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ARTICLE

Macrophage migration inhibitory factor predicts an unfavorable outcome after transarterial chemoembolization for hepatic malignancies

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Abstract

Transarterial chemoembolization (TACE) is a therapeutic option for patients with intermediate-stage hepatocellular carcinoma (HCC) or metastatic liver cancers. Identifying those patients who particularly benefit from TACE remains challenging. Macrophage migration inhibitory factor (MIF) represents is an inflammatory protein described in patients with liver cancer, but no data on its prognostic relevance in patients undergoing TACE exist. Here, we evaluate MIF serum concentrations as a potential biomarker in patients undergoing TACE for primary and secondary hepatic malignancies. MIF serum concentrations were measured by multiplex immunoassay in 50 patients (HCC: $n = 39$, liver metastases: $n = 11$) before and 1 day after TACE as well as in 51 healthy controls. Serum concentrations of MIF did not differ between patients and healthy controls. Interestingly, in the subgroup of patients with larger tumor size, significantly more patients had increased MIF concentrations. Patients with an objective tumor response to TACE therapy showed comparable concentrations of serum MIF compared to patients who did not respond. MIF concentrations at day 1 after TACE were significantly higher compared to baseline concentrations. Importantly, baseline MIF concentrations above the optimal cutoff value (0.625 ng/ml) turned out as a significant and independent prognostic marker for a reduced overall survival (OS) following TACE: patients with elevated MIF concentrations showed a significantly reduced median OS of only 719 days compared to patients below the cutoff value (median OS: 1430 days, $p = 0.021$). Baseline MIF serum concentrations are associated with tumor size of intrahepatic malignancies and predict outcome of patients with liver cancer receiving TACE.

Theresa H. Wirtz and Sven H. Loosen share first authorship.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

During the last years, minimally ablative procedures, such as transarterial chemoembolization (TACE), have broadened the therapeutic spectrum to treat patients with primary or secondary liver cancer. Yet, as response rates to TACE are heterogeneous, pre-interventional stratification and optimal patient selection remains challenging.

WHAT QUESTION DID THIS STUDY ADDRESS?

Here, we evaluate pre- and postinterventional serum concentrations of macrophage migration inhibitory factor (MIF) as a potential biomarker in patients undergoing TACE for primary and secondary hepatic malignancies.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Higher baseline MIF serum concentrations are associated with an increased tumor size and predict outcome after TACE.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

We provide evidence for a previously unrecognized role of MIF as a biomarker in patients receiving TACE.

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the fifth most common malignancy worldwide and is accompanied with a 5-year survival rate of only 5.1%.[1,2] Besides HCC, as the most common primary cancer of the liver, secondary hepatic malignancies (i.e., metastases), account for about 90% of all liver cancers. Of those, solid tumors leading to hepatic metastases, gastrointestinal tumors, and in particular colorectal cancers (CRCs) are the most frequent.[3] Liver metastases limit long-term survival, therefore display an important target during cancer therapy.[4] As most patients are diagnosed at advanced tumor stages and might additionally show a reduced liver synthesis capacity due to the underlying liver disease, surgical resection is only possible in selected cases of patients. Hence, palliative treatment often remains the only available therapeutic option.[5] However, during the last years, minimally ablative procedures have broadened the therapeutic spectrum. Of these, transarterial chemoembolization (TACE) has evolved as a treatment option providing an acceptable balance between antitumor effect and toxicity in patients with intermediate stage, unresectable HCC (Barcelona Clinic liver cancer stage B) as well as in patients with CRC, when surgery or systemic therapy is considered inappropriate.[6–8] Yet, as response rates to TACE are heterogeneous, pre-interventional stratification and optimal patient selection remains challenging.[7,9]

Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine that has been characterized in different pathological conditions, including malignant diseases, such as HCC and CRC.[10,11] MIF was identified to drive carcinogenesis as it, for example, influences tumor cell proliferation and apoptosis as well as metastasis formation.[12,13] MIF furthermore has been described as a promising novel biomarker reflecting disadvantageous characteristics of tumor biology as well as patients' outcome after surgical resection.[14,15] In the present study, we aimed at evaluating serum concentrations of MIF as predictive and/or prognostic marker for patients undergoing TACE for primary and secondary liver cancer, independent of disease etiology.

MATERIALS AND METHODS

Study design and patient selection

The aim of this exploratory observational cohort study was to investigate a potential role of circulating MIF as a new prognostic and/or predictive biomarker in patients undergoing TACE. Therefore, we enrolled 49 patients with primary and secondary liver cancer (HCC: $n = 39$, liver metastasis: $n = 11$) who were admitted to the Department of Gastroenterology, Digestive Diseases and Intensive Care Medicine, and who underwent TACE at the Department of Diagnostic and Interventional Radiology at University Hospital RWTH Aachen between 2013 and 2017. We collected blood samples the day before TACE as well as at day 1 after the procedure. Full blood samples were centrifuged for 10 min at 2000 g and serum aliquots were stored at −80°C until use. We also included a total of 51 healthy, cancer-free blood donors who are medically examined on a regular basis as a control population. Ethical approval was granted by the ethics committee of the University Hospital RWTH Aachen, Germany (EK 206/09). The study was conducted in accordance with the standards of the Declaration

of Helsinki. Written informed consent was obtained from all patients.

Measurement of circulating MIF concentrations

Serum concentrations of MIF were measured by multiplex immunoassay according to the manufacturer's instruction using a Bio-Plex 200 system and Bio-Plex Manager 6.0 software (Bio-Plex Pro Human Chemokine Panel, #171AK99MR2; Bio Rad).

