JGH Open Open Access An open access journal of gastroenterology and hepatology

JGHF Journal of Gastroenterology and Hepatology Foundation

doi:10.1002/jgh3.12501

ORIGINAL ARTICLE

Elevated soluble urokinase plasminogen activator receptor serum levels indicate poor survival following transarterial chemoembolization therapy for hepatic malignancies: An exploratory analysis

Sven H Loosen,*,† Max Schulze-Hagen,‡ Mihael Vucur,* Joao Gorgulho,† Pia Paffenholz,§ Fabian Benz,¶ Raphael Mohr,¶ Münevver Demir,¶ Alexander Wree,¶ Christiane Kuhl,‡ Christian Trautwein,† Frank Tacke,¶ Philipp Bruners,‡ Tom Luedde* and Christoph Roderburg¶

*Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University Düsseldorf, Düsseldorf, Departments of †Medicine III, ‡Diagnostic and Interventional Radiology, University Hospital RWTH Aachen, Aachen, §Department of Urology, University Hospital Cologne, Cologne and ¶Department of Hepatology and Gastroenterology, Charité University Medicine Berlin, Berlin, Germany

Key words
biomarker, cancer, hepatocellular carcinoma, prognosis, transarterial chemoembolization.

Accepted for publication 16 January 2021.

Correspondence
Christoph Roderburg, Department of Hepatology and Gastroenterology, Charité University Hospital Berlin, Berlin, Germany.
Email: christoph.roderburg@charite.de

Tom Luedde and Christoph Roderburg share senior authorship.

Declaration of conflict of interest: The authors declare no conflict of interest.
Author contribution: Tom Luedde, Christiane Kuhl, Philipp Bruners, Sven H Loosen, and Christoph Roderburg designed the study; Sven H Loosen, Christoph Roderburg, Fabian Benz, Christiane Kuhl, and Philipp Bruners recruited the patients; Sven H Loosen performed experiments; Max Schulze-Hagen and Philipp Bruners performed assessment of radiological TACE response; Sven H Loosen performed statistical analysis and generated figures and tables; Christian Trautwein, Frank Tacke, Pia Paffenholz, and Mihael Vucur provided intellectual input; Sven H Loosen and Tom Luedde drafted the manuscript; and all authors approved the paper.

Abstract

Background and Aim: Transarterial chemoembolization (TACE) represents a standard of care for patients with intermediate-stage hepatocellular carcinoma (HCC) or liver metastases. However, identification of the ideal candidates for TACE therapy remains challenging. The soluble urokinase plasminogen activator receptor (suPAR) has recently evolved as a prognostic marker in patients with cancer; however no data on suPAR in the context of TACE exists.
Methods: Serum levels of suPAR were measured by an enzyme-linked immunosorbent assay in $n = 48$ TACE patients (HCC: $n = 38$, liver metastases: $n = 10$) before intervention and 1 day after TACE, as well as in 20 healthy controls.
Results: Serum levels of suPAR were significantly elevated in patients with liver cancer compared to healthy controls. Patients with or without an objective tumor response to TACE therapy had comparable levels of circulating suPAR. Importantly, baseline suPARs above the ideal prognostic cut-off value (5.39 ng/mL) were a significant prognostic marker for reduced overall survival (OS) following TACE. As such, patients with initial suPAR levels >5.39 ng/mL showed a significantly reduced median OS of only 256 days compared to patients with suPAR serum levels below the cut-off value (median OS: 611 days). In line with previous data, suPAR serum concentrations correlated with those of creatinine but were independent of tumor entity, leukocyte count, and C-reactive protein in multivariate analysis.
Conclusion: Baseline suPAR serum levels provide important information on the postinterventional outcome of liver cancer patients receiving TACE.

Introduction

Hepatocellular carcinoma (HCC) is a major global health burden, and its incidence has increased such that it has become the fifth most common malignancy worldwide.[1] Despite HCC being the most common etiology of primary liver cancer, secondary liver malignancies—metastases from other cancers—are much more frequent and represent about 90% of all liver cancers.[1] Gastrointestinal tumors, particularly colorectal cancers (CRCs), represent the most frequent tumors leading to liver metastases.[2] For many patients, liver metastasis is a limiting factor for long-term survival, thus representing an important therapeutic target in the

oncological management of cancer patients.[3,4] In most cases, for both primary and secondary hepatic malignancies, complete tumor resection is the only curative option. However, as many patients are diagnosed at advanced tumor stages and/or display impaired liver synthesis capacity, surgery is only possible in selected cases, and palliative treatment often remains the only available therapeutic option.[4] In this context, transarterial chemoembolization (TACE) has evolved as a standard treatment option providing an acceptable balance between antitumor effect and toxicity.[5] In patients with HCC, TACE represents the standard therapeutic option for patients with intermediate-stage unresectable tumors (Barcelona Clinic Liver Cancer stage B).[6] Interestingly, TACE has also evolved as an additional therapeutic option in CRC patients when surgery or systemic therapy is considered not appropriate.[7] Both in primary and secondary liver malignancies, response rates to TACE and toxicity of TACE are heterogeneous. Despite many different preinterventional stratification algorithms, such as the ART or SNACOR, optimal patient selection has remained challenging.[6,8] Soluble urokinase plasminogen activator receptor (suPAR) has been established as a promising novel biomarker reflecting the tumor biology in terms of grading or prognosis and allows us to guide preoperative treatment decisions regarding patients' outcomes in manifold cancers.[9,10] In the present study, we aimed at evaluating serum concentrations of suPAR as predictive and/or prognostic markers for patients undergoing TACE for primary and secondary liver cancer, independent of the disease etiology.

