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CLINICAL INVESTIGATION INTERVENTIONAL ONCOLOGY

Retrospective Evaluation and Significance of Neutrophil-to-Lymphocyte Ratio Prior to and 1 month Following Microwave Ablation of Hepatocellular Carcinoma

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Abstract

Purpose Neutrophil-to-lymphocyte ratio (NLR) recently demonstrated predictive value for hepatocellular carcinoma (HCC) recurrence after thermal ablation. Microwave ablation (MWA) has been shown to induce changes in the immune landscape after HCC treatment. This study aims at identifying predictors of local tumor progression (LTP) and post-treatment NLR kinetics after MWA.

Materials and Methods Data from 108 consecutive patients who underwent percutaneous MWA of 119 HCCs with a 2450 Hz/100 W generator in two institutions from October 2014 to September 2021 were retrospectively reviewed. Forty-five HCCs (42 patients) met inclusion criteria for analysis (technique efficacy, pre- and post-treatment NLR availability, follow-up > 6 months, absence of complications). NLR was analyzed prior to therapy and at 1-month follow-up; difference between the two time points was defined as ΔNLR_{1stFU}.

Results After a median follow-up of 25 months, LTP occurred in 18 HCCs (40%) and 18 patients (42.9%). Multivariate competing risk regression comprising $\Delta NLR_{1stFU} > 0$, cirrhosis etiology and subcapsular location showed that the only independent predictor of LTP was $\Delta NLR_{1stFU} > 0$, on both a per-patient (HR = 2.7, $p = 0.049$) and per-tumor (HR = 2.8, $p = 0.047$) analysis. $\Delta NLR_{1stFU} > 0$ occurred in 24/42 patients (57.1%). In this subgroup, higher rates of female patients ($p = 0.026$), higher mean baseline NLR ($p < 0.0001$) and lower mean energy/size ($p = 0.006$) were observed. Upon ROC curve analysis, energy/size < 1414 J/mm predicted $\Delta NLR_{1stFU} > 0$ with 76% sensitivity and 70% specificity (AUC = 0.74).

Conclusion NLR increase after ablation was the only independent predictor of LTP, supporting the role of balance between systemic inflammation and immunity in recurrence after MWA. Ablation energy/tumor size predicted NLR increase, reinforcing the concept of immune ablation.

Level of Evidence III.

Keywords Hepatocellular carcinoma · Thermal ablation · Microwave · Inflammation · NLR · Adaptive immune response

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver tumor and represents the sixth most common cancer worldwide [1, 2].

Thermal ablation is recognized as a treatment option for very early and early-stage HCC, along with surgery and liver transplantation. In this regard, radiofrequency ablation (RFA) is the most extensively studied, with proven advantage over liver resection in terms of complications and length of stay [3]. However, concern still exists given the relatively high recurrence rate compared with surgical resection [4]. Known independent risk factors for local tumor progression (LTP), i.e., disease recurrence occurring at the margins of the ablation zone, are insufficient ablation margins and heat sink effect of contiguous vessels [5, 6], while subcapsular location has a debated influence on local recurrence given the non-univocal literature results [7, 8]. On top of that, patient baseline inflammatory status, easily obtained by measurement of neutrophil-to-lymphocyte ratio (NLR), has recently shown to have an important predictive value for recurrence after RFA [9].

Microwave ablation (MWA) offers the possibility to achieve higher temperatures in shorter time and has lower sensitivity to heat sink effect compared with RFA. These theoretical advantages have been recently confirmed on large populations showing a better tumor control on perivascular [10] and > 2.5 cm HCCs [11].

Recent studies have shown added value by locoregional therapies in triggering tumor-specific immune response by promoting oncolysis, wherein dying cells are left in place and antigens are recognized, triggering the immune cascade [12, 13]. In particular, cell death occurring after RFA was shown to activate innate and adaptive immunity [14, 15].

Little evidence exists on how the dynamics of systemic inflammatory markers after MWA influence local tumor progression, as well as which MWA parameters may influence systemic inflammation in the clinical setting.

The primary aim of the study was to investigate value of circulating pre- and post-treatment inflammatory markers in predicting local tumor progression after percutaneous MWA.

The secondary aim of the study was to identify which MWA technical features may influence inflammatory markers kinetics.

Materials and Methods

Study Design

A retrospective analysis was performed on 109 consecutive patients with HCC who underwent percutaneous MWA in two institutions between October 2014 and August 2021. The population under study had to fulfill the following inclusion criteria: biopsy-proven or imaging-proven HCC (preoperative dynamic contrast-enhanced CT or MRI with a liver-specific acquisition protocol meeting diagnostic criteria for probable/definite HCC according to Liver Imaging Reporting and Data System version 2018 [16]); Barcelona clinic liver cancer (BCLC) disease stage 0, A, B deemed amenable of curative treatment; preoperative imaging acquired within 30 days from ablation. Exclusion criteria were occurrence of complications, disease persistence at 1 month, follow-up < 6 months, unavailability of preoperative or 1 month post-ablation NLR. A complete exclusion flow diagram is provided in Fig. 1.

