; 95% CI 0.747–0.912) and 0.8 mg/dl (AUC = 0.835; $r = 0.039$; 95% CI 0.759–0.911).

Explant Histopathologic Analysis

All explanted livers were thoroughly analyzed by an experienced team of pathologists. Diagnosis of HCC was confirmed in all cases. Frequencies of aggressive tumor features, such as MVI, tumor grading, and lymphovascular tumor invasion (LVI), were determined.

Immunosuppression and Post-LT Surveillance

Posttransplant maintenance immunosuppression consisted of a calcineurin inhibitor-based dual treatment by cyclosporine A or tacrolimus augmented with mycophenolate mofetil. Corticosteroids were withdrawn latest 3 months post-LT, except for patients with autoimmune liver cirrhosis. Surveillance post-LT consisted of liver ultrasound and AFP level determination every 3 months. Furthermore, thoracoabdominal CT scan was performed twice during the first post-LT year and minimum yearly thereafter, or in the case of tumor-related symptoms. Suspicious tumor nodules underwent biopsy in the case of inconclusive imaging.

Statistical Analysis

Data are expressed as mean ± standard deviation or median and interquartile range, where appropriate. Student *t* test or Mann–Whitney *U* test was used for data comparison. Overall survival (OS), recurrence-free survival (RFS), and cumulative risk of HCC recurrence were calculated using the Kaplan–Meier method. Cox's proportional hazards model was used for identifying significant and independent predictors of posttransplant HCC recurrence, OS, and MVI. A p value < 0.05 was defined as statistical significant for all investigations. All statistical analyses were performed by SPSS 24.0 software (IBM Inc., Munich, Germany).

Results

Clinicopathologic Characteristics

The clinicopathologic characteristics of the study cohort are listed in Table 1. Ethyltoxic cirrhosis (54.6%) and chronic viral hepatitis (31.1%; $n = 25$ hepatitis C; $n = 12$ hepatitis B) were the major transplant indications. At final pretransplant radiographic staging, tumors were meeting and exceeding the MC in 69 patients (58%) and 50 patients (42%), respectively. Metric tumor data, CRP values, and frequencies of MVI were significantly different between both subgroups (Table 1).

Overall Outcome

Posttransplant follow-up was ranging between 5 and 184 months (median 74 months). Actuarial OS and RFS rates of the entire study group at 3 and 5 years post-LT were 83.2% and 74.2%, and 77.8% and 75%, respectively. Stratified by MC, OS was significantly better in Milan In patients (88.4%; 82.5%) compared to patients with Milan Out tumors (74%, 62.7%; $p < 0.001$). The corresponding cumulative risks of HCC relapse were 13.2% and 13.2% within, and 34.6% and 41.6% beyond MC ($p = 0.001$). In total, 29 patients experienced HCC recurrence, 9 in the Milan In (13%) and 20 in the Milan Out subset (40%; $p = 0.001$).

Predictors of Outcome

In univariate analysis including 16 pre- and posttransplant variables, AFP level > 100 ng/ml, CRP value > 0.8 mg/dl, multifocal tumor manifestation, total tumor diameter > 10 cm, beyond 3 tumor nodules, MC Out status, presence of MVI, poor tumor differentiation, and presence of LVI were significant risk factors. Apart from MVI, only elevated serum AFP and CRP levels remained as significant and independent predictors of HCC relapse (Table 2). In addition, AFP level ≤ 100 ng/ml, solitary tumor manifestation, and absence of LVI were identified as independent and significant promoters of OS, whereas CRP level ≤ 0.8 mg/dl almost reached statistical significance (Table 3).

Table 1 Clinicopathologic characteristics of the study population ($n=119$)

