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HIF-1 α and VEGF as prognostic biomarkers in hepatocellular carcinoma patients treated with transarterial chemoembolization

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ABSTRACT

Background: Neoangiogenesis plays a crucial role in the progression of hepatocellular carcinoma (HCC), and concerns have been raised about the role of neoangiogenesis on the effectiveness of transarterial chemoembolization (TACE).

Aim: In this study, we aimed to evaluate Vascular Endothelial Growth Factor (VEGF) and Hypoxia-Inducible Factor-1 α (HIF-1 α) as circulating prognostic biomarkers in HCC patients treated with TACE.

Methods: Blood samples were collected from 163 patients before (t_0) and four weeks after TACE (t_1).

Results: Higher levels of VEGF after TACE were demonstrated (264.0 [78.7–450.8] vs. 278.6 [95.0–576.6] pg/mL; $p < 0.0001$). Responders to TACE had lower levels of VEGF than non-responders both at t_0 (200.0 [58.9–415.8] vs. 406.6 [181.4–558.6] pg/mL; $p = 0.006$) and at t_1 (257.3 [68.5–528.6] vs. 425.9 [245.2–808.3] pg/mL; $p = 0.003$), and in both groups there was an increase in VEGF compared to measurements before treatment ($p = 0.001$ and $p = 0.005$, respectively). VEGF was not associated with overall survival (OS), while patients with HIF-1 $\alpha \leq 0.49$ ng/mL showed better prognosis (median OS 28.0 months [95% CI 19.7–36.3] vs. 17.0 months [95% CI 11.1–22.9]; $p = 0.01$). Moreover, HIF-1 α was identified as an independent prognostic parameter.

Conclusions: VEGF and HIF-1 α can be considered useful prognostic biomarkers in HCC patients treated with TACE.

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1. Introduction

Angiogenesis is one of the hallmarks of cancer [1] and plays a crucial role in the development and progression of hepatocellular carcinoma (HCC) [2,3].

HCC displays a high level of neoangiogenic activity during its growth, leading to a unique vascular derangement, as the tumor tends to be primarily fed by arterial inflow, unlike the surrounding liver tissue that receives the majority of blood supply through the portal system [4]. However, primary liver tumors have significant vascular abnormalities, such as vessels covered by smooth muscle cells and/or capillaries without fenestration and with laminin base-

ment membrane deposition [2]. As a result of the abnormal blood flow, despite being a highly angiogenic cancer, HCC seems to be characterized by hypoxia [2]. Hypoxia can promote tumor growth and progression and resistance to therapies [5]. In fact, it has been shown that overactivation of angiogenesis in HCC is associated with a worse prognosis. A transcriptomic signature of five genes involved in the angiogenic process (ANGPT2, NETO2, ESM1, NR4A1, DLL4) was found to accurately identify rapidly growing tumors and was associated with shorter survival [6]. Additionally, several studies have shown that overexpression of Vascular Endothelial Growth Factor (VEGF) and its transcription factor Hypoxia-Inducible Factor (HIF)-1 α , the two key mediators of angiogenesis, is a negative prognostic factor, particularly in patients treated with surgery and systemic therapies [7–18].

Neoangiogenesis activation has been called into question when the issue of resistance to transarterial treatments is considered. Indeed, the release of angiogenic factors, prompted by ischemia

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caused by embolization, may be one of the reasons for the high rates of recurrence after transarterial chemoembolization (TACE) [3,19]. This treatment relies on both local intra-arterial administration of a chemotherapeutic agent and embolization of tumor feeding arteries. Ischemia, hypoxia, and necrosis caused by embolization can stimulate neo-angiogenesis. However, due to structural and functional defects, newly formed tumor blood vessels may further aggravate hypoxia and thereby form a vicious cycle leading to tumor recurrence and metastasis [20]. As a result, it may be hypothesized that HIF-1 α and VEGF could represent valuable biomarkers in identifying patients with poor response to TACE and shorter overall survival (OS). Some studies have already suggested that high VEGF levels are associated with less effective treatment and poorer prognosis, while few data are available for circulating HIF-1 α in this setting [21,22].

In this study we aimed to evaluate, in a group of HCC patients treated with TACE, the ability to predict response to treatment and the prognostic accuracy of the two most important molecules involved in angiogenesis, HIF-1 α and VEGF.

2. Materials and methods

2.1. Study population

Blood samples collected from 163 patients with HCC consecutively admitted to the Gastroenterology Unit of Padova University Hospital for TACE treatment from January 2012 to December 2020 were evaluated. Each subject provided written informed consent to participate to the study, which was conducted in accordance to the Declaration of Helsinki and was approved by the Ethics Committee of the Padova University Hospital.