Methods

Design of the study. This observational cohort study was designed to evaluate the potential role of circulating suPAR before and after TACE therapy as a potential novel prognostic biomarker. We included a total of $n = 48$ patients with primary and secondary liver cancer (HCC: $n = 38$, liver metastasis: $n = 10$) who were admitted to the Department of Medicine III and who received TACE at the Department of Diagnostic and Interventional Radiology at University Hospital RWTH Aachen between 2013 and 2017 (detailed characteristics are shown in Tables 1 and S1). Serum samples were collected prior to TACE therapy and at day 1 after the procedure. After collection, blood samples were centrifuged for 10 min at 2000*g*, and serum was stored at −80°C until use. We included $n = 20$ healthy, cancer-free blood donors who are medically examined on a regular basis. 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. A commercially available enzyme-linked immunosorbent assay (ELISA) was used to measure suPAR serum concentrations according to the manufacturer's instructions (Nr. A001, suPARnostic, ViroGates, Birkerød, Denmark).

Evaluation of TACE response. For the evaluation of TACE response, we needed to carry out a multidetector computed tomography (CT) with multiphasic acquisitions in non-contrast, arterial portal venous, and late-venous phases, or multiphasic, contrast-enhanced liver magnetic resonance imaging (MRI) (1,5 T, Philips, Hamburg, Germany) was performed not earlier than 4 weeks prior and at approximately 4 weeks after TACE. All CT and MRI scans were assessed according to RECIST 1.1 criteria for nonarterially enhanced tumor entities[11] and mRECIST criteria for HCC.[12] Tumor response at 1 month after TACE was classified using the standard nomenclature for RECIST 1.1 and mRECIST: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR and PR were considered an objective response (OR).[13]

Table 1 Description of study population

	Study cohort
Patients undergoing TACE	$n = 48$
Gender (%): male–female	79.2–20.8
Age (years, median and range)	66 (37–89)
BMI (kg/m^2, median and range)	24.97 (17.16–36.72)
Hepatic malignancy (%)	
HCC	79.1
Liver metastasis (CRC)	12.5
Liver metastasis (gastric cancer)	2.1
Liver metastasis (pancreatic)	4.2
Liver metastasis (CCA)	2.1
Cause of HCC	
Alcoholic	27.0
HCV	21.6
HBV	13.5
Cryptogenic	21.6
Others (e.g. NASH)	16.2
Stage of liver cirrhosis (HCC only)	
CHILD A	83.3
CHILD B	16.7
OR to TACE therapy (%)	
Yes–No	41.5–58.5
Deceased during follow-up (%)	
Yes–No	74.5–25.5
Maximum tumor diameter (cm, median and range)	2.8 (1.0–12.9)

BMI, body mass index; CCA, cholangiocarcinoma; CHILD, Pugh-Child score; CRC, colorectal carcinoma; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; OR, objective response; TACE, transarterial chemoembolization.

Statistical analysis. Statistical analysis was performed as recently described in detail.[14] The prognostic role of suPAR was confirmed in uni- and multivariate Cox regression analyses. All statistical analyses were performed with SPSS 23 (SPSS, Chicago, IL, USA).[15] A P-value of <0.05 was considered statistically significant ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Results

Baseline suPAR levels are elevated in liver cancer patients. To evaluate the regulation of circulating suPAR levels in liver cancer patients, we first compared baseline suPAR levels of TACE patients with healthy controls. suPAR serum levels were significantly elevated in patients with HCC or liver metastases compared to healthy controls (Fig. 1a). The area