Transarterial chemoembolization

Primary as well as secondary hepatic malignancies were treated using an emulsion of a chemotherapeutic agent and an embolic agent diluted with iodized contrast (Ultravist 300, Bayer Vital GmbH). HCCs were treated with Doxorubicin and ethiodized oil (Lipiodol, Guerbet LLC). Intrahepatic metastases from other solid tumor entities, such as colorectal, gastric, or pancreatic cancer, were treated using a chemotherapeutic agent in accordance with the specific guidelines and Lipiodol, degradable starch microspheres (EmboCept S, PharmaCept GmbH), or drug eluting beads (DcBeads, BTG International Ltd). All chemoembolization procedures were performed via the right femoral artery. A hepatography as well as a contrast-enhanced cone-beam computed tomography (CT) in late arterial contrast phase were performed using a 2.4F- or 2.7F-microcatheter. A superselective (subsegmental), selective (segmental), or nonselective (lobar) approach was performed depending on the type, number, size, localization, and arterial supply of the respective tumor.

Assessment of TACE response

To evaluate tumor response, all patients underwent either a multidetector CT with multiphasic, contrast-enhanced acquisitions in native, arterial, portal venous, and late-venous phase, or a multiphasic, contrast enhanced liver magnetic resonance imaging (MRI; 1,5T; Philips Medical Systems DMC GmbH, Hamburg, Germany) not earlier than 4 weeks prior and ~ 4 weeks after TACE. All CT and MRI scans were analyzed according to RECIST 1.1 criteria for nonarterially enhanced tumor entities[16] and mRECIST criteria for HCC.[17] Overall tumor response was classified using the standard nomenclature for RECIST 1.1 and mRECIST as follows: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR and PR were subsequently summarized into objective response (OR).[18]

Statistical analysis

Statistical analysis was performed as previously described.[19] Contingency data were analyzed using Fisher's exact test. Receiver operating characteristic (ROC) curves were generated by plotting sensitivity against 1-specificity. The optimal cutoff values for ROC curves were established using the Youden index.[20] Binary logistic regression, including predicted probabilities, was performed for establishing prognostic models. The prognostic role of MIF was confirmed by univariate and multivariate Cox-regression analysis. All statistical analyses were performed with SPSS 23 (SPSS, Chicago, IL). A p value of less than 0.05 was considered statistically significant ($^{*}p < 0.05$; $^{**}p < 0.01$; $p < 0.001$).

RESULTS

Cohort characteristics

In our analysis, 50 patients with primary or secondary liver cancer who underwent TACE were included. Seventy-eight percent of patients were male and 22% were female. The median age of the study cohort was 65 years with a range from 37 to 89 years. Approximately four of five of all included patients suffered from HCC, whereas 22% had been diagnosed with liver metastasis due to a nonhepatic solid tumor. In this subgroup, most of the patients had metastatic colorectal carcinoma (12% of the whole study cohort); eight (73%) patients of those with secondary liver cancer only had hepatic metastasis, two (18%) patients had hepatic and pulmonary metastasis, and one (9%) patient had hepatic, pulmonary, and renal metastasis. The median tumor size was 27 mm with a range from 10 to 129 mm. All patients with HCC had underlying cirrhosis, which was related to alcoholism in 26%, whereas 23% of patients had cirrhosis induced by chronic hepatitis C virus (HCV) infection. The median model of end stage liver disease (MELD) score of the cohort was 13. 41.5% of all patients undergoing TACE showed an objective response (CR or PR), 58.5% did not. Mortality rate was 73.5% during follow-up. Further detailed characteristics of the study cohort are summarized in Table 1.

Larger tumor sizes are associated with higher baseline MIF serum concentrations

To get first insights in the regulation of serum MIF concentrations in patients with liver cancer, we compared baseline MIF concentrations of patients undergoing TACE with healthy, noncancer controls. MIF concentrations were not elevated in patients with HCC or liver metastases compared

TABLE 1 Description of study population at baseline

	Study cohort, $n = 50$
Sex, *n* (%)	
Male	39 (78)
Female	11 (22)
Age, median (range) (years)	65 (37–89)
BMI, median (range) (kg/m^2)	25.23 (17.16–36.72)
Hepatic malignancy, *n* (%)	
HCC	39 (78)
Liver metastasis (CRC)	6 (12)
Liver metastasis (gastric cancer)	1 (2)
Liver metastasis (pancreatic)	2 (4)
Liver metastasis (CCA)	2 (4)
Number and localization of metastatic sites, *n* (%)	
1: hepatic metastasis only	8 (73)
2: hepatic + pulmonary metastasis	2 (18)
3: hepatic, pulmonary, renal metastasis	1 (9)
Tumor size, median (range) (mm)	27 (10–129)
Cause of HCC, *n* (%)	
Alcoholic	10 (26)
HCV	9 (23)
HBV	5 (13)
Cryptogenic	9 (23)
Others (e.g., NASH)	6 (15)
Child Pugh stage of cirrhosis (HCC only), *n* (%)	
Child Pugh A	33 (85)
Child Pugh B	6 (15)
MELD Score, median (range)	13 (5–26)
Liver function parameters at baseline, median (range)	
Bilirubin (mg/dl)	0.71 (0.26–2.43)
AST (U/L)	39 (20–180)
ALT (U/L)	38 (11–309)
gGT (U/L)	137 (8–2268)
AP (U/L)	137 (45–618)
OR to TACE therapy, %	
Yes	41.5
No	58.5
Deceased during follow-up, %	
Yes	73.5
No	26.5
MIF serum concentration, median (range) (ng/ml)	
Before TACE	0.78 (0.31–4.32)
At day 1 after TACE	1.11 (0.35–11.67)

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CCA, cholangiocarcinoma; CHILD, Pugh-Child score; CRC, colorectal carcinoma; gGT, gamma-glutamyltransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MELD, model of end stage liver disease; MIF, migration inhibitory factor; NASH, non-alcoholic steatohepatitis; OR, objective response; TACE, transarterial chemoembolization.