Variable	All patients ($n=119$)	Milan In ($n=69$)	Milan Out ($n=50$)	p value
Mean recipients' age in years ± STD	58.5 ± 6.8	58.5 ± 7.3	58.5 ± 6.1	0.952
Gender				0.753
Male	71 (59.7%)	42 (60.9%)	29 (58%)	
Female	48 (40.3%)	27 (39.1%)	21 (42%)	
Liver disease				0.076
Ethyltoxic	65 (54.6%)	33 (47.8%)	32 (64%)	
Viral hepatitis	37 (31.1%)	28 (40.6%)	9 (18%)	
Autoimmune	4 (3.4%)	3 (4.3%)	1 (2%)	
Cholestatic	2 (1.7%)	0 (0%)	2 (4%)	
Other	11 (9.2%)	5 (7.2%)	6 (12%)	
Median calculated Meld score (range)	16 (9–35)	18 (9–35)	16 (9–33)	0.456
Child–Pugh status				0.785
A	53 (44.5%)	30 (43.5%)	23 (46%)	
B/C	66 (55.5%)	39 (56.5%)	27 (54%)	
Median AFP level at LT in ng/ml (range)	70 (2.7–46930)	50 (3.3–13300)	112 (2.7–46930)	0.2
Median CRP level at LT in mg/dl (range)	0.8 (0.1–9.5)	0.6 (0.1–5)	1.0 (0.1–9.5)	0.008
TACE prior LT				0.751
No	40 (33.6%)	24 (34.8%)	16 (32%)	
Yes	79 (66.4%)	45 (65.2%)	34 (68%)	
Solitary HCC manifestation[a]	58 (48.7%)	45 (65.2%)	13 (26%)	< 0.001
Median largest HCC nodule in cm (range)[a]	4 (1–20)	3 (1–6)	6 (3–20)	< 0.001
Median total tumor diameter in cm (range)[a]	5 (1–20)	5 (1–14)	10 (5.8–20)	< 0.001
Median number of tumor nodules (range)[a]	1.5 (1–8)	1 (1–5)	3 (1–8)	< 0.001
Microvascular invasion				0.02
No	74 (62.2%)	49 (71%)	25 (50%)	
Yes	45 (37.8%)	20 (29%)	25 (50%)	
Tumor differentiation				0.122
Well/moderate	98 (83.4%)	60 (87%)	38 (76%)	
Poor	21 (17.6%)	9 (13%)	12 (24%)	
Lymphovascular invasion				0.493
No	94 (79%)	53 (76.8%)	41 (82%)	
Yes	25 (21%)	16 (23.2%)	9 (18%)	
HCC recurrence	29 (24.4%)	9 (13%)	20 (40%)	0.001

AFP alpha-fetoprotein; *CRP* C-reactive protein; *HCC* hepatocellular carcinoma; *LT* liver transplantation; *MELD* Model for End-Stage Liver Disease; *STD* standard deviation; *TACE* transarterial chemoembolization

[a]According to pretransplant radiographic staging

Serological Tumor Activity Index

Outcome stratified by AFP and CRP is demonstrated in Fig. 1a–d. Based on multivariable analysis (Table 2), we defined the following serological tumor activity (STA) indices: low STA index: AFP ≤ 100 ng/ml + CRP ≤ 0.8 mg/dl ($n=44$); intermediate STA index: AFP > 100 ng/ml or CRP > 0.8 mg/dl ($n=50$); high STA index: AFP > 100 ng/ml + CRP > 0.8 mg/dl ($n=25$).

Tumor-specific characteristics of the different risk cohorts are listed in Table 4. Tumor size criteria and numbers of unfavorable tumor features were significantly larger in high STA patients compared to low and intermediate STA patients, respectively. In contrast, we found no significant differences between low and intermediate STA patients, although AFP level tended to and CRP level proved to be significantly lower in the low STA subset (Table 4).

Posttransplant HCC recurrence rates were 2.3%, 14%, and 84% in patients with low, intermediate, and high STA index (Table 4), respectively.

The actuarial post-LT 3- and 5-year OS rates were 100% and 95% in low, 88% and 79.8% in intermediate, and 44% and 26.3% in high STA index (Fig. 2a). The corresponding

Table 2 Uni- and multivariable analysis of clinical factors predicting post-LT HCC recurrence

Variable	*p* value	OR (95% CI)	*p* value
Female vs. male	0.761		
Age recipients' > vs. ≤ 60 years	0.430		
Viral vs. non-viral liver disease	0.352		
No TACE vs. TACE prior LT	0.309		
(Lab.)MELD > vs. ≤ 15	0.158		
Child C vs. Child A/B cirrhosis	0.210		
AFP at LT > vs. ≤ 100 ng/ml	< 0.001	13.31 (3.625–48.864)	< 0.001
CRP at LT > vs. ≤ 0.8 mg/dl	< 0.001	13.97 (2.450–79.623)	0.003
Multiple vs. solitary tumor[a]	0.009		
Maximum tumor nodule size > vs. ≤ 5 cm[a]	0.292		
Total tumor diameter > vs. ≤ 10 cm[a]	0.031		
Number HCC nodules > vs. ≤ 3[a]	0.019		
Milan Out vs. Milan In[a]	0.001		
MVI vs. no MVI	< 0.001	15.77 (5.355–46.454)	< 0.001
Poor vs. well/moderate grading	< 0.001		
LVI vs. no LVI	< 0.001		

AFP alpha-fetoprotein; *CRP* C-reactive protein; *HCC* hepatocellular carcinoma; *LT* liver transplantation; *LVI* lymphovascular invasion; *MVI* microvascular; *MELD* Model for End-Stage Liver Disease; *Odds ratio* odds ratio; *TACE* transarterial chemoembolization