Presence of HCC was defined according to guidelines available at the time of the diagnosis. HCC was histologically confirmed in 53 patients (32.5%), while in the remaining cases the diagnosis was based on the typical features at imaging (computed tomography [CT] or magnetic resonance imaging [MRI]) [23].

TACE was performed, after super selective catheterization of the tumor-feeding artery, either administering a mixture of chemotherapeutic drug and Lipiodol followed by embolization (conventional TACE) or drug-eluting beads loaded with doxorubicin (DEB-TACE). In all patients, two blood samples were collected: the first immediately before TACE procedure (t_0) and the second after four weeks, at the time of the control imaging (abdomen CT scan or MRI) routinely performed to evaluate the efficacy of treatment (t_1).

The following clinical and tumoral parameters were recorded: sex, age, etiology of the underlying liver disease, main serological parameters (total bilirubin, INR, albumin and AFP, this latter both at t_0 and at t_1) and Child-Pugh class. In addition, tumor characteristics such as number and size of liver lesions, evaluated before TACE with dynamic CT or MRI, were recorded. Patients were staged according to the Barcelona Clinic Liver Cancer (BCLC) system [24]. Baseline vascularization ("low" vs. "high") was arbitrarily defined by an expert radiologist who judged the contrast enhanced CT scan performed as a staging procedure before TACE and the angiographic phase of the TACE procedure. The efficacy of TACE was evaluated with dynamic CT or MRI performed four weeks after the treatment and the modified Response Evaluation Criteria In Solid Tumors (mRECIST) were used to classify the radiological response in complete (CR), partial (PR), stable disease (SD), or progressive disease (PD) [25]. Adverse events to TACE were also registered.

2.2. HIF-1 α and VEGF assay

Ten millilitres of venous blood were collected from each patient, 5 mL of which were used for serum separation. Samples were preserved at -20°C till the assay of biochemical markers.

VEGF (pg/mL) and HIF-1 α (ng/mL) were determined on serum by using specific ELISA kits (Human VEGF Platinum ELISA, Affymetrix eBioscience; ELISA kit for HIF-1 α , Cloud-Clone Corp.).

2.3. Statistical analysis

Quantitative variables were reported as median and interquartile range (IQR), while categorical variables as absolute frequency and percentage. Mann-Whitney test, Wilcoxon matched-pairs signed rank test, chi-square test or Fischer's exact test were used in the comparison between groups, as appropriate. The Spearman correlation coefficient was calculated in order to establish correlations between quantitative variables. Survivals were expressed as median and 95% confidence interval (CI). Overall survival (OS) was calculated from the date of TACE to the date of death for any reason, last follow-up evaluation, or data censoring (31st December 2021). The prognostic cut-off of the markers was established using the ROC curve method, taking as threshold value the one with maximal sensitivity and specificity (Youden J test). Survival curves were estimated with the Kaplan-Meier method and the difference between curves was assessed by the log-rank test. Multivariate Cox analysis was used to identify independent prognostic predictors, including in the model only variables significantly or borderline ($p \leq 0.1$) associated with survival at univariate analysis. The p value (two-tail) was considered statistically significant when <0.05 . IBM SPSS Statistics (Version 25.0, IBM Corp. Armonk, NY, USA) and GraphPad Prism (version 8.3.1, GraphPad Software, La Jolla, CA, USA) were used for all the calculations in this study.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the study population are summarized in Table 1.

The patients were mostly men (79.8%), with a median age of 69 years (IQR 63–75). Most of them (54.6%) had HCC developing on a viral liver disease, preserved liver function (Child-Pugh A in 79.8%) and an intermediate stage tumor (BCLC B in 51.5%). The median AFP level was 15 ng/mL. c-TACE was performed in 77 patients, while the others received DEB-TACE. The majority of the patients had a partial or complete response to TACE (PR: 47.9%; CR: 30.1%), without adverse events (which occurred in 19.6% of cases).

3.2. VEGF and HIF-1 α levels

Significantly higher levels of VEGF were demonstrated at t_1 (278.6 pg/mL, IQR 95.0–576.6) compared to t_0 (264 pg/mL, IQR 78.7–450.8; $p < 0.0001$) (Fig. 1a). On the contrary, no statistically significant differences were found between HIF-1 α levels at t_0 and t_1 (0.25 ng/mL [IQR 0.11–0.49] vs. 0.23 ng/mL [IQR 0.12–0.38]; $p = 0.37$) (Fig. 1b). A positive correlation was demonstrated between HIF-1 α and VEGF levels at t_0 ($r = 0.47$, 95% CI 0.28–0.62; $p < 0.0001$) and at t_1 ($r = 0.43$, 95% CI 0.23–0.58; $p < 0.0001$) (Fig. 2a and b). In addition, VEGF levels at t_0 were positively correlated with t_1 levels ($r = 0.86$, 95% CI 0.80–0.89; $p < 0.0001$) and HIF-1 α levels at t_0 were positively correlated with HIF-1 α levels at t_1 ($r = 0.75$, 95% CI 0.64–0.83; $p < 0.0001$) (Fig. 2c and d).