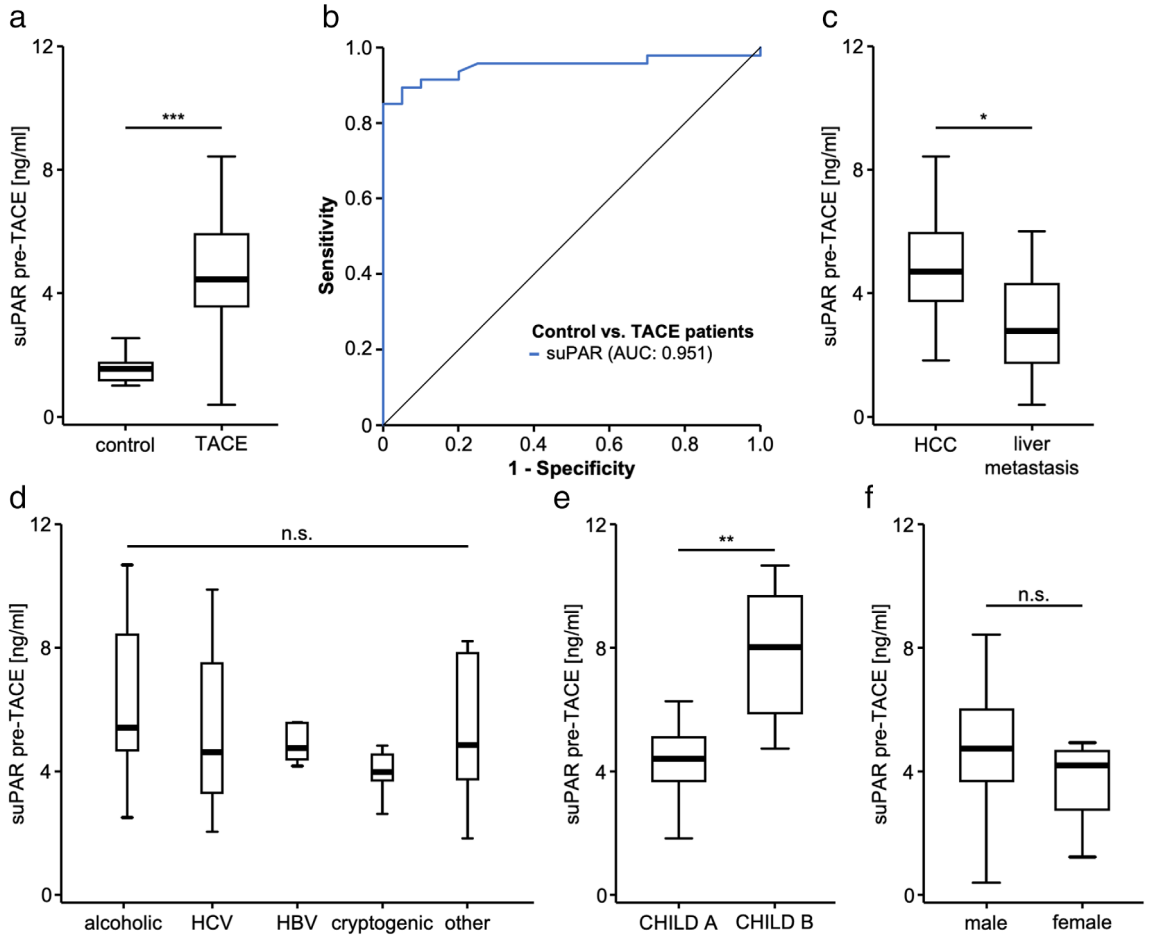

Figure 1 Circulating levels of soluble urokinase plasminogen activator receptor (suPAR) are elevated in patients with liver cancer. (a) Baseline suPAR levels are significantly elevated in patients with liver cancer. (b) receiver operating characteristics (ROC) curve analysis reveals an area under the curve (AUC) value of 0.951 for the discrimination between transarterial chemoembolization patients and healthy controls. (c) suPAR levels are significantly higher in hepatocellular carcinoma (HCC) patients compared to patients with liver metastases. (d) suPAR levels are comparable between the underlying disease etiologies (HCC only). (e) Patients with Child-Pugh score (CHILD) B liver cirrhosis have significantly higher suPAR values compared to CHILD A patients. (f) suPAR levels are unaltered between male and female patients.

under the curve (AUC) value of circulating suPAR for the discrimination between liver cancer patients and healthy controls was 0.951 (Fig. 1b). To gain further insights into the regulation of suPAR in our study cohort, we subsequently compared circulating suPAR levels between subgroups. Here, suPAR levels were significantly higher in HCC patients compared to patients with liver metastases (Fig. 1c), while the underlying disease etiology (alcoholic, hepatitis C virus, hepatitis B virus, cryptogenic or other, HCC patients only) had no significant impact on serum suPAR levels (Fig. 1d). Moreover, we observed significantly higher suPAR serum levels in patients with Child-Pugh score (CHILD) B liver cirrhosis compared to CHILD A patients (HCC patients only, Fig. 1e). Finally, suPAR levels were comparable between male and female patients (Fig. 1f).

To further dissect potential underlying mechanisms that trigger elevated suPAR serum levels in patients with HCC and liver metastases, we then performed extensive correlation analyses between baseline suPAR levels and various laboratory parameters. While suPAR did not correlated with alanine aminotransferase, Gamma-Glutamyltransferase (GGT), or bilirubin (Table S2), we observed a strong positive correlation between suPAR and creatine serum levels (r_S: 0.416, $P = 0.005$, Fig. S1A), as well as a significant negative correlation between suPAR and albumin levels (r_S: −0.500, $P < 0.001$, Fig. S1B). Moreover, suPAR positively correlated with C-reactive protein (CRP) (r_S: 0.309, $P = 0.041$) and lactate dehydrogenase serum levels (r_S: 0.359, $P = 0.027$, Table S2).

Baseline suPAR serum levels and tumor response to TACE therapy. In the next step, we aimed to evaluate if preinterventional suPAR levels might have a predictive value in terms of the individual response to 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. However, suPAR serum levels were comparable between these groups (Fig. 2a).

Moreover, receiver operating characteristics (ROC) curve analysis revealed a low AUC value of 0.546 regarding the discrimination between OR and non-OR patients based on initial suPAR serum levels. In line, binary logistic regression analysis did not reveal circulating suPAR levels as a predictor for OR after TACE (OR: 1.056, 95% CI: 0.816–1.367, $P = 0.677$).