[a]According to pretransplant radiographic staging

Table 3 Uni- and multivariable analysis of clinical factors promoting post-LT overall survival

Variable	*p* value	OR (95% CI)	*p* value
Female vs. male	0.385		
Age recipients' ≤ vs. > 60 years	0.175		
Non-viral vs. viral liver disease	0.934		
TACE vs. no TACE prior LT	0.963		
(Lab.)MELD ≤ vs. > 15	0.139		
Child A/B cirrhosis vs. Child C	0.025		
AFP at LT ≤ vs. > 100 ng/ml	< 0.001	2.79 (1.456–5.325)	0.002
CRP at LT ≤ vs. > 0.8 mg/dl	0.003	1.99 (1.001–3.964)	0.05
Solitary vs. multiple tumor[a]	0.001	2.04 (1.047–4.009)	0.037
Maximum tumor nodule size ≤ vs. > 5 cm[a]	0.308		
Total tumor diameter ≤ vs. > 10 cm[a]	0.109		
Number HCC nodules ≤ vs. > 3[a]	0.04		
Milan In vs. Milan Out[a]	< 0.001		
No MVI vs. MVI	< 0.001		
Moderate/well vs. poor grading	0.001		
No LVI vs. LVI	0.001	2.34 (1.167–4.705)	0.017

AFP alpha-fetoprotein; *CRP* C-reactive protein; *HCC* hepatocellular carcinoma; *LT* liver transplantation; *LVI* lymphovascular invasion; *MVI* microvascular; *MELD* Model for End-Stage Liver Disease; *OR* odds ratio; *TACE* transarterial chemoembolization

[a]According to pretransplant radiographic staging

risks of cumulative HCC relapse were 2.3% and 2.3%, 14.2% and 14.2%, and 79.1% and 91.6% (Fig. 2b), respectively.

Upon adjusted analysis, predictive capability for HCC recurrence was greatest for high STA index (OR 54.44, 95% CI 15.511–205.347), followed by CRP > 0.8 mg/dl (OR 24.47, 95% CI 5.461–109.635), AFP > 100 ng/dl (OR 11.74, 95% CI 4.365–31.6), and MC (OR 4.44, 95% CI 1.806–10.937).