VEGF levels at t_0 were associated with the number of liver lesions (Supplementary Figure 1a), with higher values in multifocal (303.0 pg/mL, IQR 91–557.8) compared to monofocal HCC (116.6 pg/mL, IQR 18.7–295.0; $p < 0.0001$), but not with tumor size (Supplementary Figure 1b). Intermediate stage tumors had higher levels of VEGF compared to very early and early stage (308.0 pg/mL [IQR 104.0–586.9] vs. 185.1 pg/mL [IQR 46.7–328.3], respectively; $p = 0.006$) (Supplementary Figure 1c). Although patients with

Table 1
Baseline characteristics.

Variables		Study population (n = 163)
Sex-males		130 (79.8)
Age		69 (63–75)
Etiology	Viral	89 (54.6)
	Not viral	67 (41.1)
	Viral + other	7 (4.3)
Child-Pugh class	A	130 (79.8)
	B	33 (20.2)
Multifocal tumors		116 (71.2)
Size of the largest liver lesion (cm)	<3	76 (46.6)
	3–5	53 (32.5)
	>5	34 (20.9)
AFP (ng/mL)		15.0 (5.5–65.2)
Vascularization grade	Low	84 (51.5)
	High	79 (48.5)
BCLC stage	0-A	79 (48.5)
	B	84 (51.5)
Type of TACE	c-TACE	77 (47.2)
	DEB-TACE	86 (52.8)
Radiological response (mRECIST)	CR	49 (30.1)
	PR	78 (47.9)
	SD	26 (15.9)
	PD	10 (6.1)
Adverse events	No	131 (80.4)
	Post-embolic syndrome	16 (9.8)
	Abdominal pain	11 (6.8)
	Cholecystitis	2 (1.2)
	Liver abscess	1 (0.6)
	Pancreatitis	1 (0.6)
	Liver decompensation	1 (0.6)

Data are presented either as median and interquartile range or number and percentage.

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; c-TACE, conventional transarterial chemoembolization; DEB-TACE, drug-eluting beads transarterial chemoembolization; mRECIST, modified Response Criteria In Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

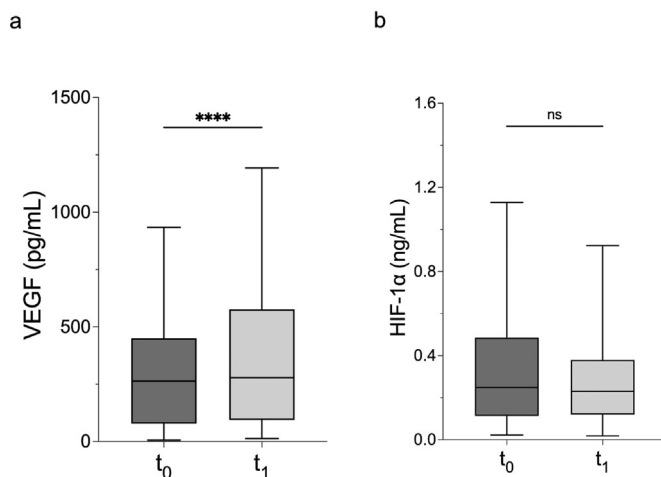


Fig. 1. Comparison of VEGF and HIF-1 α levels before (t_0) and after (t_1) TACE. (a) VEGF levels were significantly higher after TACE compared to the levels measured before the treatment. (b) No significant differences were demonstrated in HIF-1 α levels before and after TACE.

highly vascularized tumors had higher levels of VEGF (297.9 pg/mL, IQR 86.3–472.4) than patients with a low vascularization grade (203.0, IQR 74.6–418.7), the difference was not statistically significant ($p = 0.12$) (Supplementary Figure 1d). VEGF levels were not different according to the other variables considered (sex, age, etiology, Child-Pugh class). None of the variables considered showed a correlation with HIF-1 α (Supplementary Figure 1e–h). Nevertheless, despite not statistically significant, HIF-1 α levels were higher in patients with multifocal tumors, with lesion ≥ 3 cm in diameter and in BCLC B patients.