to healthy controls (Figure 1a). To evaluate whether the entity of the hepatic malignancy might influence MIF serum concentrations, we compared patients with HCC to patients with metastatic liver cancer. MIF concentrations did not differ between both groups (Figure 1b). Nevertheless, the correlation of serum MIF concentration with the size of the intrahepatic malignancy revealed a significant result—here, patients with increasing tumor size were revealed to have higher MIF serum concentrations at baseline (Figure 1c). Nevertheless, when subdividing patients into subgroups with a tumor size above or below the median tumor size of 27 mm, patients with larger tumors showed a significantly higher proportion of patients with increased baseline MIF serum concentrations above the cohort's median of 0.78 ng/ml (17 of 27 patients, 63%) compared to the proportion of patients with MIF serum concentrations below this cutoff (10 of 27 patients, 37%, $p = 0.033$; Figure 1c). Next, MIF concentrations were compared between further subgroups of our cohort to gain insights into possible demographic and clinical factors that influence MIF concentrations in the included patients. MIF concentrations did not differ between male and female patients (Figure 1d). In those patients who suffered from HCC as a complication of cirrhosis, we observed comparable serum concentrations of MIF in patients with Child Pugh stage B liver cirrhosis compared to Child Pugh A patients (patients with HCC only; Figure 1e). The underlying etiology of liver disease (alcoholic, HCV, HBV, cryptogenic, or others, including non-alcoholic steatohepatitis) had no significant impact on serum MIF concentrations (Figure S1).

To further dissect potential underlying mechanisms that influence MIF concentrations associated with tumor size, we next performed correlation analyses between baseline MIF concentrations and demographic as well as laboratory markers reflecting systemic inflammation as well as liver function. Although MIF did not correlate with the patients' age or body mass index (BMI; Table 2), we observed a strong positive correlation between MIF and serum concentrations of inflammatory markers, such as leukocytes (r_s: 0.319, $p = 0.027$) and tumor necrosis factor α (TNFα; r_s: 0.388, $p = 0.006$), as well as anti-inflammatory markers, such as interleukin 10 (IL-10; r_s: 0.445, $p = 0.001$). Baseline MIF serum concentrations did not correlate with markers of liver function, such as bilirubin and the MELD score but strongly correlated with lactate dehydrogenase (r_s: 0.539, <0.001).

Baseline MIF serum concentrations and tumor response to TACE therapy

Subsequently, we aimed at evaluating if pre-interventional MIF concentrations might have a predictive value with