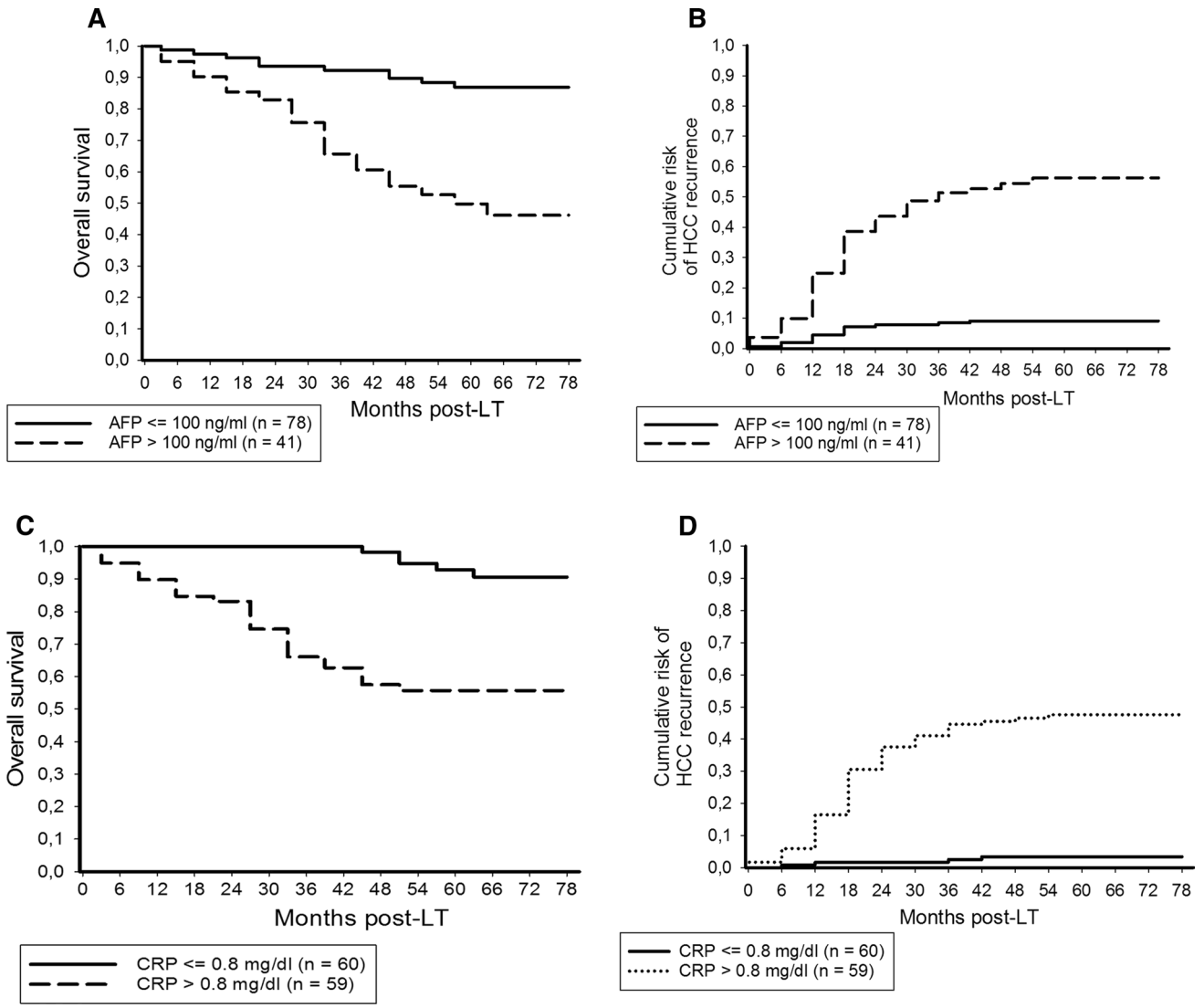

Fig. 1 Outcome according to AFP and CRP values. **a** Actuarial OS rates at 3 and 5 years post-LT were 92.3% and 86.9% in low and 65.7% and 49.8% in high AFP patients, respectively ($p < 0.001$). **b** The cumulative risks of HCC relapse were 7.8% and 9.1% in low, and 50.1% and 56.3% in high AFP patients ($p < 0.001$). **c** Based on CRP level, actuarial 3- and 5-year OS rates were 100% and 90.6% (low CRP), and 66.1% and 55.7% (high CRP), respectively ($p = 0.003$). **d** The 3- and 5-year cumulative risks of HCC recurrence were 1.7% and 3.4% in the low CRP group, and 43.7% and 47.6% in the high CRP subset ($p < 0.001$)

Outcome Stratification According to MC and Serological Parameters

Risk stratification based on MC and serological tumor markers is described in Table 5.

In the Milan In subset, seven patients demonstrated high STA, and 5 of them subsequently developed HCC recurrence (71.4%). In contrast, none of 35 MC In patients with low (0%) and 4 of MC In patients with intermediate (12.9%) STA developed tumor relapse.

Thirty-two Milan Out patients (64%) exhibited low ($n = 13$) or intermediate ($n = 19$) STA, of which only 4 (12.5%) experienced HCC recurrence. Conversely, 16 of 18 Milan Out patients developed tumor recurrence when demonstrating high STA index at LT (88.9%; Table 5).

In the Milan In group, RFS rates at 3 and 5 years post-LT were 100% and 100%, 87.1% and 87.1%, and 17.1% and 17.1% in low, intermediate, and high STA index (Fig. 3a). Corresponding outcome data in the Milan Out cohort were 100% and 91.7%, 83.6% and 83.6%, and 22.2% and 0% (Fig. 3b), respectively. There were no significant differences in tumor-specific survival between Milan In patients and MC Out patients with low or intermediate STA index (Fig. 3c).

Table 4 Tumor-specific differences according to STA index

Variable	Low STA ($n=44$)	Intermediate STA ($n=50$)	High STA ($n=25$)	p value Low *vs.* intermediate STA	p value Low *vs.* high STA	p value Intermediate *vs.* high STA
Median AFP level at LT in ng/ml (range)	19 (3.3–100)	74.9 (2.7–5580)	450 (150–46930)	0.06	0.038	0.05
Median CRP level at LT in mg/dl (range)	0.5 (0.1–0.8)	1 (0.1–4)	1.5 (1–9.5)	< 0.001	< 0.001	< 0.001
Solitary HCC manifestation[a]				0.269	0.003	0.031
Yes	27 (61.4%)	25 (50%)	6 (24%)			
No	17 (38.6%)	25 (50%)	19 (76%)			
Median largest HCC nodule in cm (range)[a]	3 (1–9.5)	3,5 (1–10)	5 (2–20)	0.652	0.029	0.046
Median total tumor diameter in cm (range)[a]	5 (1–17)	6 (1–18)	9 (2–20)	0.993	0.002	< 0.001
Median number tumor nodules (range)[a]	1 (1–8)	1.5 (1–5)	3 (1–6)	0.727	0.014	0.001
Milan status[a]				0.388	0.001	0.005
In	31 (70.5%)	31 (62%)	7 (28%)			
Out	13 (29.5%)	19 (38%)	18 (72%)			
Microvascular invasion				0.290	< 0.001	< 0.001
No	35 (79.5%)	35 (70%)	4 (16%)			
Yes	9 (20.5%)	15 (30%)	21 (84%)			
Tumor differentiation				0.881	< 0.001	< 0.001
Well/moderate	40 (90.9%)	45 (90%)	13 (52%)			
Poor	4 (9.1%)	5 (10%)	12 (48%)			
Lymphovascular invasion				0.748	0.005	0.009
No	38 (86.4%)	42 (84%)	14 (56%)			
Yes	6 (13.6%)	8 (16%)	11 (44%)			
HCC recurrence	1 (2.3%)	7 (14%)	21 (84%)	0.042	< 0.001	< 0.001