When patients treated with different TACE techniques were analyzed separately, we found a statistically significant increase in VEGF at t_1 both in patients receiving c-TACE (t_0 level: 91.0 pg/mL [IQR 30.1–344.0] vs. t_1 level: 114.4 pg/mL [IQR 38.0–375.1]; $p = 0.01$) and in patients receiving DEB-TACE (t_0 level: 325.0 pg/mL [IQR 182.8–599.2] vs. t_1 level: 417.5 pg/mL [IQR 250.3–679.0]; $p = 0.0007$). By contrast, no statistically significant differences in HIF-1 α levels were found: it was 0.14 (IQR, 0.07–0.35) at t_0 vs. 0.12 ng/mL (IQR, 0.09–0.23) at t_1 in patients receiving c-TACE ($p = 0.37$), and 0.33 ng/mL (IQR, 0.14–0.52) at t_0 vs. 0.26 ng/mL (IQR, 0.16–0.42) at t_1 in those treated with DEB-TACE ($p = 0.60$) (Supplementary Figure 2).

3.3. Survival analysis

The median OS in the whole patient population was 25.0 months (95% CI 20.6–29.4), with a 3-year survival rate of 33.2%.

With the ROC curve method, the prognostic cut-off for VEGF and HIF-1 α at t_0 able to maximizing sensitivity and specificity was identified at a value of 177 pg/mL and 0.49 pg/mL, respectively.

According to the cut-off established, VEGF was not associated with survival. The median OS for patients with VEGF ≤ 177 pg/mL was 24.0 months (95% CI 18.9–29.1) compared to 23.0 months (95% CI 15.1–30.9) in patients with VEGF >177 pg/mL ($p = 0.34$) (Fig. 3a). On the contrary, at the identified cut-off of 0.49 ng/mL, HIF-1 α measured at t_0 proved to be a useful prognostic predictor. Patients with HIF-1 α levels below the cut-off had a statistically significant longer OS compared to patients with marker levels above the cut-off (median OS 28.0 months [95% CI 19.7–36.3] vs. 17.0 months [95% CI 11.1–22.9], respectively; $p = 0.01$) (Fig. 3b). The prognostic relevance of the biomarkers was also investigated considering the dynamic change of VEGF and HIF-1 α levels after the treatment. In particular, delta-VEGF (Δ VEGF) was arbitrarily defined as positive if there was an increase of the marker level af-