Elevated suPAR serum levels are a prognostic factor for overall survival following TACE therapy. Next, we hypothesized that circulating suPAR levels might be indicative of the patients' overall survival (OS) rather than predicting the direct tumor response to TACE therapy. To test this hypothesis, we divided our cohort into two groups according to the baseline suPAR concentration using the 75th percentile (5.94 ng/mL) as a cut-off value. In Kaplan–Meier curve analysis, TACE patients with initial suPAR levels >5.94 ng/mL showed a strong trend toward an impaired OS, but statistical significance was not reached ($P = 0.086$, Fig. 3a). We therefore established an ideal prognostic cut-off value of 5.39 ng/mL that best identifies patients with an impaired outcome after TACE (see Methods section for details). When applying this cut-off value, Kaplan–Meier curve analysis revealed a significantly impaired OS for patients with baseline suPAR levels above the cut-off value (Fig. 3b). The median OS for patients with initial suPAR levels >5.39 ng/mL was only 256 days compared to 611 days for patients who had a suPAR level below the optimal cut-off value (Fig. 3b).

To further substantiate the prognostic potential of circulating suPAR and to exclude potential confounders, we subsequently performed uni- and multivariate Cox regression analyses (Table 2). Univariate Cox regression analysis revealed baseline suPAR levels above 5.39 ng/mL as a significant prognostic factor for OS (HR: 2.451, 95% CI: 1.219–4.930, $P = 0.012$). We then included parameters with a potential prognostic relevance in univariate analyses ($P < 0.200$) into multivariate analysis (tumor entity, leukocyte count, CRP). Here, suPAR serum levels stood out as an independent prognostic factor for OS (HR: 2.295, 95% CI: 1.090–4.832, $P = 0.029$). Importantly, the prognostic relevance of suPAR was also independent of the tumor entity, meaning that the prognostic role of circulating suPAR after TACE was relevant for both HCC and liver metastasis patients (Table 2).

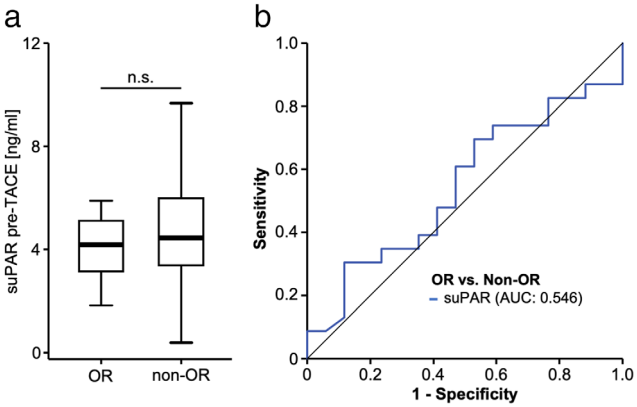

Figure 2 Preinterventional soluble urokinase plasminogen activator receptor (suPAR) serum levels and tumor response to transarterial chemoembolization (TACE). (a) suPAR levels before TACE are comparable between liver cancer patients who show an objective response (OR) and nonresponding (non-OR) patients. (b) receiver operating characteristics (ROC) curve analysis for the discrimination between OR and non-OR patients.

Postinterventional suPAR serum levels and patients' outcome. Based on the promising role of baseline suPAR levels to predict outcome following TACE therapy, we

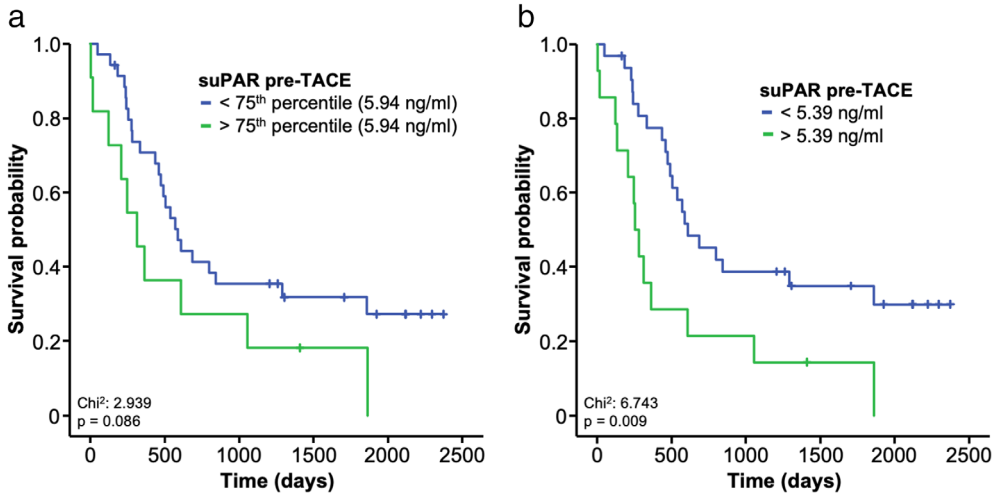

Figure 3 Elevated baseline soluble urokinase plasminogen activator receptor (suPAR) levels predict an unfavorable outcome after transarterial chemoembolization. (a) Liver cancer patients with baseline suPAR levels above the 75th percentile (5.94 ng/mL) show a strong trend toward an impaired postinterventional survival. (b) Patients with suPAR serum levels above the ideal prognostic cut-off value (5.39 ng/mL) have a significantly impaired overall survival compared to patients with baseline suPAR levels below this cut-off.