AFP alpha-fetoprotein; *CRP* C-reactive protein; *HCC* hepatocellular carcinoma; *LT* liver transplantation; *STA* serological tumor activity

[a]According to radiographic staging

Predictors of MVI

The actuarial 3- and 5-year OS rates with and without the presence of MVI were 98.6% and 91.4%, and 57.8% and 46.7%, respectively. The cumulative risk of HCC recurrence at 3 and 5 years post-LT was significantly higher in patients with (50.9% and 56.4%) compared to those without MVI (5.4% and 6.8%; $p<0.001$).

In univariate analysis including 14 pre-LT available clinical variables, MC Out status and increased levels of AFP and CRP were significantly correlated with the presence of MVI. In multivariable analysis, only elevated AFP and CRP levels remained as independent and significant predictors of MVI (Table 6). Upon adjusted analysis, high STA (versus low/intermediate) demonstrated more predictive power for indicating MVI (OR 15.31; 95% CI 4.774–49.112) than AFP or CRP level (Table 6).

Discussion

To the best of our knowledge, this is the first study demonstrating that serum levels of AFP and CRP combined in a pretransplant easy to determine serological risk index optimizes posttransplant outcome prediction in HCC patients. Five-year OS was excellent in low (95%) and still appropriate in intermediate STA index (79.8%), whereas it proved to be inacceptable in patients with pre-LT elevation of both serological markers (26.3%; Fig. 2a). We suppose that the combination of preoperatively elevated AFP and CRP levels indicates a very aggressive tumor phenotype and may, therefore, predict oncologically futile LT.

Of note, the introduced serological parameter was able to discriminate the cancer outcome independently from the MC (Table 5). For example, we identified 7 Milan In patients with high STA and 5 of them were finally suffering from