Ablation Technique

Ablation was free-hand ultrasound-guided under deep sedation. All ablations were performed by experienced IRs (> 100 ablation procedures) using a 2450 MHz/100 W microwave generator (Emprint, Medtronic). All ablations were performed at 100 W; ablation time was tailored to tumor size according to manufacturer instructions (Instructions for Use, Emprint^{TM} percutaneous Antenna with Thermosphere^{TM} technology, Ablation Zone Charts. R0065469).

Follow-up

Institutional follow-up defines CT/MRI using a liver-specific acquisition protocol 1 month after the procedure, then every 3 months for the first year and every 6 months thereafter. Standardized terminology and reporting criteria for tumor ablation were used to determine ablation endpoints according to Ahmed et Al. [17]. LTP was defined as appearance of foci of vital disease at the margins of the ablation zone at any of the follow-up time points.

Data Considered

For each patient, data regarding gender, age, history of previous HCC and liver interventions, Child–Pugh Class, etiology of cirrhosis, BCLC stage, number of lesions and all imaging (pre- and post-procedure) were collected. For each tumor, data regarding tumor size, location, imaging characteristics, amount of delivered energy over tumor size (J/mm), technique efficacy and LTP were collected. Subcapsular location was defined as ≤ 1 mm distance between tumor margin and liver capsule; proximity to high-risk areas was defined as < 5 mm distance between tumor margin and heart, lungs, gastrointestinal tract, gallbladder, first and second portal branches, base of hepatic veins, inferior vena cava or right kidney [18]. Tumor characteristics according to Liver Imaging Reporting and Data System (LI-RADS) version 2018 criteria were also collected for each tumor, including arterial/portal enhancement pattern, presence of capsule and threshold growth,

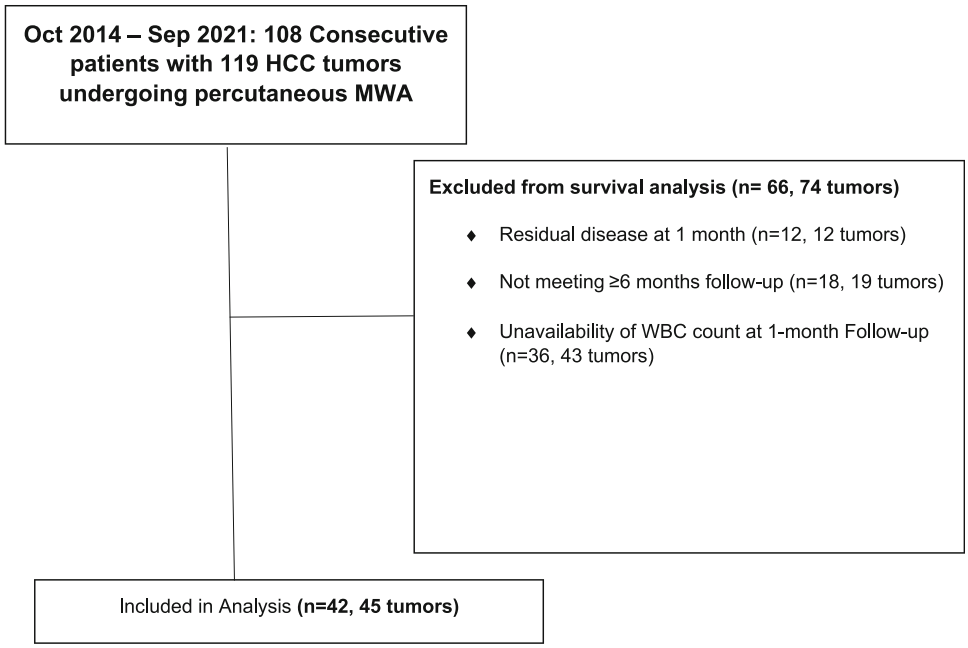

Fig. 1 Flowchart diagram for patient selection

i.e., $\geq$ 50% tumor diameter increase in $\leq$ 6 months [16]. Actual tumor size was re-determined on the day of procedure by real-time US. Minimal ablation margins were evaluated on the three orthogonal planes on 1-month contrast-enhanced imaging follow-up, as previously described [19]. Peripheral blood samples with complete white blood cell count collected upon hospital admission and at 1-month follow-up were reviewed to evaluate neutrophil count, lymphocyte count and NLR (obtained dividing the absolute neutrophil count by the absolute lymphocyte count). Difference in NLR between the first follow-up visit and preoperative period was calculated and indicated as ΔNLR_{1stFU}.

Statistical Analysis

Continuous variables were calculated as mean and standard deviation (SD) and categorical variables as frequencies. Statistical analyses were performed using R Statistical Software (version 4.0.4; R Foundation for Statistical Computing, Vienna, Austria); categorical variables were compared through Chi-square analysis; continuous variables were compared through Mann–Whitney U-test. LTP-free survival (LTPFS) was analyzed per tumor and per patient with univariate and multivariate competing risk regression analysis. In accordance with recent international consensus guidelines [20], death occurring before local progression was considered a competing risk. Hazard ratios (HR) and 95% confidence intervals (95% CI) were calculated. The significance level for all parameters was set at $p \leq 0.05$.