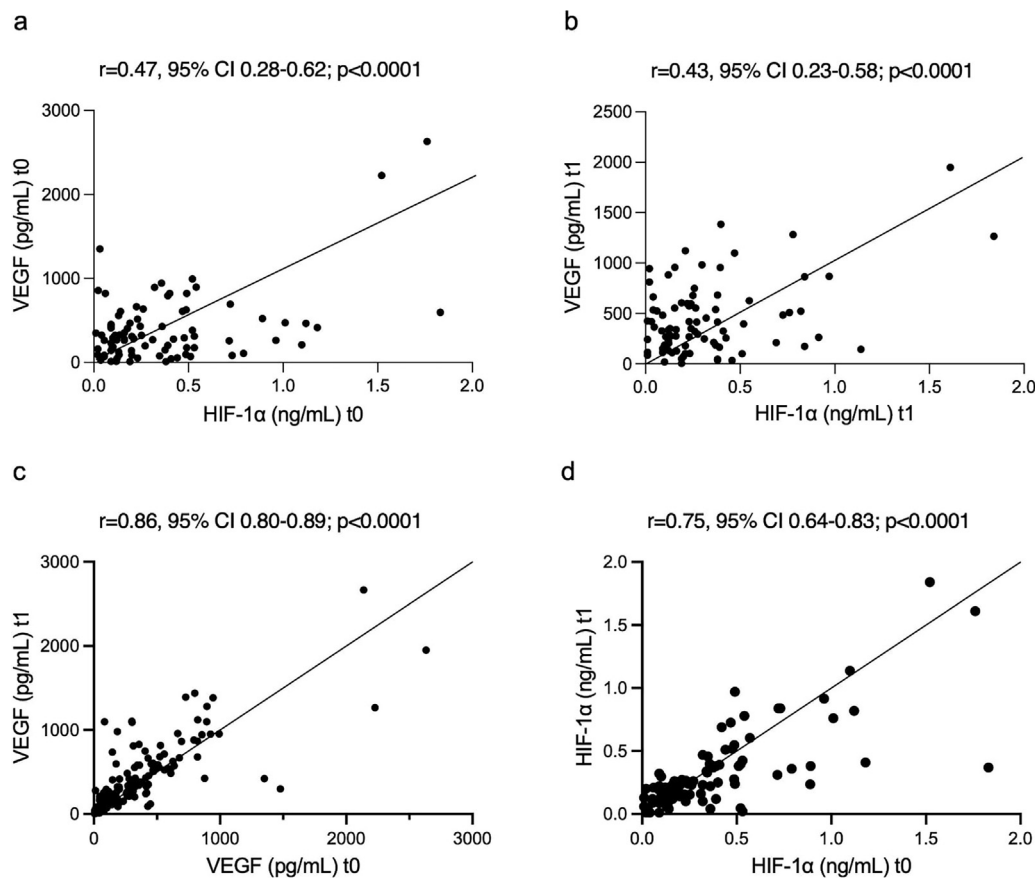


Fig. 2. Correlations between VEGF and HIF-1 α levels. VEGF levels were positively correlated with HIF-1 α levels at t_0 (a) and at t_1 (b). VEGF levels at t_0 and t_1 (c), and HIF-1 α levels at t_0 and t_1 (d) were also positively correlated.

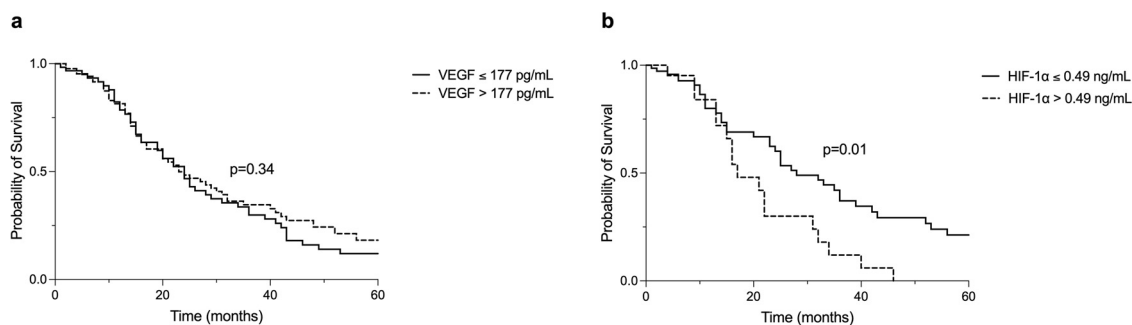


Fig. 3. Kaplan-Meier survival curves according to the level of VEGF and HIF-1 α . (a) No statistically significant differences in survival were demonstrated between patients with levels of VEGF below and above the cut-off (177 pg/mL). (b) Patients with levels of HIF-1 α below the identified cut-off (0.49 ng/mL) demonstrated significantly longer survival.

ter TACE > 25% or negative if there was a decrease of the marker level after TACE > 25%, otherwise the marker was considered stable. Delta-HIF-1 α (Δ HIF-1 α) was similarly defined. No statistically significant differences in OS were demonstrated between the Δ VEGF negative/stable group and the Δ VEGF positive group, and Δ HIF-1 α also was not associated with survival (Supplementary Figure 3).

The univariate analysis demonstrated that, beyond high HIF-1 α level, factors associated with an increased mortality risk were Child-Pugh class B (HR=2.28, 95% CI 1.46–3.56), multifocality (HR=1.60, 95% CI 1.03–2.46), larger tumors (HR=1.73 [95% CI 1.13–2.66] for 3–5 cm nodules and HR=1.66 [95% CI 1.03–2.67] for >5 cm nodules), BCLC B stage (HR=2.02, 95% CI 1.35–3.03) and high vascularization grade (HR=1.50, 95% CI 1.02–2.21). There were no differences in survival related to type of TACE (c-TACE vs. DEB-

TACE). After adjustment for confounders, HIF-1 α was singled out at multivariate Cox analysis as an independent prognostic factor (HR=2.03, 95% CI 1.05–3.94), together with Child-Pugh class (HR=2.97, 95% CI 1.55–5.68) and BCLC stage (HR=1.98, 95% CI 1.04–3.77) (Table 2).