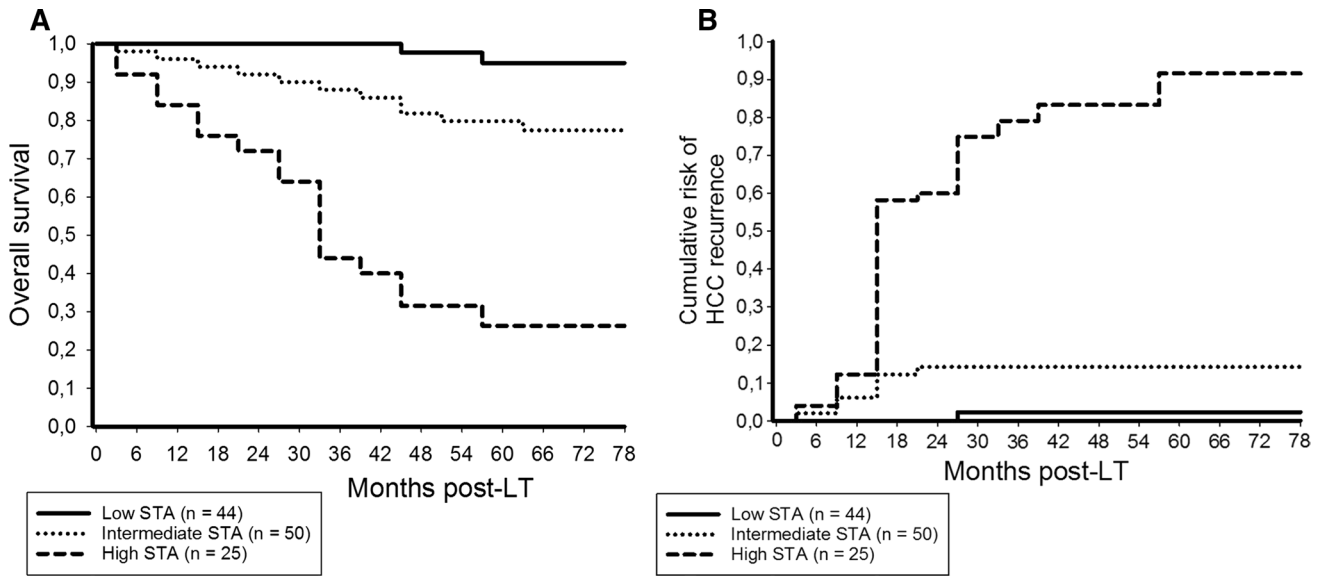

Fig. 2 Outcome according to STA index. **a** Three and 5-year OS rates were 100% and 95% in low, 88% and 79.8% in intermediate, and 44% and 26.3% in high STA patients, respectively (low vs. intermediate: $p=0.144$; low vs. high: $p<0.001$; intermediate vs. high: $p<0.001$). **b** The corresponding cumulative risks of HCC relapse were 2.3% and 2.3% in low, 14.2% and 14.2% in intermediate, and 79.1% and 91.6% in high STA patients (low vs. intermediate: $p=0.038$; low vs. high: $p<0.001$; intermediate vs. high: $p<0.001$), respectively

Table 5 Risk stratification by MC and serological biomarkers

	Milan In ($n=69$)	p value	Milan Out ($n=50$)	p value
AFP		0.001		0.001
≤ 100 ng/ml	53/3 (5.7%)		25/4 (16%)	
> 100 ng/ml	16/6 (37.5%)		25/16 (64%)	
CRP		0.002		< 0.001
≤ 0.8 mg/dl	40/1 (2.5%)		20/1 (5%)	
> 0.8 mg/dl	29/8 (27.6%)		30/19 (63.3%)	
STA		< 0.001		< 0.001
Low	31/0 (0%)		13/1 (7.7%)	
Intermediate	31/4 (12.9%)		19/3 (15.8%)	
High	7/5 (71.4%)		18/16 (88.9%)	
	Total/recurrences		Total/recurrences	

AFP alpha-fetoprotein; *CRP* C-reactive protein; *STA* serological tumor activity

tumor relapse (71.4%). This result confirms previous studies that a considerable number of Milan In patients (≈15–20%) develop post-LT HCC recurrence [10, 11]. Our data clearly suggest that patients meeting the MC should not receive a liver transplant in the case of pre-LT elevated AFP and CRP values, since they are subject to a high oncological risk (Fig. 3a). Even more important, STA index was able to identify beyond MC patients with excellent prognosis (Fig. 3c), which in retrospect would not have justified exclusion from LT. In contrast, the combination of MC Out status and elevated serological tumor markers clearly predicted futile LT, since calculated 5-year RFS was disastrous (0%) in this specific subset (Fig. 3b).

Currently, there is overwhelming evidence that biological factors beyond radiographic tumor load correlate with poor outcome in the transplant setting [13–16, 41]. In this context, poor grading and MVI are considered as most important predictors of aggressive tumor behavior [13–15, 42]. Also in our present analysis, both were identified as important prognostic factors, with MVI being the most powerful predictor of HCC relapse, whereas in contrast, tumor size criteria did not play an independent prognostic role (Table 2). This result of our study clearly underlines that tumors expressing such features of biological invasiveness have to reliably be identified, in order to emancipate from rigid macromorphological tumor burden limits. Preoperative tumor biopsy seems to be inappropriate, since it carries a considerable risk of tumor cell seeding and, besides, sample errors may lead to inaccurate decision-making [16, 17]. Thus, with special regard to the dramatic organ shortage in our transplant area [43], we are rather favoring a "tumor no-touch" policy, which increases the need for accurate noninvasive surrogate markers of tumor aggressiveness.

Similar to prior reports [18–20, 23–25], we have identified AFP as an independent prognostic marker of unfavorable histopathology (Table 6), post-LT HCC relapse (Table 2) and OS (Table 3). Apart from that, AFP level increase was shown to be linked with risk of patients drop out from the waiting [44]. Nonetheless, it has not yet been implemented as standard biomarker in public organ sharing systems [21]. The lack of a consensual prognostic cutoff value (range