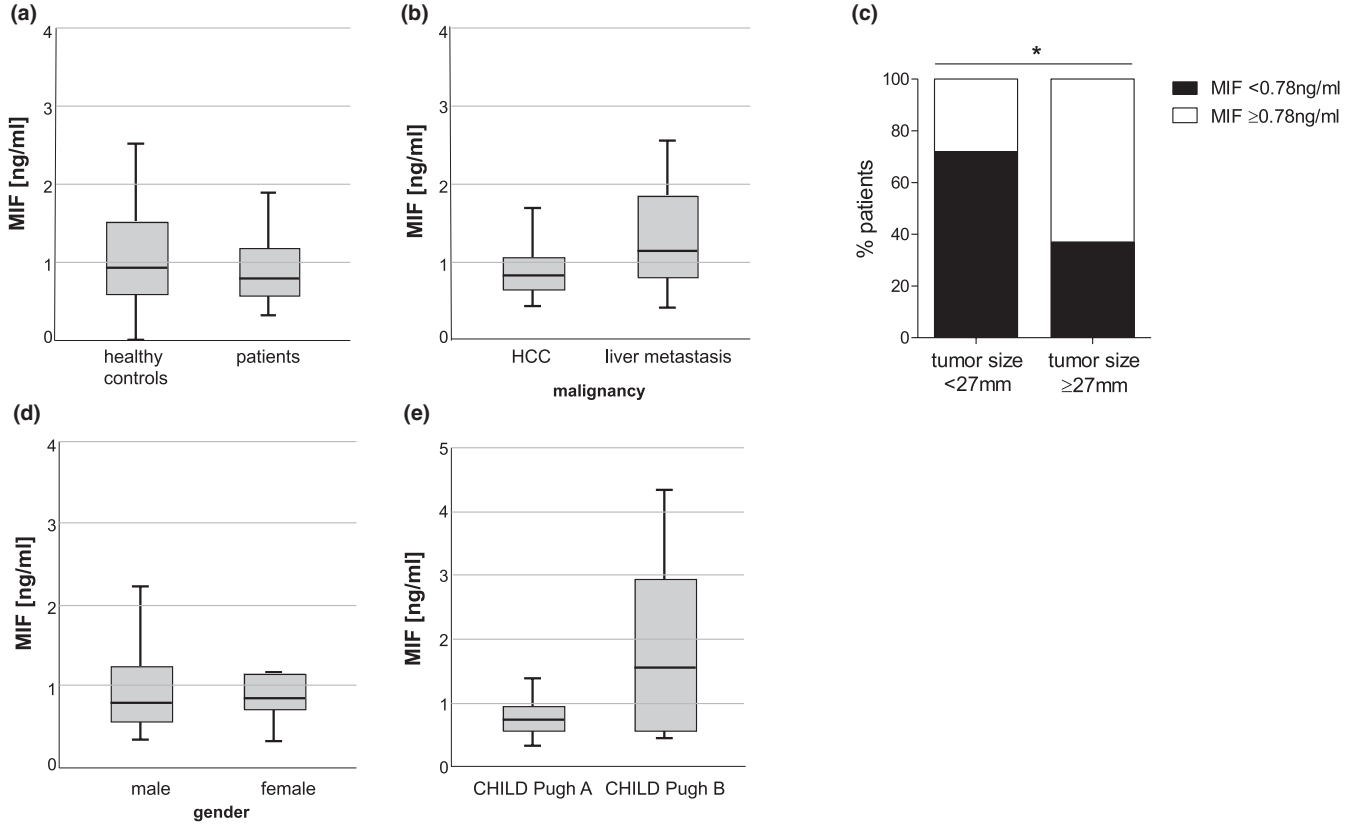

FIGURE 1 The proportion of patients with augmented MIF serum concentration is significantly increased in patients with larger tumor size. (a, b) Baseline MIF serum concentrations neither differ between patients with hepatic malignancies and healthy controls nor between patients with HCC compared to patients with liver metastases. (c) Serum MIF at baseline correlates with the size of intrahepatic malignancy. (d) The proportion of patients with increased baseline MIF serum concentrations above the median cutoff (0.78 ng/ml) is significantly higher within the group of patients with a tumor size above the median cutoff of 27 mm as Fisher's exact test reveals ($p = 0.033$). (d) MIF serum concentrations are unaltered between male and female patients. (e) Patients with Child Pugh stage B liver cirrhosis have comparable MIF serum concentrations compared to Child Pugh A patients. HCC, hepatocellular carcinoma; MIF, migration inhibitory factor

regard to the individual therapeutic response after TACE. Patients were stratified into two subgroups either showing an OR (including complete and partial tumor response) or showing no OR (non-OR, including SD and PD) following TACE. MIF serum concentrations did not differ between these groups (Figure 2a). In line with this, ROC curve analysis for the discrimination between OR and non-OR patients revealed area under the curve (AUC) values of 0.515 for MIF, whereas serum lactate dehydrogenase (LDH) and the tumor size as known prognostic markers in liver cancer reached an AUC value of 0.443 or 0.618, respectively (Figure 2b). Moreover, an established model combining all three parameters only reached an AUC of 0.647 (Figure 2c). Hence, neither MIF nor LDH or the combination of MIF with established parameters for treatment response was able to discriminate between OR and non-OR patients within our study cohort. Next, we aimed at investigating whether TACE would influence circulating MIF concentrations. Post-interventional MIF concentrations at day 1 after TACE were available for 43 patients. When compared to the respective pre-interventional MIF concentrations, serum concentrations at day 1 after TACE were significantly higher (median MIF concentration of 1.11 ng/ml compared to 0.78 ng/ml at baseline; Table 1 and Figure 2d) and strongly correlated with markers of systemic inflammation including CRP and IL-6 as well as liver function tests (i.e., aspartate aminotransferase [AST] and alanine aminotransferase [ALT]; (Table S1). However, also those MIF serum concentrations at day 1 after TACE did not differ between the two subgroups of patients after stratification for OR (Figure 2e).

An elevated MIF serum concentration represents a prognostic factor for overall survival following TACE therapy

We next hypothesized that circulating MIF concentrations might be indicative for the patients' overall survival (OS) rather than predicting the direct tumor response to TACE therapy. To test the predictive capability of baseline MIF concentrations to discriminate between overall survivors

TABLE 2 Correlations of MIF with patients' baseline characteristics, markers of inflammation, and liver function before TACE

	r	*p* value
Baseline characteristics		
Age	−0.115	0.431
BMI	0.048	0.745
Markers of inflammation		
Leukocytes	0.319	0.027*
CRP	0.199	0.191
TNFα	0.388	0.006*
IL-10	0.445	0.001**
Markers of organ function		
Bilirubin total	−0.103	0.490
AST	0.155	0.503
ALT	0.034	0.833
gGT	−0.059	0.698
AP	0.166	0.269
LDH	0.536	<0.001***
MELD score	0.204	0.189

Note: Spearman rank correlation test was used to test significance; the Spearman's rho correlation coefficient is depicted as "*r*" with $*p < 0.05$; $**p < 0.005$, $***p < 0.001$.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; gGT, gamma-glutamyltransferase; IL-10, interleukin 10; LDH, lactate dehydrogenase; MELD, model of end stage liver disease; MIF, migration inhibitory factor; TACE, transarterial chemoembolization; TNFα, tumor necrosis factor α.

and nonsurvivors, ROC curve analysis was performed first. Here, ROC curve analysis of baseline MIF serum concentrations reached an AUC of 0.708 (Figure 3a), whereas the AUC for tumor size was 0.663 (Figure 3b) and 0.614 for LDH (Figure 3c). When combining the ROC curve analysis for all three parameters, including all patients with available measurements for MIF, LDH, and tumor size, we revealed an even increased AUC value of 0.731 (Figure 3d).

To further test the prognostic relevance of baseline MIF serum concentrations, Kaplan-Meier curve analysis was complemented. Our cohort was divided into two groups according to baseline MIF concentration using the 50th percentile (0.785 ng/ml) as a cutoff value. Here, patients undergoing TACE with initial MIF concentrations greater than 0.785 ng/ml showed an impaired OS ($p = 0.013$, Figure 4a). We furthermore used the Youden index (see Material and Method section for details) to establish the optimal prognostic cutoff value of 0.625 ng/ml. This cutoff value reached the highest sensitivity of 0.77 and specificity of 0.62. Initial MIF concentrations above this cutoff identify patients with an impaired outcome after TACE in Kaplan-Meier analysis ($p = 0.021$; Figure 4b). The median OS for patients with initial MIF concentrations greater than 0.625 ng/ml was only 719 days compared to 1430 days for patients who had an MIF concentration below the optimal cutoff value.