Table 2 Uni- and multivariate Cox regression analysis for overall survival

Parameter	Univariate Cox regression		Multivariate Cox regression	
	P-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)
suPAR >5.39 ng/mL	0.012	2.451 (1.219–4.930)	0.029	2.295 (1.090–4.832)
Tumor entity (HCC *versus* liver metastasis)	0.193	1.663 (0.774–3.576)	0.335	1.604 (0.614–4.187)
Age	0.560	1.009 (0.978–1.042)		
Gender	0.904	0.950 (0.414–2.181)		
Leukocytes	0.006	1.203 (1.053–1.373)	0.815	0.975 (0.793–1.200)
ALT	0.377	0.997 (0.990–1.004)		
LDH	0.491	1.001 (0.999–1.003)		
Bilirubin	0.635	1.117 (0.600–2.308)		
CRP	0.002	1.025 (1.009–1.042)	0.020	1.027 (1.004–1.051)

ALT, alanine transaminase; CRP, C-reactive protein; HCC, hepatocellular carcinoma; LDH, lactate dehydrogenase; suPAR, soluble urokinase plasminogen activator receptor.

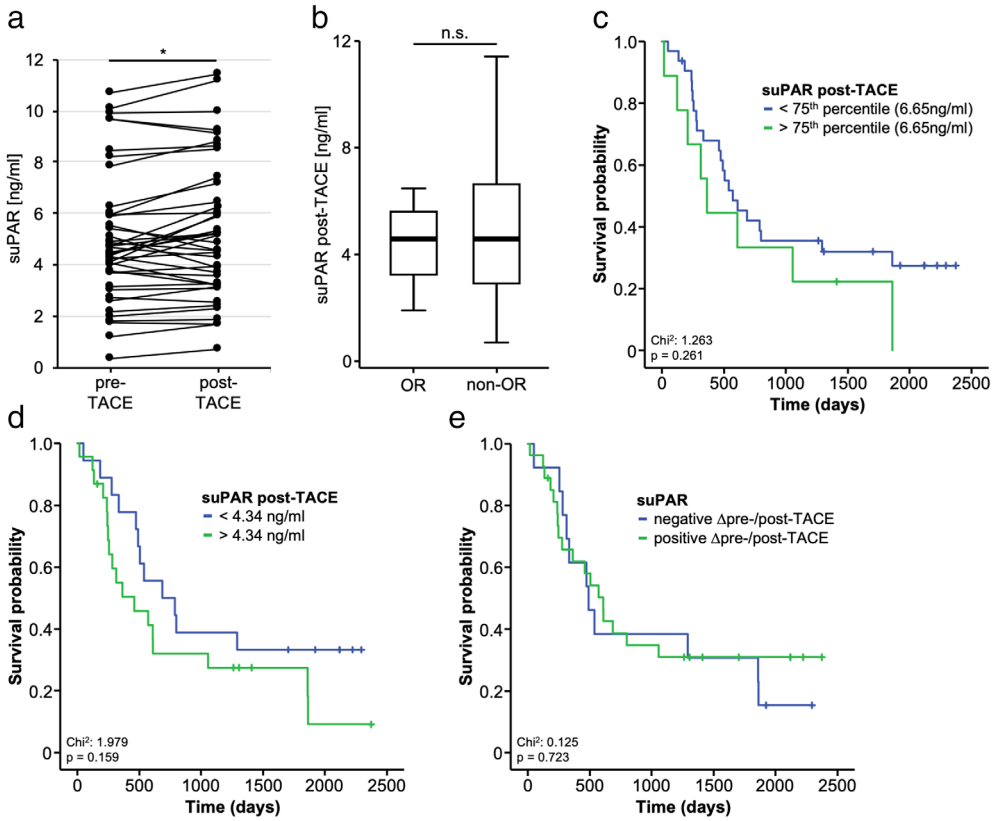

Figure 4 Postinterventional soluble urokinase plasminogen activator receptor (suPAR) levels and outcome to transarterial chemoembolization (TACE). (a) suPAR levels are significantly higher at day 1 after TACE compared to the respective pre-interventional levels. (b) suPAR levels after TACE are comparable between patients who show an objective response (OR) and non-responding (non-OR) patients. (c) Patients with baseline suPAR levels above the 75th percentile (6.65 ng/mL) have a trend toward an impaired postinterventional survival. (d) Patients with suPAR serum levels above the ideal prognostic postinterventional cut-off value (4.34 ng/mL) have a strong trend toward an impaired overall survival compared to patients with baseline suPAR levels below this cut-off.