Results

After exclusion, a study cohort consisting of 42 patients with 45 HCC tumors was identified. Two patients received ablation of more than one tumor in a single session, of whom one received treatment of three nodules and one received treatment of two nodules. Mean age of the patient cohort was 73.1 $\pm$ 8.4 years. Thirty-two patients were male (76.2%), whereas 10 patients were female (23.8%). Only three patients (7.1%) presented with a Child–Pugh stage B. Etiology of cirrhosis had a viral component in 24 patients (57.1%), of whom eight patients were infected by hepatitis B (19%) and 16 patients by hepatitis C (38.1%). Eighteen patients had non-viral etiology of cirrhosis (42.9%), of whom 10 had history of heavy alcohol consumption (23.8%), whereas eight had no obvious causes and were thus classified to have idiopathic cirrhosis (19%). One patient (2.4%) had both viral hepatitis and history of heavy alcohol consumption and was thus classified to have a mixed cirrhosis.

Twenty-four patients (57.1%) had received other treatments for HCC prior to ablation, of whom 10 received TACE (23.8%), 3 thermal ablation (7.14%) and 8 surgical resection (19%). Three patients (7.1%) had received both resection and ablation. Mean tumor size was 18.2 $\pm$ 6 mm. Other patient and tumor-related baseline characteristics are summarized in Tables 1 and 2, respectively.

Local Tumor Progression

After a median follow-up of 25 months (9–74), LTP occurred in 18 tumors (40%), with a mean time to LTP of

15 months. 6-, 12-, 18- and 24-month LTP-free survival rates were 91.1%, 77.5%, 69.9% and 63.2%, respectively.

Per-tumor Analysis

At univariate per-tumor competing risks analysis (Table 3), risk factors for LTP included $\Delta NLR_{1stFU} > 0$, i.e., increase in NLR 1 month after ablation (HR = 2.72 $p = 0.044$, Fig. 2).

The amount of delivered ablation energy over tumor size (J/mm) was not associated with LTPFS (HR = 1, $p = 0.34$). Minimal ablation margins < 5 mm were observed in 14/45 HCCs (31.1%) and were not associated with LTPFS (HR = 1.72, $p = 0.26$). In a multivariate model comprising $\Delta NLR_{1stFU} > 0$, etiology of cirrhosis and subcapsular location, the only independent predictor of LTPFS was $\Delta NLR_{1stFU} > 0$ (HR = 2.7, $p = 0.049$).

Per-patient Analysis

LTP eventually occurred in 13/24 patients showing NLR increase (54.2%) and 5/18 HCCs with decreased/stable NLR (27.8%). Per-patient analysis showed shorter mean time to LTP in patients with increased NLR after ablation (NLR increase: mean time to LTP 18.6 months; NLR stable/decreased: mean time to LTP 26 months, Fig. 2b). 6-month to 2-year cumulative LTPFS was lower in NLR increasers (6-month LTPFS: 83.3% vs. 100%; 1-year LTPFS 61.6% vs. 94.4%; 2-year LTPFS 46.9% vs. 78.7%); LTPFS at 3 years was similar in the two groups (46.9% vs. 43.7%).

Table 1 Patient baseline characteristics, total and divided by subgroups based on NLR change at 1 month

Patient variables	Total ($n = 42$)	Group A ($n = 24$)	Group B ($n = 18$)	p
Male	32 (76.2%)	15 (62.5%)	17 (94.4%)	0.026
Female	10 (23.8%)	9 (37.5%)	1 (5.6%)	
Age (years)	73.1 ± 8.4 (53–87)	72.4 ± 9.2 (53–84)	73.9 ± 7.3 (60–87)	0.98
Aetiology of cirrhosis				
Viral component	24 (57.1%)	14 (58.3%)	10 (55.6%)	0.55
Absence viral component	18 (42.9%)	10 (41.7%)	8 (44.4%)	
Hep B	8 (19%)	5 (20.8%)	3 (16.7%)	0.75
Hep C	16 (38%)	9 (37.5%)	7 (38.9%)	0.92
Alcoholic	10 (23.8%)	6 (25%)	4 (22.2%)	0.57
Mixed	1 (2.4%)	0 (0%)	1 (5.6%)	
Idiopathic	8 (19%)	4 (16.7%)	4 (22.2%)	
Child–Pugh class				
A	39 (92.9%)	21 (87.5%)	18 (100%)	0.25
B	3 (7.1%)	3 (12.5%)	0 (0%)	
BCLC stage				
0	11 (26.2%)	5 (20.8%)	6 (33.3%)	0.46
A1	19 (45.2%)	13 (54.2%)	6 (33.3%)	
A2	2 (4.8%)	1 (4.2%)	1 (5.6%)	
A3	2 (4.8%)	2 (8.4%)	0 (0%)	
A4	7 (16.7%)	3 (12.5%)	4 (22.2%)	
B	1 (2.4%)	0 (0%)	1 (5.6%)	
Multifocal disease	9 (21.4%)	4 (16.7%)	5 (27.8%)	0.46
Previous therapy for HCC	24 (57.1%)	13 (54.2%)	11 (61.1%)	0.76
Previous TACE	8 (19%)	4 (16.7%)	4 (22.2%)	0.26
Previous resection	10 (23.8%)	4 (16.7%)	6 (33.3%)	
Previous thermal ablation	4 (9.5%)	3 (12.5%)	1 (5.6%)	
Resection + ablation	2 (4.8%)	2 (8.4%)	0 (0%)	
NLR (baseline)	3.8 ± 3.5(1.3–24)	2.6 ± 0.94 (1.3–4.7)	5.4 ± 4.9 (2.12 – 24)	< 0.00001
NLR (first follow-up)	3.7 ± 2.1 (1.3–14)	3.95 ± 2.5 (1.5–14)	3.5 ± 1.5 (1.3–6)	0.83
ΔNLR_{1stFU}	− 0.5 ± 3.6 (− 18.4–11.7)	1.3 ± 2.3 (0.17–11.7)	-1.9 ± 4.2 (-18.4–0)	< 0.00001