To further substantiate the prognostic potential of serum MIF, we subsequently performed univariate Cox-regression analyses using MIF serum concentrations at baseline as a continuous parameter. Univariate Cox-regression analysis revealed baseline MIF concentrations as a significant prognostic factor for OS (hazard ratio [HR]: 1.957, 95% confidence interval [CI]: 1.268; 3.022, $p = 0.002$). We next evaluated a wide range of clinicopathological parameters (age, sex, BMI, tumor size, and tumor type) as well as various laboratory parameters of liver function (MELD score, bilirubin, AST, ALT, gGT, AP) and previously known prognostic factors for survival in patients with liver cancer (LDH) in univariate Cox-regression analysis. In multivariate Cox-regression analysis, including parameters with a potential prognostic relevance in univariate testing ($p < 0.100$), baseline MIF serum concentrations turned out as an independent prognostic marker for OS (HR: 3.391, 95% CI: 1.229–9.353, $p = 0.018$).

Post-interventional MIF serum concentrations and patients' outcome

Based on our finding that baseline MIF concentrations predict outcome following TACE therapy, we next hypothesized that postinterventional MIF concentrations might reflect an immediate response to TACE which might in turn be indicative for the tumor response. We therefore evaluated a potential impact of postinterventional MIF serum concentrations on the patients' OS after TACE. Again, we first compared the OS in patients with very high postinterventional MIF concentrations above the 50th percentile (1.115 ng/ml) and patients with day 1 serum concentrations below less than 1.115 ng/ml. Here, we observed a trend toward an impaired OS in the MIF high group ($p = 0.155$; Figure 4c). When using the optimal prognostic cutoff value for postinterventional MIF concentrations (0.803 ng/ml) revealed by ROC curve analysis and establishment of Youden index (Figure S2; see Materials and Methods section for further details), this prognostic trend was further increased but statistical significance was not reached ($p = 0.074$; Figure 4d). Finally, we tested whether the individual kinetic of MIF before and after TACE might reflect the patients' outcome and compared the OS of patients with increasing or decreasing MIF concentrations after TACE. However, we did not observe a significant difference in OS between these groups (Figure 4e).

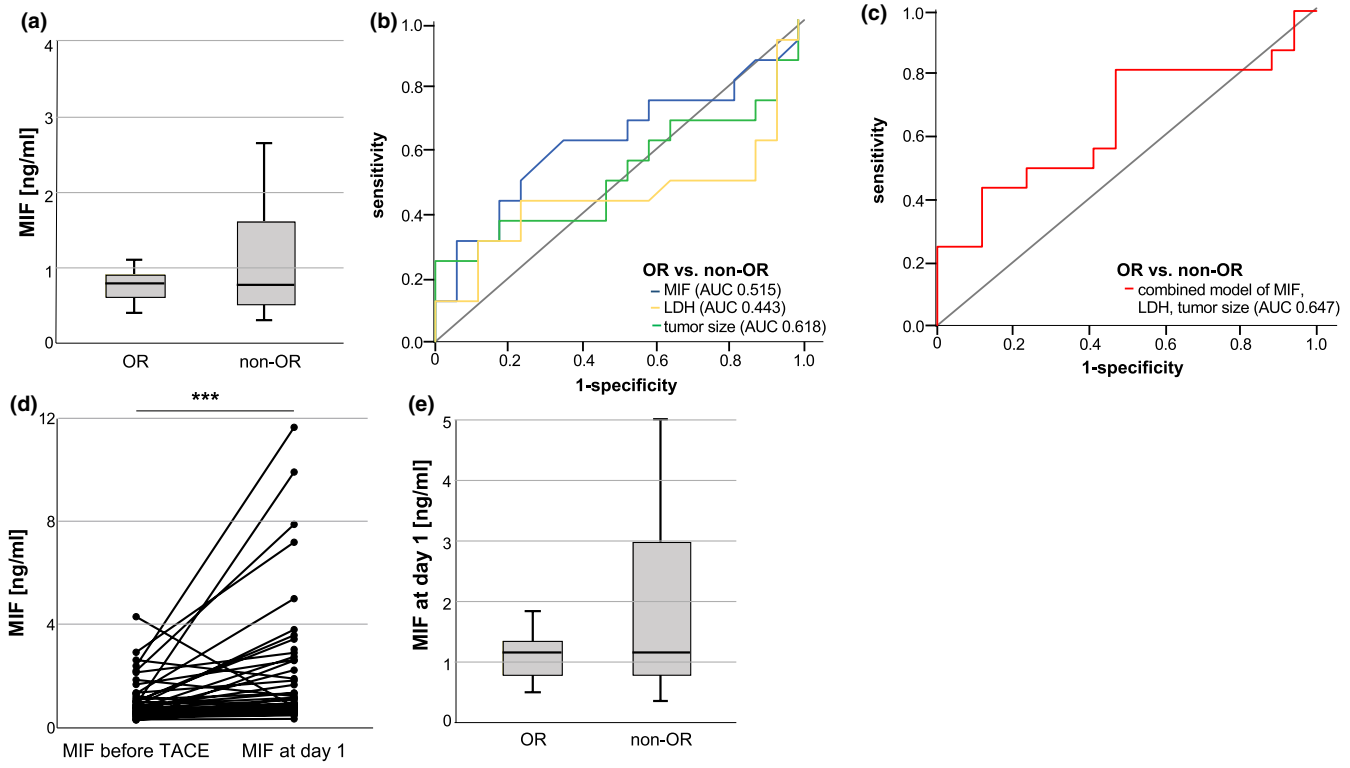

FIGURE 2 Pre- and postinterventional MIF serum concentrations and tumor response to TACE. (a) MIF concentrations before TACE are comparable between patients with liver cancer who show an objective response (OR) compared to non-responding (non-OR) patients. (b) ROC curve analysis for the discrimination between OR and non-OR patients by MIF, LDH, and the size of the target tumor lesion. (c) Combined model for the discrimination between OR and non-OR patients by MIF, serum LDH, and tumor size. (d) At day 1, after TACE paired analysis reveals significantly higher MIF serum concentrations compared to baseline values. (e) MIF concentrations determined at day 1 after TACE do not differ in patients with OR compared to non-responders (non-OR). AUC, area under the curve; LDH, lactate dehydrogenase; MIF, migration inhibitory factor; ROC, receiver operating characteristic; TACE, transarterial chemoembolization