finally evaluated the individual course of suPAR levels after TACE. Postinterventional suPAR levels at day 1 after TACE were available for $n = 42$ patients. When compared to the respective preinterventional suPAR concentrations, serum levels at day 1 after TACE were significantly higher (Fig. 4a). Similar to baseline levels, postinterventional suPAR levels were significantly elevated in HCC patients compared to liver metastases patients and CHILD B patients compared to CHILD A patients (HCC

only) but were unaltered between the underlying disease etiologies, as well as male and female patients (Fig. S2A–D).To asses if circulating suPAR levels at day 1 after TACE reflect an immediate response to TACE that might in turn be indicative of the tumor response at 1 month, we compared suPAR levels at day 1 between OR and non-OR patients. However, similar to the preinterventional results, we did not see a significant difference in suPAR levels between these groups (Fig. 4b). In line with this, ROC curve analysis revealed an AUC value of only 0.546 for the discrimination between OR and non-OR patients for day 1 suPAR levels. We then evaluated the potential impact of postinterventional suPAR serum levels on the patients' OS after TACE. Again, we first compared the OS in patients with very high postinterventional suPAR levels above the 75th percentile (6.65 ng/mL) and patients with day 1 serum levels below <6.65 ng/mL. Here, we observed a trend toward an impaired OS in the high suPAR group (Fig. 4c). When using the ideal prognostic cut-off value for postinterventional suPAR levels (4.34 ng/mL), this prognostic trend was further increased, but statistical significance was not reached ($P = 0.159$, Fig. 4d). Finally, we tested whether the individual kinetic of suPAR before and after TACE might reflect patients' outcome and compared the OS of patients with increasing or decreasing suPAR levels after TACE. However, we did not observe a significant difference in OS between these groups (Fig. 4e).

Discussion

The rise of multimodal therapeutic concepts has changed our view on how to treat cancer.[2,16] Advances in systemic chemotherapy, as well as novel surgical or locally ablative techniques, have led to a continuous and significant improvement in the survival of patients with primary and secondary liver cancer.[2,17] Here, we analyzed the prognostic and predictive role of suPAR in patients undergoing TACE for different tumor entities, with HCC representing the most important etiology, and showed that patients with elevated baseline suPAR serum concentration face a dismal prognosis. Interestingly, the results of our analysis remained unchanged when only HCC patients were considered. According to the current EASL Clinical Practice Guidelines for the management of HCC, TACE is "the most widely used primary treatment for unresectable HCC" and is the recommended first-line therapy for patients with intermediate-stage disease.[18] The intense arterial neoangiogenic activity during its progression builds the rationale for TACE, which relies on the intra-arterial infusion of a cytotoxic agent followed by embolization of the tumor-feeding blood vessels.[19] The indication for TACE should consider tumor burden, underlying liver disease, and performance status.[19,20] It is well known that, for example, patients with low performance status or an impaired liver function (Child-Pugh C or B) are unlikely to benefit from TACE, which is often detrimental in such patients.[6,19] Moreover, inadequate hepatic function, such as serum bilirubin >2 mg/dL and a tumor burden >50% of total liver volume, increases the risk of hepatic decompensation after TACE.[6,19] Current guidelines recommend discussing indications for TACE in multidisciplinary tumor boards in light of alternative treatments [Quelle]. In routine clinical practice, the individual decision for or against TACE would be significantly facilitated if reliable preinterventional stratification tools reflecting the tumor biology are available. In this context, the data presented here, as well as previous data from our[21,22] and other groups,[6,8] have the potential to change clinical decision-making in patients with both primary and secondary liver tumors.

suPAR is the cleavage product of the membrane-bound plasminogen activator (uPA) receptor (uPAR), which is expressed on the cell surface of a variety of cells including endothelial cells and has been associated with several clinical conditions such as systemic inflammation and cancer.[23–25] In different cancers, elevated levels of suPAR were indicative of an advanced disease stage and impaired patients´ prognosis.[14] Several studies have shown the role of the uPA/uPAR pathway in cancer development. As an example, uPA-deficient patients demonstrated an impaired progression of melanoma.[26] On a functional level, this might be explained by the fact that the uPA/uPAR system regulates cell apoptosis through caspase-3-dependent mechanisms.[27,28] In HCC, suPAR levels were found to be elevated even in the absence of underlying cirrhosis compared to patients with non-alcoholic fatty liver disease.[24] In a previous study, elevated levels of suPAR were found to be an excellent predictive marker for the development of HCC.[24] In line with these data, we show elevated suPAR serum concentrations in patients with HCC before TACE. Interestingly, suPAR serum levels were similar in patients who responded to therapy and those who did not. Nevertheless, suPAR levels were significantly higher in patients who succumbed to death early during long-term follow-up compared to survivors. These data suggest that a tumor-independent mechanism might be responsible for the prognostic function of suPAR in patients receiving TACE. Supporting this hypothesis, patients with more severe liver dysfunction (Child-Pugh B) displayed higher suPAR concentrations compared to those with a preserved liver function (Child-Pugh A). Similarly, Zimmermann et al. demonstrated that suPAR levels are elevated in decompensated cirrhosis and indicate an immune cell activation and an elevated mortality.[29] Of note, the exact source of elevated serum suPAR levels in cancer patients is unknown. While no data on uPAR expression in HCC are available, it is well known that primary CRCs, as well as metastases of CRC, express uPAR in immunohistochemical analyses. Notably, both infiltrating immune cells and tumor cells, as well as the stromal tissue, show positive uPAR expression,[30,31] which is in line with our own data.[32] It therefore appears possible that elevated suPAR serum concentrations in patients with CRC, as well as in patients with HCC, are caused by increased shedding of tumor cells, which has recently been suggested to reflect immune activation in the microenvironment of tumors.[33,34] The exact molecular link between high suPAR levels and a poor prognosis is presently not fully understood and beyond the scope of this manuscript. Nevertheless, the previous suggested link between uPAR and cell apoptosis, adhesion, and migration, representing essential processes in the development of cancer, may provide an explanation for this link. Therefore, our results should trigger further molecular research, for example, using *uPAR*$^{-/-}$ mice,[35] to further understand the role of suPAR in patients receiving TACE therapy.