BCLC BarcelonaClinic Liver Cancer, HCC Hepatocellular Carcinoma, NLR Neutrophil-to-lymphocyte ratio.

Table 2 Tumor baseline characteristics

Tumor variables	Total (N = 45)	Group A (n = 25)	Group B (n = 20)	p
Size (mm)	18.2 ± 6.1 (9–35)	18.4 ± 5.5 (9–31)	18 ± 6.9 (9–35)	0.51
Amount of delivered energy (J/mm)	1526.2 ± 612.5 (639–3529.4)	1308.5 ± 495.7 (639–2700)	1798.5 ± 646.5 (900–3529)	0.006
Subcapsular location	19 (42.2%)	14 (56%)	5 (25%)	0.07
Proximity to high-risk areas	27 (60%)	10 (40%)	7 (35%)	0.77
Proximity to vessels > 3 mm	7 (15.6%)	3 (12%)	4 (20%)	0.68
Non-rim arterial phase hyper-enhancement (APHE)	41 (91.1%)	21 (84%)	20 (100%)	0.12
Enhancing "capsule"	16 (35.6%)	7 (28%)	9 (45%)	0.24
Non-peripheral "washout"	39 (86.7%)	22 (88%)	17 (85%)	0.77
Threshold growth	8 (17.8%)	7 (28%)	1 (5%)	0.06
LI-RADS classification				
LI-RADS 3	3 (6.7%)	2 (8%)	1 (5%)	0.35
LI-RADS 4	3 (6.7%)	2 (8%)	1 (5%)	
LI-RADS 5	39 (86.6%)	21 (84%)	18 (90%)	
Minimal ablation margin < 5 mm	14 (31.1%)	7 (28%)	7 (35%)	0.75

T total and divided by subgroups based on NLR change at one month, *LI-RADS* Liver Imaging Reporting and Data System

Table 3 Results of per-tumor univariate and multivariate competing risk analysis on local tumor progression-free survival

Variable	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Gender (male)	1.58	0.5–4.96	0.43			
Age (years)	0.97	0.92–1.03	0.36			
Non-viral cirrhosis	2.33	0.94–5.79	0.068	2.1	0.83–5.37	0.12
Multifocal disease	0.7	0.225–2.21	0.55			
Previous therapy for HCC	0.6	0.25–1.46	0.27			
NLR (baseline)	0.99	0.9–1.02	0.15			
NLR (first follow-up)	0.996	0.94–1.03	0.12			
ΔNLR_{1stFU}	1.12	1.03–1.44	0.13			
NLR increase (first follow-up)	2.72	1.03–7.19	0.044	2.7	1.004–7.18	0.049
Size (mm)	0.99	0.94–1.06	0.89			
Amount of delivered energy (J/mm)	1	0.998–1.001	0.34			
Subcapsular location	2.15	0.87– 5.31	0.098	1.4	0.59–3.4	0.45
Proximity to high-risk areas	0.8	0.318–2.02	0.64			
Proximity to vessels > 3 mm	0.485	0.15–1.57	0.23			
Minimal ablation margin < 5 mm	1.72	0.676–4.36	0.26			
Non-rim arterial phase hyper-enhancement (APHE)	0.7	0.178–2.63	0.58			
Enhancing "capsule"	0.51	0.179–1.47	0.22			
Non-peripheral "washout"	0.7	0.18–2.77	0.6			
Threshold growth	0.67	0.18–2.47	0.55			
Li-RADS 5	0.79	0.22–2.8	0.7			

HCC Hepatocellular Carcinoma, *NLR* Neutrophil-to-lymphocyte ratio, *LI-RADS* Liver Imaging Reporting and Data System

Per-patient univariate competing risk analysis (Table 4) showed that risk factors for LTP were ΔNLR_{1stFU} (HR = 1.02, p = 0.038) and $\Delta NLR_{1stFU} > 0$, i.e., increase in NLR 1 month after ablation (HR = 2.5 p = 0.05, Fig. 2b). A multivariate model confirmed the results of the per-patient analysis, with $\Delta NLR_{1stFU} > 0$ as the only independent predictor (HR = 2.8, p = 0.047, Table 4).