DISCUSSION

Multimodal therapeutic concepts have changed clinical management of many oncologic diseases.[3,21] In particular, locally ablative techniques, such as the TACE, were introduced into treatment algorithms of primary and secondary liver cancer.[3,16] In the present analysis, we studied the prognostic and predictive potential of MIF serum concentrations in patients that were allocated to TACE for different tumor entities, with HCC representing the most important etiology. Most importantly, we provide evidence for a prognostic role of preinterventional MIF serum concentrations because patients who displayed elevated baseline MIF concentrations showed a significantly impaired prognosis compared to patients who had decreased MIF serum concentrations.

In the past, most studies dealing with MIF have analyzed the role of this pleiotropic, inflammatory protein in inflammatory diseases, including autoimmune hepatitis and alcohol-related liver injury.[22,23] Subsequently, it became increasingly clear that MIF might have a unique function that is beyond its relevant impact on chronic inflammation, as it was shown to moreover interconnect inflammatory and malignant diseases.[24] In line, we demonstrate that MIF serum concentrations in patients with intrahepatic malignancies are significantly correlated with leukocyte count as well as TNFα and IL-10 serum concentrations representing well-characterized actors during systemic inflammation. Thus, our data support the hypothesis that MIF might be part of a "malignant cycle of inflammation and renovation" promoting carcinogenesis.

Supporting this hypothesis, Zhao et al. demonstrated in a large and convincing analysis that serum concentrations of MIF are significantly elevated in patients with HCC when compared to patients with chronic HCV infection or healthy controls. In these analyses, MIF serum concentrations had higher diagnostic accuracy to diagnose even early stage HCC than serum alpha-fetoprotein.[25] In our study, we found a significant higher proportion of patients with increased MIF serum concentrations within the group of patients with larger tumor sizes providing a possible link to the previously published data revealing MIF to correlate with prognostic relevant disease characteristics, such as vascular invasion and tumor, node, and metastasis stage.[15] Larger clinical trials are necessary to confirm this association of baseline MIF serum concentrations and size of target lesion also in patients