Elevated suPAR serum concentrations have been described in the context of numerous acute inflammatory reactions and after tissue damage. We show a further increase in median suPAR concentrations at day 1 after TACE. Interestingly,

this increase had no prognostic significance. Similarly, post-TACE suPAR levels did not reflect the patient's overall outcome. As the pathophysiology behind elevated serum concentrations in the context of cancer is only poorly understood, we cannot fully explain these observations. As who that responded to TACE demonstrate similar serum levels than those who did not, it seems likely that extra tumoral factors such as systemic inflammation might influence post-TACE suPAR levels.

Notably, our study has several important limitations. First, the number of analyzed patients is rather low when compared to large clinical trials. Second, we included a rather heterogeneous patient cohort featuring both patients with primary and secondary hepatic malignancies. Despite the fact that this heterogeneity could negatively affect entity-specific conclusions, it is important to remember that the prognostic relevance of suPAR was independent of the tumor entity. Thus, the prognostic effects of suPAR seem to represent a method specific to TACE therapy and are not tumor specific—a finding that was only possible by including both patients with primary and secondary liver lesions in this study. suPAR is an inflammatory cytokine involved in many different processes and reflects manifold tumor-related processes such as tumor regeneration and proliferation, arguing that elevated suPAR might be rather method-specific for TACE than being truly tumor specific. Currently established TACE scoring systems[36] mainly rely on HCC-specific parameters. Therefore, preinterventional measurements of suPAR might be a valuable addition to future tumor entity-independent stratification algorithms for TACE, which could further improve the clinical applicability of these scores. However, larger confirmatory clinical studies including different treatment approaches [e.g. radio frequency ablation (RFA), tumor resection, or liver transplantation], as well as longitudinal postinterventional suPAR measurements, are warranted to fully elucidate the role of suPAR in the context of TACE for primary and secondary liver cancers.

Acknowledgment

The suPAR ELISA kits were provided by Virogates (Denmark).

References

1 Tang A, Hallouch O, Chernyak V, Kamaya A, Sirlin CB. Epidemiology of hepatocellular carcinoma: target population for surveillance and diagnosis. *Abdom. Radiol.* 2018; **43**: 13–25.
2 Rashidian N, Alseidi A, Kirks RC. Cancers metastatic to the liver. *Surg. Clin. North Am.* 2020; **100**: 551–63.
3 Fiorentini G, Sarti D, Aliberti C, Carandina R, Mambrini A, Guadagni S. Multidisciplinary approach of colorectal cancer liver metastases. *World J. Clin. Oncol.* 2017; **8**: 190–202.
4 Shamimi-Noori S, Gonsalves C, Shaw C. Metastatic liver disease: indications for locoregional therapy and supporting data. *Semin. Intervent. Radiol.* 2017; **34**: 145–66.
5 Sieghart W, Hucke F, Pinter M *et al*. The ART of decision making: retreatment with transarterial chemoembolization in patients with hepatocellular carcinoma. *Hepatology*. 2013; **57**: 2261–73.
6 Sacco R, Tapete G, Simonetti N *et al*. Transarterial chemoembolization for the treatment of hepatocellular carcinoma: a review. *J. Hepatocell. Carcinoma.* 2017; **4**: 105–10.
7 Massmann A, Rodt T, Marquardt S *et al*. Transarterial chemoembolization (TACE) for colorectal liver metastases—current status and critical review. *Langenbecks Arch. Surg.* 2015; **400**: 641–59.
8 Finn RS, Zhu AX, Wigdan F *et al*. Therapies for advanced stage hepatocellular carcinoma with macrovascular invasion or metastatic disease: a systematic review and meta-analysis. *Crit. Care*. 2011; **15**: R63.
9 Loosen SH, Breuer A, Tacke F *et al*. Circulating levels of soluble urokinase plasminogen activator receptor predict outcome after resection of biliary tract cancer. *JHEP Rep.* 2020; **2**: 100080.
10 Loosen SH, Tacke F, Püthe N *et al*. High baseline soluble urokinase plasminogen activator receptor (suPAR) serum levels indicate adverse outcome after resection of pancreatic adenocarcinoma. *Carcinogenesis*. 2019; **40**: 947–55.
11 Eisenhauer EA, Therasse P, Bogaerts J *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur. J. Cancer*. 2009; **45**: 228–47.
12 Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin. Liver Dis.* 2010; **30**: 52–60.
13 Edeline J, Boucher E, Rolland Y *et al*. Comparison of tumor response by response evaluation criteria in solid tumors (RECIST) and modified RECIST in patients treated with sorafenib for hepatocellular carcinoma. *Cancer*. 2012; **118**: 147–56.
14 Loosen SH, Roderburg C, Kauertz KL *et al*. Elevated levels of circulating osteopontin are associated with a poor survival after resection of cholangiocarcinoma. *J. Hepatol.* 2017; **67**: 749–57.
15 Roy S, Hooiveld GJ, Seehawer M *et al*. microRNA 193a-5p regulates levels of nucleolar- and spindle-associated protein 1 to suppress hepatocarcinogenesis. *Gastroenterology*. 2018; **155**: 1951–1966.e26.
16 Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018; **391**: 1301–14.
17 Dörr NM, Bartels M, Morgul MH. Current treatment of colorectal liver metastasis as a chronic disease. *Anticancer Res*. 2020; **40**: 1–7.
18 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu PR, European Association for the Study of the Liver A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J-L, et al. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J. Hepatol*. 2018; **69**: 182–236.
19 Sangro B, D'Avola D, Iñarrairaegui M, Prieto J. Transarterial therapies for hepatocellular carcinoma. *Expert Opin. Pharmacother.* 2011; **12**: 1057–73.
20 Galle PR, Forner A, Llovet JM *et al*. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J. Hepatol.* 2018; **69**: 182–236.
21 Loosen SH, Schulze-Hagen M, Leyh C *et al*. IL-6 and IL-8 serum levels predict tumor response and overall survival after TACE for primary and secondary hepatic malignancies. *Int. J. Mol. Sci.* 2018; **19**: 1766.
22 Loosen SH, Schulze-Hagen M, Bruners P *et al*. Sarcopenia is a negative prognostic factor in patients undergoing transarterial chemoembolization (TACE) for hepatic malignancies. *Cancers*. 2019; **11**: 1503.
23 Koch A, Voigt S, Kruschinski C *et al*. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. *Crit. Care*. 2011; **15**: R63.
24 Chounta A, Ellinas C, Tzanetakou V *et al*. Serum soluble urokinase plasminogen activator receptor as a screening test for the early diagnosis of hepatocellular carcinoma. *Liver Int.* 2015; **35**: 601–7.
25 Fidan E, Mentese A, Ozdemir F *et al*. Diagnostic and prognostic significance of CA IX and suPAR in gastric cancer. *Med. Oncol.* 2013; **30**: 540.
26 Shapiro RL, Duquette JG, Roses DF *et al*. Induction of primary cutaneous melanocytic neoplasms in urokinase-type plasminogen activator (uPA)-deficient and wild-type mice: cellular blue nevi invade but do not progress to malignant melanoma in uPA-deficient animals. *Cancer Res.* 1996; **56**: 3597–604.