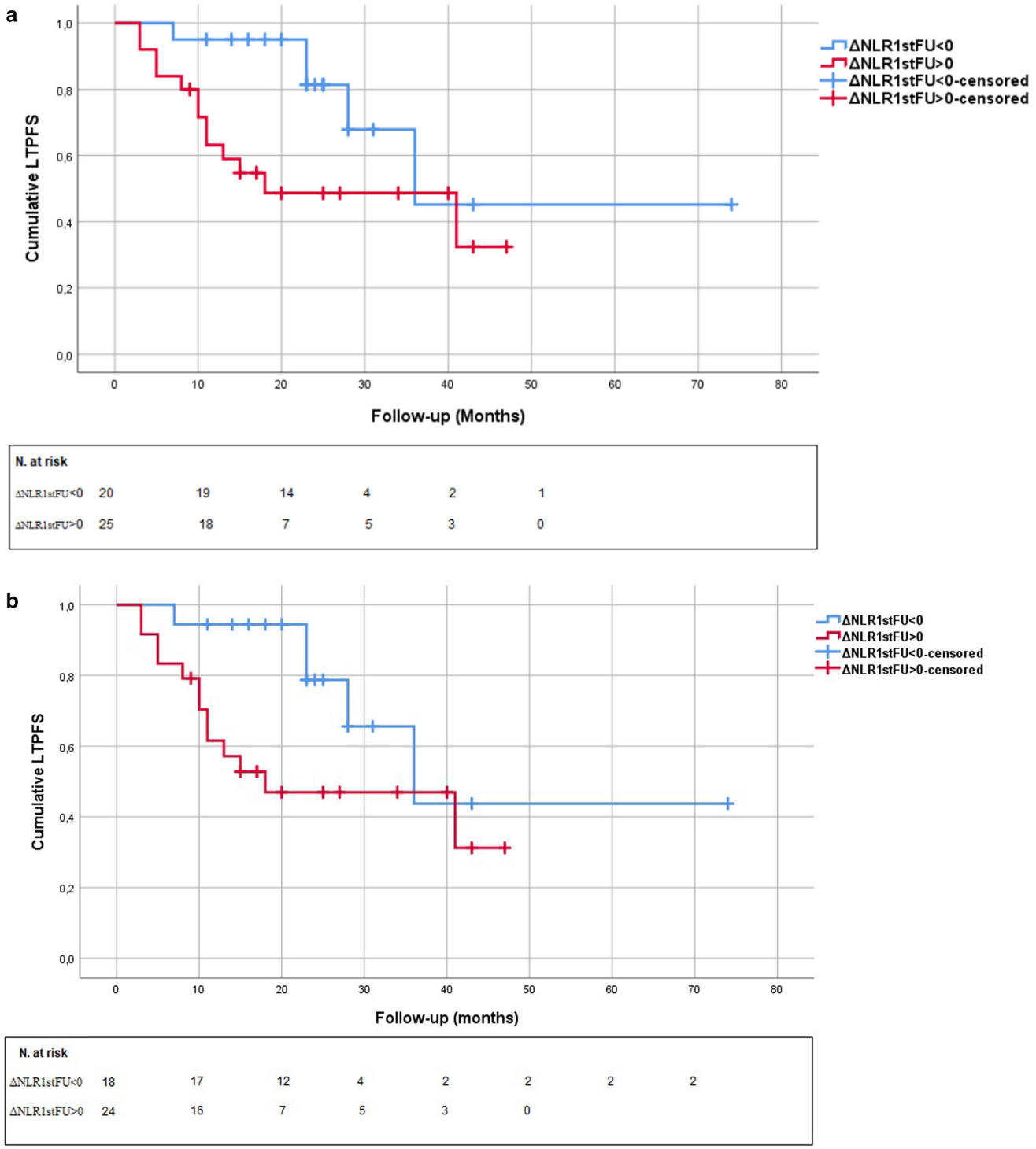

Fig. 2 **a** Kaplan–Meyer curve for LTPFS per tumor according to NLR change at 1 month and **b** Kaplan–Meyer curve for LTPFS per patient according to NLR change at 1 month

Factors Associated with NLR Increase

Baseline NLR was 3.8 ± 3.5, whereas NLR at 1 month was 3.7 ± 2.1 (Fig. 3). NLR increase at 1 month occurred in 24 out of 42 patients (57.1%).

Based on NLR change observed 1 month after ablation, the study population was divided into group A (NLR increase, $n = 24$) and group B (NLR decrease, $n = 18$). Group A showed higher rates of female patients [Group A: 9 (37.5%) vs. Group B: 1 (5.6%), $p = 0.026$], lower mean

baseline NLR (Group A: 2.6 ± 0.94 vs. Group B: 5.4 ± 4.9, $p < 0.0001$) and higher ΔNLR_{1stFU} (Group A: 1.3 ± 2.3 vs. Group B: − 1.9 ± 4.2, $p < 0.0001$). Other patient-related variables were homogeneous among the two groups, notably NLR at 1 month (Group A: 3.95 ± 2.5 vs. Group B, 3.5 ± 1.5 $p = 0.83$) and non-viral etiology of cirrhosis [Group A: 10 (41.7%) vs. Group B: 8 (44.4%), $p = 0.55$]. Regarding the characteristics of the HCC tumors in the two subgroups (Table 2), mean delivered energy over size was significantly lower in group A (Group A: 1308.5 ± 495.7 J/mm vs. Group B: 1798.5 ± 646.5 J/mm, $p = 0.008$). Minimal ablation margins < 5 mm were homogeneous among the two groups [Group A: 7 (28%) vs. Group B: 7 (35%), $p = 0.75$]. Per-patient and per-tumor characteristics of the two subgroups are summarized in Tables 1 and 2, respectively.