3.4. VEGF and HIF-1 α as markers of radiological response to TACE

The objective response rate (complete response [CR] + partial response [PR]) after TACE was 78.0% (127 patients). VEGF levels were associated with radiological response, as patients with stable disease (SD) and progressive disease (PD) showed significantly higher levels of the marker. At t_0 , patients without radiological response to TACE had significantly higher levels of VEGF (406.6 pg/mL, IQR 181.4–558.6) compared to patients with CR

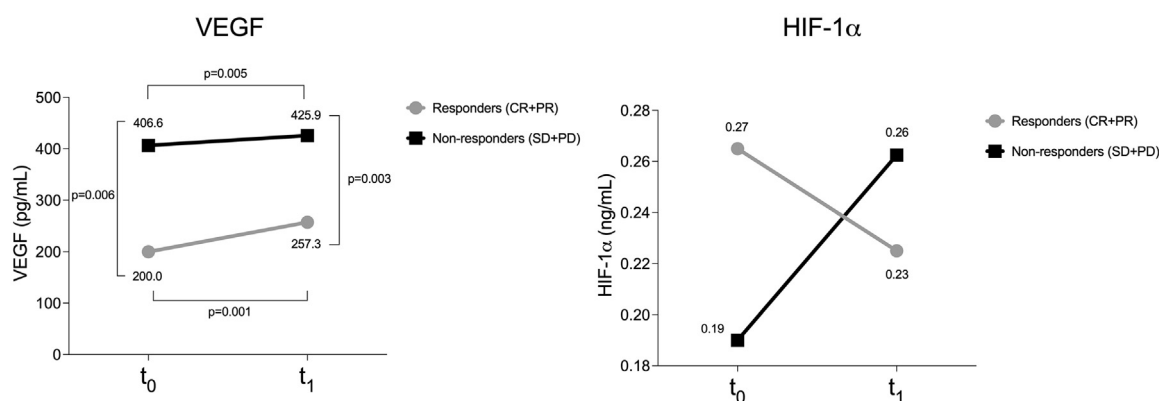
Table 2

Univariate and multivariate Cox models for factors independently associated with survival.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Sex				
Male	Ref	–	–	–
Female	0.80 (0.49–1.27)	0.33	–	–
Age (years)	1.01 (0.99–1.03)	0.21	–	–
Etiology				
Viral	Ref	–	–	–
Not viral	0.97 (0.66–1.42)	0.87	–	–
Viral + other	1.28 (0.55–2.95)	0.57	–	–
Child-Pugh class				
A	Ref	–	Ref	–
B	2.28 (1.46–3.56)	0.0003	2.97 (1.55–5.68)	0.001
Multifocality				
Monofocal	Ref	–	– ^a	– ^a
Multifocal	1.60 (1.03–2.46)	0.03	–	–
Diameter (cm)				
<3 cm	Ref	–	– ^a	– ^a
3–5 cm	1.73 (1.13–2.66)	0.01	–	–
>5 cm	1.66 (1.03–2.67)	0.04	–	–
BCLC stage				
0-A	Ref	–	Ref	–
B	2.02 (1.35–3.03)	0.001	1.98 (1.04–3.77)	0.04
AFP (ng/mL)				
≤200	Ref	–	–	–
>200	1.19 (0.70–2.03)	0.53	–	–
Vascularization				
Low	Ref	–	Ref	–
High	1.50 (1.02–2.21)	0.04	0.97 (0.49–1.90)	0.92
Type of TACE				
c-TACE	Ref	–	–	–
DEB-TACE	0.86 (0.59–1.27)	0.45	–	–
Radiological response (mRECIST)				
CR+PR	Ref	–	–	–
SD+PD	1.07 (0.70–1.64)	0.76	–	–
VEGF (pg/mL)				
<177	Ref	–	–	–
≥177	0.83 (0.57–1.22)	0.35	–	–
HIF-1 α (ng/mL)				
≤0.49	Ref	–	Ref	–
>0.49	2.13 (1.17–3.86)	0.01	2.03 (1.05–3.94)	0.04

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; c-TACE, conventional transarterial chemoembolization; DEB-TACE, drug-eluting beads transarterial chemoembolization; mRECIST, modified Response Criteria In Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; VEGF, vascular endothelial growth factor; HIF-1 α , hypoxia inducible factor 1 α .

^a Not included in the multivariate model in order to avoid collinearity with BCLC stage.

**Fig. 4.** VEGF (a) and HIF-1 α (b) levels in responders (CR+PR) and non-responders (SD+PD) at t_0 and t_1 .

or PR (200.0 pg/mL, IQR 58.9–415.8; $p = 0.006$). Similar results were obtained when VEGF levels were considered at t_1 , with SD or PD patients having a significantly higher levels of the marker compared to responders to treatment (425.9 pg/mL [IQR 245.2–808.3] vs. 257.3 pg/mL [IQR 68.5–528.6]; $p = 0.003$). Both in responders and in non-responders, there was an increase in VEGF compared to the levels measured before treatment ($p = 0.001$

and $p = 0.005$, respectively), with a slightly higher relative increase in patients with CR and PR (Fig. 4a). These results were not confirmed for HIF-1 α . In responders, HIF-1 α levels decreased after TACE, although this difference was not statistically significant (0.27 ng/mL [IQR 0.12–0.47] vs. 0.23 ng/mL [IQR 0.12–0.38]; $p = 0.30$). By contrast, HIF-1 α levels increased after the treatment in non-responders, but again no statistically significant differences

were demonstrated (0.19 ng/mL [IQR 0.09–0.67] vs. 0.26 ng/mL [IQR 0.11–0.42]; $p = 0.95$) (Fig. 4b).