27 Chen Y, Kelm RJ, Budd RC, Sobel BE, Schneider DJ. Inhibition of apoptosis and caspase-3 in vascular smooth muscle cells by plasminogen activator inhibitor type-1. *J. Cell. Biochem.* 2004; **92**: 178–88.
28 Soeda S, Oda M, Ochiai T, Shimeno H. Deficient release of plasminogen activator inhibitor-1 from astrocytes triggers apoptosis in neuronal cells. *Mol. Brain Res.* 2001; **91**: 96–103.
29 Zimmermann HW, Reuken PA, Koch A *et al*. Soluble urokinase plasminogen activator receptor is compartmentally regulated in decompensated cirrhosis and indicates immune activation and short-term mortality. *J. Intern. Med.* 2013; **274**: 86–100.
30 Kim T-D, Song K-S, Li G *et al*. Activity and expression of urokinase-type plasminogen activator and matrix metalloproteinases in human colorectal cancer. *BMC Cancer*. 2006; **6**: 211.
31 Illemann M, Bird N, Majeed A *et al*. Two distinct expression patterns of urokinase, urokinase receptor and plasminogen activator inhibitor-1 in colon cancer liver metastases. *Int. J. Cancer*. 2009; **124**: 1860–70.
32 Loosen SH, Tacke F, Binnebosel M *et al*. Serum levels of soluble urokinase plasminogen activator receptor (suPAR) predict outcome after resection of colorectal liver metastases. *Oncotarget*. 2018; **9**: 27027–38.
33 Koch A, Zimmermann HW, Gassler N *et al*. Clinical relevance and cellular source of elevated soluble urokinase plasminogen activator receptor (suPAR) in acute liver failure. *Liver Int.* 2014; **34**: 1330–9.
34 Backes Y, van der Sluijs KF, Mackie DP *et al*. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. *Intensive Care Med.* 2012; **38**: 1418–28.
35 Genua M, D'Alessio S, Cibella J *et al*. The urokinase plasminogen activator receptor (uPAR) controls macrophage phagocytosis in intestinal inflammation. *Gut*. 2015; **64**: 589–600.
36 Kadalayil L, Benini R, Pallan L *et al*. A simple prognostic scoring system for patients receiving transarterial embolisation for hepatocellular cancer. *Ann. Oncol.* 2013; **24**: 2565–70.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1 (A) SuPAR serum levels before TACE positively correlate with serum creatinine levels. (B) SuPAR serum levels before TACE negatively correlate with serum albumin levels.

Figure S2 (A) SuPAR levels at day 1 after TACE are significantly higher in HCC patients compared to patients with liver metastases. (B) Postinterventional suPAR levels are comparable between the underlying disease etiologies (HCC only). (C) Patients with CHILD B liver cirrhosis have significantly higher suPAR levels at day 1 compared to CHILD A patients. (D) Postinterventional suPAR levels are unaltered between male and female patients.

Table S1. Serum levels of laboratory markers
Table S2. Correlation analyses of baseline suPAR levels with various laboratory parameters of organ dysfunction.