Upon ROC curve analysis (Figs. 4 and 5), ablation energy < 1414 J/mm allowed prediction of NLR increase 1 month after ablation with 76% sensitivity and 70% specificity (AUC = 0.74).

Discussion

In our study cohort, neutrophil-to-lymphocyte ratio increase occurring 1 month after microwave ablation was the only independent predictive factor for local tumor progression. No confounding factors were identified at baseline and univariate analysis; notably, even though lower microwave ablation energy was able to predict NLR increase, no effect was exerted by energy alone on LTPFS. Factors traditionally associated with local tumor progression after thermal ablation, such as perivascular and subcapsular location [5, 6], were not predictive of local recurrence. Furthermore, lower energy levels were able to predict NLR increase at 1-month follow-up.

White blood cell counts (neutrophil, lymphocyte, monocyte, platelet, globulin) are a readily available surrogate marker of the systemic inflammatory response, and several studies have demonstrated the role of the NLR (Neutrophil–lymphocyte ratio) in predicting the prognosis in solid tumors [21]. In recent studies, NLR has proven to be a convenient, repeatable and reliable prognostic tool in patients with HCC treated by either curative or palliative methods [22, 23]. In particular, preoperative NLR and platelet-to-lymphocyte ratio (PLR) have recently been

Table 4 Results of per-patient univariate and multivariate competing risk analysis on local tumor progression-free survival

Variable	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Gender (male)	1.67	0.532–5.2	0.38			
Age (years)	0.98	0.929–1.04	0.49			
Non-viral cirrhosis	2.1	0.85–5.1	0.11	2.21	0.78–6.21	0.13
Multifocal disease	0.86	0.28–2.64	0.79			
Previous therapy for HCC	0.64	0.264–1.55	0.32			
NLR (baseline)	0.9	0.7–1.02	0.32			
NLR (first follow-up)	0.97	0.78–1.22	0.78			
ΔNLR_{1stFU}	1.02	1.01–1.3	0.037			
NLR increase (first follow-up)	2.5	1.001–6.59	0.05	2.8	0.79–6.21	0.047
Size (mm)	0.98	0.955–6.59	0.52			
Amount of delivered energy (J/mm)	1	0.998–1.001	0.36			
Subcapsular location	2.04	0.824–5.07	0.12	1.25	0.48–3.3	0.65
proximity to high-risk areas	0.74	0.293–1.88	0.53			
Proximity to vessels > 3 mm	0.44	0.134–1.47	0.18			
Minimal ablation margin < 5 mm	1.6	0.624–4.09	0.33			
Non-rim arterial phase hyper-enhancement (APHE)	0.65	0.161–2.63	0.55			
Enhancing "capsule"	0.51	0.174–1.47	0.21			
Non-peripheral "washout"	0.68	0.165–2.79	0.59			
Threshold growth	0.68	0.181–2.56	0.57			
Li-RADS 5	0.76	0.204–2.82	0.68			

HCC Hepatocellular Carcinoma, *NLR* Neutrophil-to-lymphocyte ratio, *LI-RADS* Liver Imaging Reporting and Data System

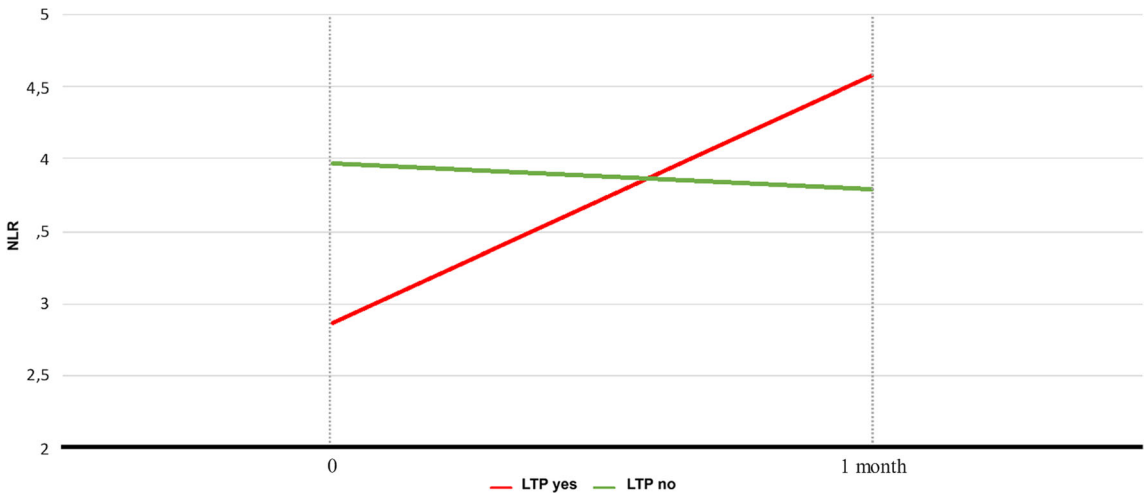

Fig. 3 Line chart representing mean NLR change from baseline to 1-month follow-up in patients with local tumor progression (red line) or not (green line) during the follow-up period. Of note, mean NLR tends to increase in patients with LTP (uphill slope), whereas it tends to decrease in patients without LTP (downhill slope)