The probability of being refractory to TACE was higher in patients with VEGF levels above the cut-off established with the ROC curve method at t_0 (21.2% vs. 78.8%; $p = 0.009$). The same was demonstrated considering VEGF levels after TACE, with a cut-off of 102 pg/mL (established with the ROC curve method). Even in this case, the probability having SD or PD after TACE was higher in patients with the marker above the cut-off (6.1% vs. 93.9%; $p = 0.003$). On the other hand, the same analysis using HIF-1 α at t_0 and t_1 was not statistically significant.

4. Discussion

Angiogenesis is of fundamental importance in the development and progression of HCC [2,3]. Considering their key role in the angiogenic process, VEGF and HIF-1 α have been evaluated as potential prognostic biomarkers, and several studies in the literature demonstrate their usefulness in patients managed with different treatments, including TACE [7–18,22,26]. In this study, we provide further evidence on the usefulness of these biomarkers in stratifying prognosis of HCC patients treated with TACE. Firstly, a statistically significant increase of VEGF levels was observed after TACE compared to the levels measured before the treatment. By contrast, no statistically significant differences were demonstrated between t_0 and t_1 levels of HIF-1 α . Some previous studies investigated the dynamic changes of these two molecules in serum of patients treated with TACE, showing different results. Jia et al. [26] found that the day after TACE both markers reach their peak value and then decline one week after the procedure, although remaining significantly higher than before TACE. According to the paper of Li et al. [27], VEGF levels increase significantly the day after treatment and then decrease gradually on the third- and seventh-day post-TACE. Other studies report a slower increase in the VEGF levels after the treatment [28,29]. The challenge in many of these studies, including our, is to measure dynamic changes of HIF-1 α and VEGF values in serum following TACE, as these biomarkers are evanescent [19]. This may contribute to explain the absence of differences in HIF-1 α comparing its level before the treatment and one month after TACE-induced ischemia. However, similarly to previous studies [22,27], we confirmed the sustained increase over time of VEGF after TACE. The increase of this latter without a correspondent variation in HIF-1 α levels was an unexpected finding, considering that the two molecules are related at a molecular level in the stimulation of angiogenesis (HIF-1 α is a transcription factor of VEGF) [3]. Indeed, a strong positive correlation between these two molecules has been demonstrated also in this study.

Among the patients included in this study, approximately half were treated with c-TACE while the others received DEB-TACE. The two techniques have a potentially different effect in inducing ischemia and in the consequent activation of angiogenesis [30,31]. Our results showed that DEB-TACE tend to cause higher tissue ischemia as reflected by the significantly more pronounced increase of VEGF at t_1 compared to patients who received c-TACE, thus confirming our previous preliminary results [31]. However, it should be acknowledged that opposite results have been found by other researchers: Schicho et al. [30] showed that DEB-TACE caused a significantly lower rise of VEGF compared to c-TACE, providing a possible explanation in postulating that the Lipiodol used in c-TACE does not cause a complete occlusion of the treated vessels; this leads to a partial perpetual reperfusion, which in turn leads to the expression of angiogenetic growth factors. The fact that in our center the c-TACE technique involves the injection of embolizing particles following the infusion of the Lipiodol + chemotherapy emulsion, thus causing a complete occlusion of the afferent vessels to the tumor, could explain the different findings. Nevertheless, other

studies in larger cohorts and with standardized procedure are necessary to achieve conclusive results.

Differently from what has been previously demonstrated in patients treated with TACE [21,22], the prognostic role of VEGF was not confirmed in this study. Nevertheless, HIF-1 α level above the identified cut-off was predictive of poorer survival and this variable maintained its independent prognostic role at the Cox multivariate analysis. HIF-1 α expression has been repeatedly associated with prognosis of HCC patients, and a metaanalysis demonstrated that its overexpression correlated with poor OS and disease-free survival [9]. However, all these studies considered the tissue expression of HIF-1 α and, in the majority of cases, evaluated its ability to predict prognosis after liver resection [7–18]. The findings from our study revealed that HIF-1 α , when assessed as a circulating marker in patients undergoing TACE, could offer valuable prognostic information.