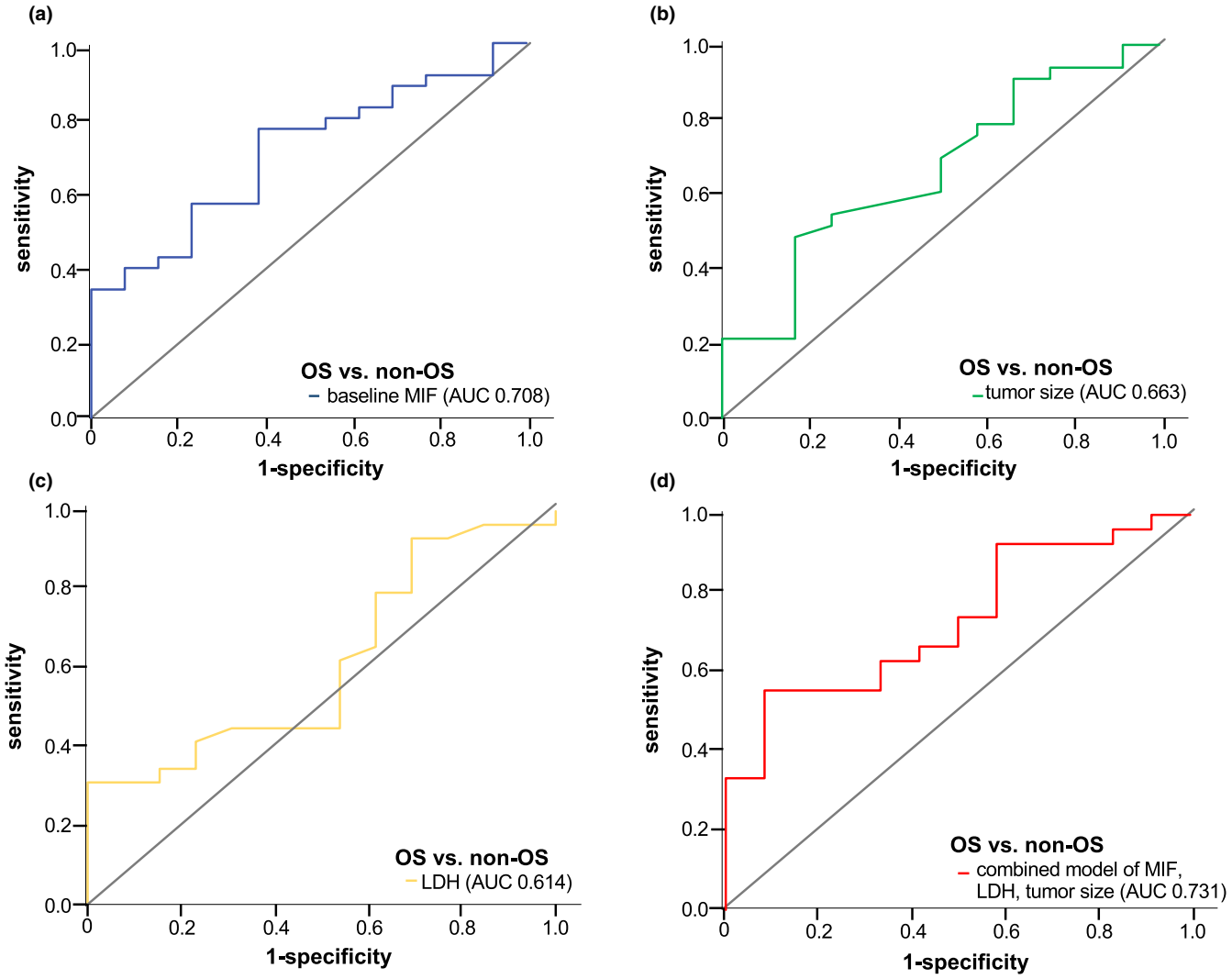

FIGURE 3 Comparison of the prognostic value of MIF, serum LDH and tumor size for overall survival. ROC curve analysis for the discrimination between overall survivors (OS) and non-survivors (non-OS) by MIF (a), tumor size (b), and serum LDH (c). A combined model of ROC curve analysis including MIF, LDH, and tumor size is depicted in (d). AUC, area under the curve; LDH, lactate dehydrogenase; MIF, migration inhibitory factor; ROC, receiver operating characteristic

undergoing TACE. In contrast, we did not detect elevated concentrations of MIF in patients with primary or secondary liver tumors, when compared to healthy controls. In detail, patients with intrahepatic malignancy displayed almost identical MIF concentrations than healthy controls and we failed to detect a correlation of patients' clinicopathological characteristics with MIF concentrations. This apparent contradiction to previous studies might have several reasons. First, we analyzed a very specific cohort of patients that received TACE for HCC or gastrointestinal cancer with liver metastases that might not be fully comparable to previous studies. Second, MIF concentrations in our cohort were lower than those found by Zhao et al. Third, we analyzed MIF concentrations in patients' serum, whereas Zhao et al. analyzed MIF concentrations in plasma. Interestingly, in the study of Akbar et al., MIF serum concentrations also did not discriminate between 66 patients with HCC and 26 patients with cirrhosis, highlighting a possible selection bias and moreover raising the question whether the analysis of MIF concentrations in serum compared to plasma might be associated with different findings.[26]

Serum concentrations and tissue expression of MIF significantly correlate—also including different states of HCC—as a recent analysis revealed.[15] We therefore compared pre- and postinterventional MIF serum concentrations in patients treated by TACE. It was previously described that patients with HCC who underwent tumor resection show a slight decline of plasma MIF concentrations on postoperative day 3, an intermediate decline on postoperative day 7, and an intense decline on postoperative day 30, suggesting that an entire tumor resection might be associated to lower MIF concentrations.[25] However, in our cohort, MIF serum concentrations were strongly elevated at day 1 after TACE. This finding might be related