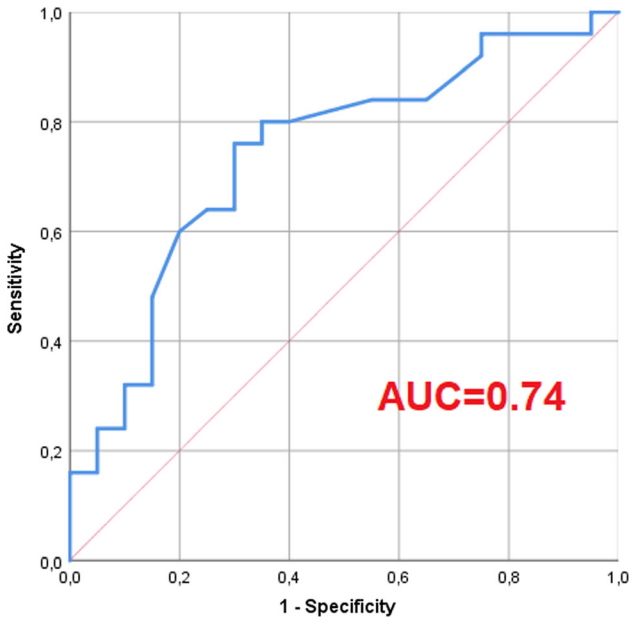

Fig. 4 ROC curve for prediction of NLR increase at 1 month at decreasing levels of ablation energy over size (J/mm)

identified as predictive factors for recurrence after radiofrequency ablation [9].

Several hypotheses have been proposed regarding the molecular mechanisms through which increased NLR may affect tumor recurrence and prognosis [24].

On the one hand, evidence from preclinical studies demonstrated that neutrophilia is able to inhibit the cytolytic activity of immune cells, such as activated *T* cells and natural killer cells [25]; furthermore, clinical studies on HCC patients found that peritumoral and intratumoral neutrophils were associated with angiogenesis progression [26] and exhibited increased autophagic activity [27]. Taken together, all these effects may contribute to influence tumor microenvironment and facilitate tumor progression.

On the other hand, since lymphocytes (particularly $CD8^+$ *T* cells and natural killer cells) are directly involved in anti-tumor immune surveillance [28], relative lymphopenia observed in these patients may further allow tumor growth and progression.

Very interestingly, in our study cohort, post-ablation NLR increase rather than baseline NLR was able to predict local tumor progression-free survival, indicating that a change in systemic inflammation had occurred and might have affected prognosis. A single study in the literature showed that an increase in NLR after RFA predicted OS, without any effect on recurrence-free survival [29].

In our cohort, 18 patients exhibited NLR decrease, whereas 24 patients experienced an increase in NLR 1 month after ablation. In this regard, lower ablation energy over tumor size was associated with NLR increase at 1 month, and values below 1414 J/mm were able to predict NLR increase.

The effect of ablative therapies on immunomodulation and inflammatory status has been the object of several preclinical and clinical studies. In particular, cell death occurring after RFA was shown to activate innate and adaptive immunity [14, 15].

Even though less studied, MWA also has shown the ability to trigger immunogenic cell death, with a significant increase in *T* cells and IL-12 [30]. Furthermore, a study by Leuchte et al. demonstrated clinical benefit in patients mounting tumor-specific *T*-cell responses in peripheral blood after MWA in terms of longer RFS [31].

On the other side, RFA has also been shown to be associated with intrasegmental recurrence or switch to an