Another interesting finding was that, although the relative increase of VEGF in non-responders was lower compared to responders to treatment, patients who achieved a complete or a partial response had significantly lower levels of VEGF both before and after TACE. This result, confirming previous studies [21], seems to suggest that treatment is less effective in patients with more activated neoangiogenic process, and supports other reports in the literature showing that increased VEGF levels has an important role in the development of collateral blood vessels nourishing the surviving residual tumor tissue [32]. The results achieved with HIF-1 α also confirm this hypothesis. Although not demonstrating a statistically significant difference between t_0 and t_1 levels both in responders and non-responders, a decrease in the level of this transcription factor in responders and an increase in non-responders was found. Considered that patients with lower levels of VEGF were more likely to have a radiological response (CR + PR), this marker could be considered as a useful predictor of the response to TACE. This result (VEGF predictor of radiological response but not associates with OS) seem to be paradoxical, also considered previous studies demonstrating that response to TACE according to mRECIST is an independent predictor of survival [33]. To explain this unexpected finding, it should be noted that in our study radiological response according to mRECIST, even if established by an expert interventional radiologist, was not associated with survival at the univariate analysis. Moreover, the surrogacy between radiologic response and radiological-based endpoints (e.g., progression-free survival) still represents a controversial topic. Our results, despite apparently paradoxical, are in line with the findings of a recent study of surrogacy validation in patients treated with TACE, demonstrating that the surrogate relationship of radiology-based endpoints with OS is poor [34]. Probably the relative small number of patients evaluated, the use in about half of the cases of TACE with lipiodol (which increases the risk of not correctly classifying the response to treatment at one month [35,36]) and the use of additional therapies impacting OS in the case of refractoriness to TACE contribute to this result obtained in our cohort of patients.

Based on the results of this study, circulating levels of VEGF and HIF-1 α could potentially be useful in stratifying the prognosis of patients treated with transarterial-therapies. Moreover, these two biomarkers could be useful in identifying patients with high-risk of poor outcomes after TACE, who could benefit from early shift to systemic therapy. This is extremely important considered that transarterial treatments could have a role in worsening liver function, thereby compromising the feasibility and safety of subsequent systemic therapies [37]. In addition, there has been a growing interest in investigating the utilization of systemic therapies in combination to TACE. Specifically, TKIs have gathered attention due to their potential to limit the release of angiogenic factors triggered by the ischemia consequent to the embolization. Despite this potential, the outcomes of these combined approaches have gener-

ated conflicting results [38–45], underscoring the need for further prospective data to validate both the efficacy and safety of these combination regimens.

Finally, VEGF circulating levels were higher in patients with multifocal tumors and in BCLC B stage, while no differences were demonstrated according to tumor size and vascularization grade. As far as HIF-1 α was evaluated in association with tumor burden, no statistically significant differences were found. Nevertheless, multifocality, larger nodule size and intermediate stage has higher levels of the marker. These results suggest that angiogenesis is particularly activated in tumors with more aggressive biology, and this was further confirmed by the association of neoangiogenic molecules with survival.

Among the inherent limitations of this study, the first and most relevant one is its retrospective design that could have introduced unintended biases. Second, the long time elapsed between the TACE procedure and the measurement of the post-treatment VEGF and HIF-1 α levels may have jeopardized the ability to identify dynamic changes in marker levels, and the association between these changes and the treatment outcome. However, we made this choice in the timing of blood sampling to match the t_1 markers measure with the control imaging (routinely performed one month after TACE). Third, this study included both patients who were naïve to the treatment, at their first TACE, and patients who had already undergone a number of procedures. This could have introduced a selection bias, but no difference in the baseline levels of any of the markers considered between naïve and experienced patients was observed. Therefore, we can consider that, if any difference between naïve and experienced patients exists, this is minimal.

This study provide evidence on the activation of angiogenesis and angiogenic molecules in HCC patients treated with TACE. We confirmed that TACE-induced ischemia is able to activate neoangiogenesis signaling pathways, as demonstrated by the increase of VEGF after treatment. Both VEGF and HIF-1 α could be considered useful circulating prognostic biomarkers in this setting. In particular, VEGF levels are increased in patients with greater tumor burden (intermediate stage multifocal tumor) and could be useful for predicting response to TACE, since patients with higher levels are more frequently non-responders to treatment. Moreover, HIF-1 α was an accurate predictor of patient overall survival. With the aim of refining patient prognosis, the evaluation of these biomarkers could be useful, but additional studies, possibly prospective, are needed to confirm these encouraging results.

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Conflict of Interest

The Authors declare no potential conflict of interest.

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Not applicable.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.09.019.

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