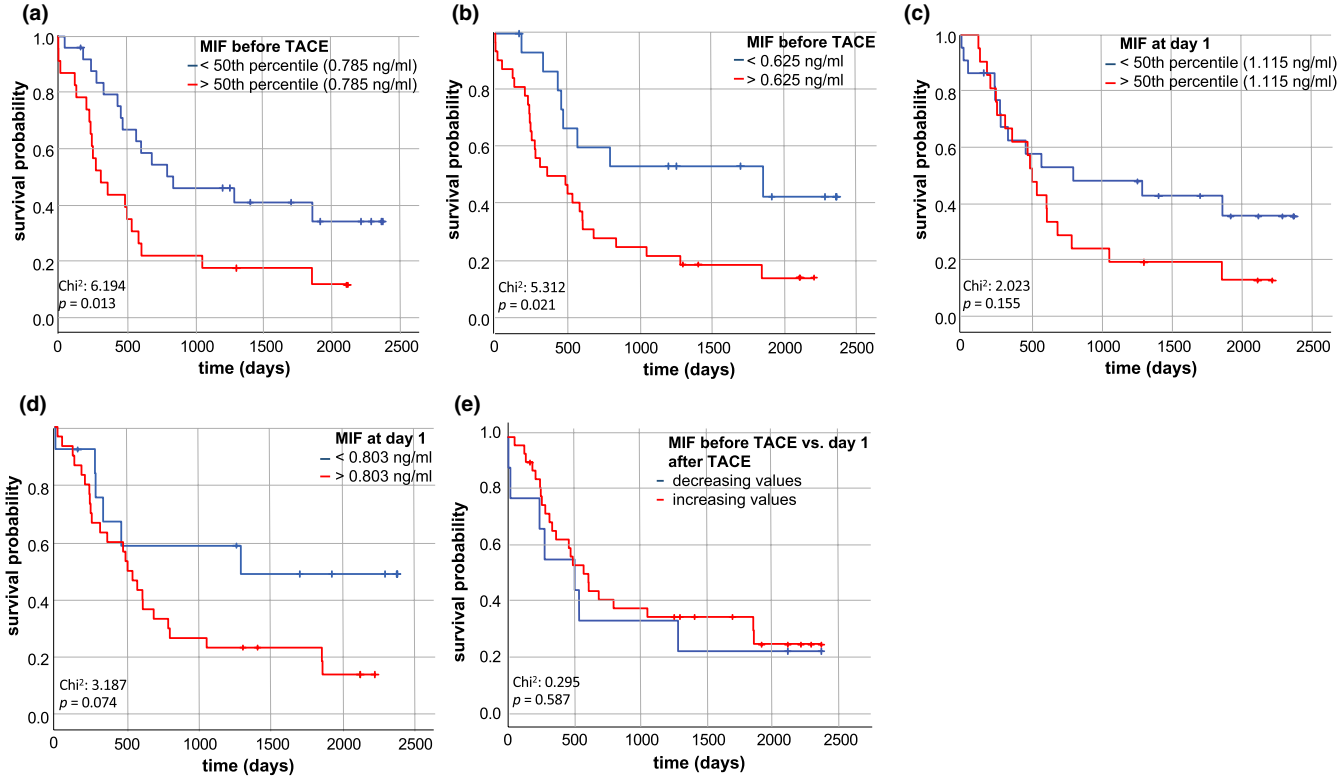

FIGURE 4 Elevated baseline MIF concentrations predict an unfavorable outcome after TACE. (a) Patients with liver cancer with baseline MIF concentrations above the 50th percentile (0.785 ng/ml) show a significantly impaired post-interventional survival. (b) Patients with MIF serum concentrations above the optimal prognostic cutoff value (≥0.625 ng/ml) show a significantly impaired overall survival compared to patients with baseline MIF concentrations below this cutoff. (c, d) Neither the 50th percentile (1.115 ng/ml) nor the optimal cutoff value (0.803 ng/ml) of MIF serum concentrations at day 1 after TACE predicts overall survival in patients with primary or secondary hepatic cancer. (e) The overall survival of patients with intrahepatic malignancy undergoing TACE is comparable in patients who show increasing to patients with decreasing MIF concentrations before and at day 1 after TACE. MIF, migration inhibitory factor; TACE, transarterial chemoembolization

to a postinterventional, inflammatory reaction due to tissue damage that might have led to an upregulation of circulating MIF next to other inflammatory markers. Therefore, we cannot exclude that a potential decrease of MIF concentrations in responders might have been masked. To investigate whether there is a prognostic significance of inflammatory biomarkers in hepatocellular carcinoma following treatment is the aim of recent studies.[27] These data are complemented by our study, as those patients who did respond to TACE therapy compared to nonresponders did not show significant differences of MIF serum concentrations, regardless, whether all patients or only patients with HCC were included into the analysis. Moreover, an increase of MIF serum concentrations after TACE did not lead to a favorable outcome of the included patients.

In contrast to the results on tumor response, we found significantly lower baseline concentrations of MIF in those patients that displayed long-term survival compared to those that succumbed to death within the observation period. In line, MIF serum concentrations turned out as an independent marker to discriminate between patients with a favorable and unfavorable prognosis in Kaplan-Meier and multivariate Cox-regression analyses. Interestingly, established prognostic markers such as the tumor size and serum LDH level did not reliably predict outcome in our cohort of patients, which might be due to the small cohort size. Nevertheless, when we combined initial MIF values with the tumor size and LDH serum levels, the prognostic relevance of MIF was further increased. Although confirmatory data are needed, this finding argues that MIF should rather be implemented into multimodal stratification tools rather than being used as a stand-alone biomarker. In contrast, baseline MIF concentrations were not capable to identify those patients who would respond to the TACE procedure. We therefore argue that larger multicentric trials are needed to further investigate the predictive value of baseline MIF concentrations and to validate the result of MIF as a representative serum-based parameter with prognostic relevance next to the established parameters. Nonetheless, because our study included both patients with primary and secondary hepatic malignancies, our data argue for an entity independent prognostic value of MIF in patients receiving TACE. Of note, these data complement previously published data from other HCC cohorts as well as from recent findings demonstrating that expression

TABLE 3 Uni- and multivariate Cox-regression analysis of baseline MIF serum concentrations

	Univariate Cox-Regression		Multivariate Cox-Regression Model 1	
Parameter	***p* value**	**HR (95% CI)**	***p* value**	**HR (95% CI)**
MIF	0.002*	1.957 (1.268–3.022)	0.018*	3.391 (1.229–9.353)
Tumor size	0.133	1.008 (0.998–1.017)		
Tumor type	0.083	1.919 (0.918–4.012)	0.297	0.346 (0.047–2.550)
Age	0.495	1.011 (0.980–1.044)		
Sex	0.524	0.764 (0.333–1.751)		
BMI	0.234	0.950 (0.873–1.034)		
MELD score	0.111	1.067 (0.985–1.155)		
Bilirubin	0.562	1.222 (0.620–2.408)		
AST	0.085	1.009 (0.999–1.019)	0.888	1.001 (0.981–1.023)
ALT	0.386	0.997 (0.990–1.004)		
gGT	0.020*	1.001 (1.000–1.002)	0.223	1.002 (0.999–1.005)
AP	0.020*	1.003 (1.001–1.006)	0.678	1.003 (0.990–1.015)
LDH	0.381	1.001 (0.999–1.003)		

The HR and the 95% CI are displayed. A p value of less than 0.05 was considered statistically significant (*$p < 0.05$).

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; gGT, gamma-glutamyltransferase; HR, hazard ratio; LDH, lactate dehydrogenase; MELD score, model of end stage liver disease score; MIF, Macrophage Migration Inhibitory Factor.

of MIF in HCC tissue is reversely proportional to patients' prognosis.[14]

Our study bears several limitations. First, our cohort is rather small, featuring only 49 patients and a monocentric design was applied. Second, we included both patients with primary and secondary hepatic malignancies, leading to a rather heterogeneous cohort and entity specific conclusions cannot be drawn. Third, serum MIF determination after TACE only took place at day 1 after TACE so our study is not capable of investigating a potential prognostic value of serum MIF concentrations at later time points after TACE. Therefore, larger clinical trials featuring a prospective multicentric design and including both MIF serum measurements before and at different time points after TACE are needed before a use of peri-interventional MIF analysis in clinical routine should be considered. Nevertheless, we provide evidence for a previously unrecognized role of MIF as a biomarker in patients receiving TACE.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

T.H.W., S.H.L., C.T., M.-L.B., F.T., T.L., and C.R. wrote the manuscript. T.H.W., S.H.L., T.L., and C.R. designed the research. T.H.W., S.H.L., J.G., J.K., M.J., M.D., R.M., P.B., C.K., C.T., M.-L.B., F.T., T.L., and C.R. performed the research. T.H.W., S.H.L., and M.S.-H. contributed analytical tools and analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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