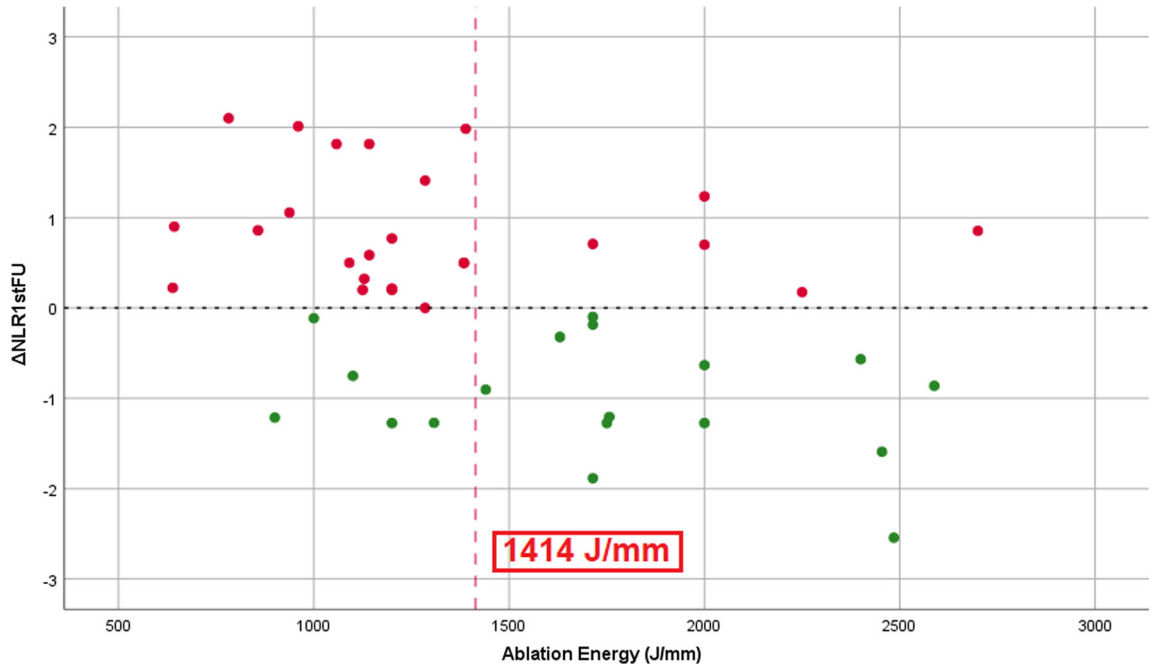

Fig. 5 Scatter plot giving graphical representation of NLR change from baseline to first follow-up as a function of ablation energy over size (J/mm). Dots representing $\Delta NLR_{1stFU} > 0$ are represented in red, whereas dots representing $\Delta NLR_{1stFU} < 0$ are represented in green. Vertical dotted line represents the energy threshold identified on ROC curve analysis for optimal prediction of NLR increase

aggressive tumor phenotype when ablation was partial [32–34]. This detrimental effect has been demonstrated to be caused by the onset of an inflammatory microenvironment in the periablational zone, with IL-6 and neutrophils described as potential contributors [35]. A study on rodent cell lines demonstrated that development of this pro-oncogenic inflammatory milieu was favored by exposure to moderate, non-lethal heating (< 50 °C) of the perilesional area [36].

MWA ability to develop higher temperatures in shorter time translates into more efficient conduction of heat from the central zone to the periphery resulting in an overall larger volume of lethal hyperthermia [37]. These advantages were shown to imply beneficial immunomodulation by Ahmad et al. who described lower serum levels of IL-6 following liver MWA compared with RFA in the rat model [38].

Even though data on the influence of MWA protocol parameters on the immune response are limited, one preclinical study by Velez et al. [39] compared pro-inflammatory signals and extrahepatic tumor growth in mice with breast adenocarcinoma treated with either a slower lower power (5 W for 120 s) MWA, a faster higher-power (20 W 15 s) MWA, RFA or a sham liver ablation; the authors found significant IL-6 elevation and increased extrahepatic tumor growth following 5 W MWA and RFA compared with 20 W MWA, concluding that a faster heating MWA protocol may mitigate the post-ablation pro-oncogenic inflammatory milieu.

In our study, all ablations were performed at high power (100 W), following manufacturers indications to achieve a satisfying ablation zone. Crucial data emerging from this study are that microwave energy/tumor size alone, while being an indicator of ablation zone size according to manufacturer indications, was not shown to influence local tumor progression-free survival. At the same time, minimal ablation margins below 5 mm were evenly distributed in the two study groups, despite the different mean energy delivered, indicating homogeneous tumor coverage. Considering the ability of Thermosphere technology to maintain a constant wavelength allowing a reliable, spherical ablation zone [40], the difference in NLR change in the two subsets of the population could be explained by the development of high lethal temperatures in the periablational area of patients treated for longer time, which hindered the development of pro-oncogenic inflammation while still favoring cell-mediated immune surveillance. With this regard, it is interesting to note that a greater difference in cumulative local tumor progression-free survival was observed in the first 2 years after ablation, eventually reaching similar levels at 3-year follow-up. This is consistent with the time-limited efficacy of the immune surveillance triggered by ablative therapies [37].

Another interesting data emerging from our population are a significant higher risk for females to have an increase

in NLR after the ablation therapy rather than males. Although it must be stressed that the relatively low number of female subjects (n = 10) limits the consistency of these data, one possible explanation could be the higher concentration of 17-beta-estradiol, previously shown to contribute to increase neutrophils in peripheral blood [41] and to enhance the production of pro-inflammatory cytokines (such as IL-1, IL-6 and TNF) when present at low doses [42].

This study has some limitations: firstly, the limited sample size limited the consistency of data regarding LTPFS; secondly, the retrospective nature of the study limited the access to other relevant covariates which were previously proven to be predictive factors of recurrence, such alfa-fetoprotein and albumin–bilirubin grade [43]. Another limitation is the lack of the histopathological specimen of the treated tumors, which may bring valuable information on microvascular invasion and intratumoral immune cell infiltrate, both of which have been shown to correlate with HCC recurrence [44, 45]. The lack of this valuable information, along with the relatively high percentage of patients who had already received other treatments for HCC prior to ablation and patients with non-viral hepatitis, may explain the relatively high occurrence of local tumor progression in our study cohort compared with the literature.

Conclusion

Despite the limitations, given the easy availability of the inflammatory markers utilized, the results of this study may have an impact on clinical practice. Firstly, the ablation protocol might be tailored toward higher energies not only according to size, but also depending on patient inflammatory status and gender. Secondly, post-ablation NLR change assessment might aid in choosing a stricter follow-up schedule or in guiding patient selection for the selection of adjuvant therapy.

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Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

Consent to Publish Patients also signed informed consent regarding publishing their data and diagnostic images.

Ethical Standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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