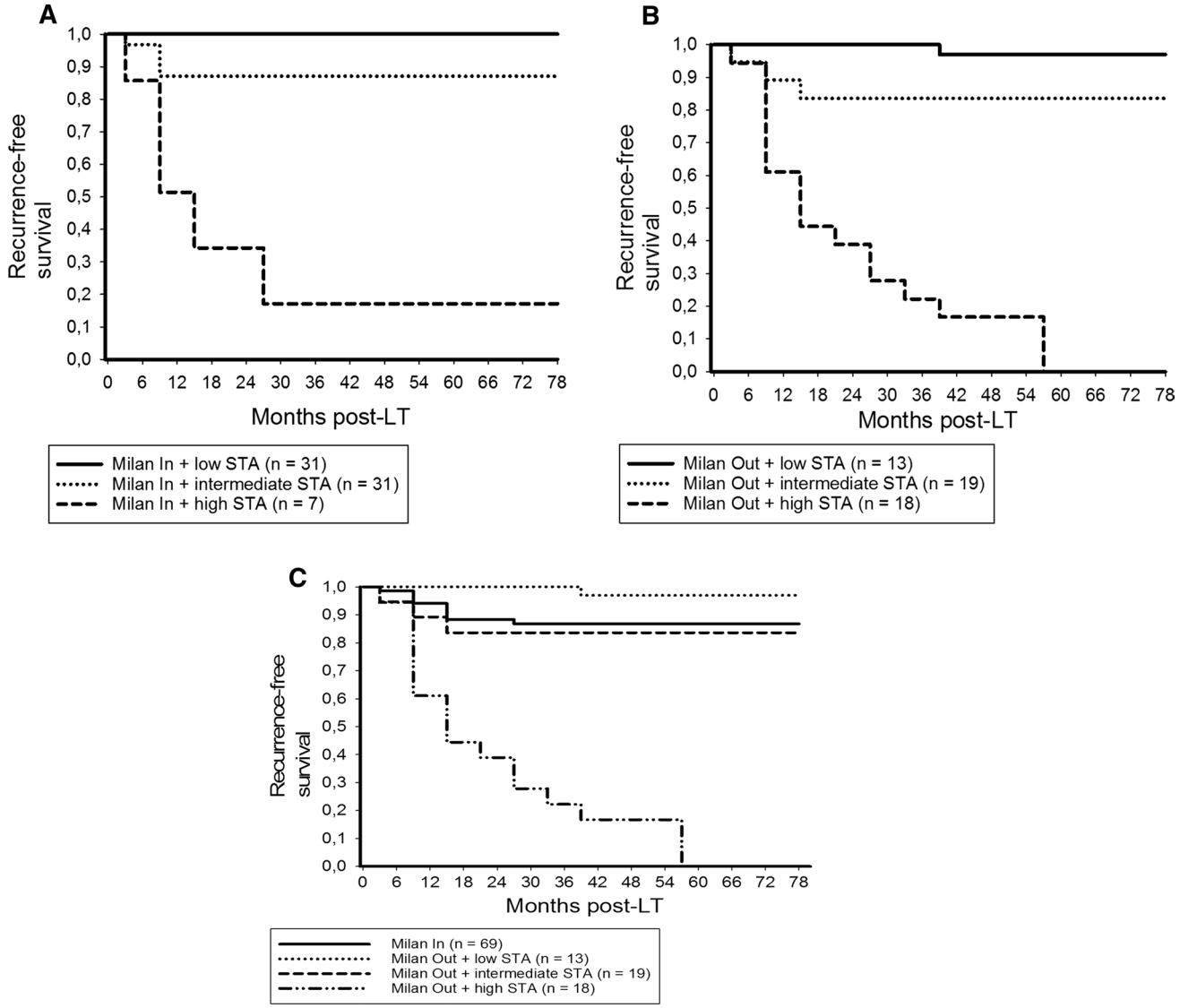

Fig. 3 Outcome stratified by MC and STA index. **a** In the Milan In cohort, actuarial RFS rates at 3 and 5 years post-LT were 100% and 100% in low STA, 87.1% and 87.1% in intermediate STA, and 17.1% and 17.1% high STA (low vs. intermediate: $p=0.04$; low vs. high: $p<0.001$; intermediate vs. high: $p<0.001$). **b** In the Milan Out subset, 3- and 5-year RFS rates were 100% and 91.7%, 83.6% and 83.6%, and 22.2% and 0% in low, intermediate, and high STA index, respectively (low vs. intermediate: $p=0.46$; low vs. high: $p<0.001$; intermediate vs. high: $p<0.001$). **c** There were no significant outcome differences between Milan In patients and Milan Out patients with low ($p=0.562$) or intermediate ($p=0.696$) STA index

15–1000 ng/ml) [23, 24], presence of non-AFP-producing tumors [25], and uncertainty whether to apply static or dynamic AFP values in the context of neoadjuvant tumor treatments [6, 45, 46] seem to limit its predictive capabilities. Moreover, AFP level increase may be related to inflammatory liver damage and not exclusively reflecting tumor progression [47, 48].

There is an increasing body of evidence that cancer biology is not only determined by tumor invasiveness but may be significantly affected by systemic pro-inflammatory and immunologic response reactions [26–28]. C-reactive protein is an acute-phase reactant that is primarily synthesized in hepatocytes in response to interleukin (IL)-1 and IL-6. Apart from its role as a universal inflammatory marker, it gained a growing clinical importance for describing biological cancer behavior [31, 49]. Increased pretreatment serum CRP values were demonstrated to correlate with outcome following non-surgical and surgical treatment of HCC [32–35]. In the transplant setting, CRP was recently suggested to correlate with risk of HCC recurrence, particularly in beyond MC stages [36–39].

Table 6 Pretransplant available predictors of MVI

Variable	*p* value	OR (95% CI)	*p* value
Male gender	0.407		
Age recipients' > 60y	0.082		
Viral liver disease	0.685		
No TACE prior LT	0.250		
(Lab.)MELD > 15	0.521		
Child B/C cirrhosis	0.247		
AFP > 100 ng/ml	0.01	2.39 (1.005–5.667)	0.049
CRP > 0.8 mg/dl	< 0.001	6.82 (2.861–16.255)	< 0.001
Multiple HCC nodules[a]	0.465		
Maximum tumor nodule size > 5 cm[a]	0.129		
Total tumor diameter > 10 cm[a]	0.185		
Number HCC nodules > 3[a]	0.396		
Milan Out[a]	0.020		

AFP alpha-fetoprotein; *CRP* C-reactive protein; *HCC* hepatocellular carcinoma; *LT* liver transplantation; *MELD* Model for End-Stage Liver Disease; *OR* odds ratio; *TACE* transarterial chemoembolization

[a]According to pretransplant radiographic staging

Apart from serum AFP, CRP emerged as the only pretransplant available independent predictor of MVI (Table 6) and HCC recurrence (Table 2), whereas it almost reached statistical significance for OS (Table 3) in our analysis. Furthermore, both tumor markers were able to stratify the oncological risk in patients meeting and exceeding the MC (Table 5). We, therefore, supposed that combining them in a pre-LT serological risk index might improve selection process independently from tumor size data. In fact, we identified STA index as most powerful predictor of MVI (OR 15.31) and HCC relapse (OR 54.44) in our study.

In the past, several expanded selection criteria including AFP have been proposed. In a series of 166 LT patients, Toso et al. [50] reported on a 20% increase of transplant eligibility when combining AFP values with total tumor volume (≤ 115 cm^3). Lai et al. [51] demonstrated an 22.2% increase when complementing total tumor diameter (≤ 8 cm) with AFP. The Hangzhou selection criteria even allow LT in patients without strict tumor burden limit when poorly differentiated tumors were excluded and AFP level was less or equal to 400 ng/ml. However, pre-LT tumor biopsy is required, which makes it not appropriate for our needs [52].

In our series, augmenting the MC with STA index increased the number of appropriate liver recipients from 69 (Milan In) to 101 (Milan In + Milan Out with low/intermediate STA; rise of 44.9%), without promoting tumor relapse risk (12.9% vs. 13%; Fig. 3c; Table 5).

When applying a purely tumor biology-based risk stratification, increased transplant eligibility (36.2%) might have been accompanied by an even lower oncological risk (8.5%; Fig. 2a; Table 5). According to our results, it seems to be the specific capability of STA index to identify a small subgroup with high-grade oncological risk, which consequently increases the transplant benefit for the remaining patients (Fig. 2a).

These data should be validated in a prospective multicenter approach. In particular, the oncological significance of intermediate STA index (pre-LT increase of either AFP or CRP level) requires further investigation, as tumor recurrence rate was significantly higher (14% vs. 2.3%) and OS tended to be lower (Fig. 2a) in this subset when compared to low STA recipients. Interestingly, both subgroups did not significantly differ regarding features of tumor invasiveness (Table 4), which suggests another biological impact of STA beyond link to histopathologic aggressiveness.

Our study has several limitations. First, it is a retrospective observational trial with all the inherent flaws of such a study design. Second, impact of STA index on patients' risk of dropout was not analyzed in this study. Apart from that, we have used final pre-LT available AFP and CRP values without considering level dynamics during waiting time. And moreover, only patients without tumor-specific symptoms were accepted for LT in this series, which probably preselected biologically favorable tumors.

We conclude that pretransplant serum AFP and CRP levels should together be incorporated into the transplant selection algorithm in HCC patients. In particular, patients with elevation of both serological biomarkers are at an inacceptable oncological risk, regardless of radiologic tumor size. This easy pre-LT available serological risk index seems to optimize individual decision making, since it is highly predictive for MVI and poor outcome. Oncologically futile LT may, thereby, be avoided, and the pool of eligible liver recipients significantly expanded.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest related to the article.

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