DNase is a prognostic marker in liver cancer patients receiving transarterial chemoembolization therapy

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Introduction

Transarterial chemoembolization (TACE) has been shown to be an effective therapy in late stages of hepatocellular cancer (HCC). Local application of cytotoxic drugs, iodized oil and embolic material leads to ischemic necrosis and tumor shrinkage \cite{1}.

Stratifying patients before treatment and early modification of the therapy are important aspects of individual patient care in order to optimize therapy efficacy and to avoid unnecessary side effects with the related morbidity. Therefore, clinical and laboratory parameters are highly valuable prognostic and predictive markers. To date, various clinical factors, such as tumor size, portal invasion, ascites, performance status and Child-Pugh score as well as the laboratory biomarkers bilirubin and $\alpha$-fetoprotein (AFP), have been reported as meaningful pre-therapeutic prognostic and predictive parameters in HCC patients undergoing TACE therapy \cite{2}. As cytotoxic drugs induce cell death, biomarkers reflecting necrotic and apoptotic processes are considered to be appropriate parameters indicating therapeutic efficacy already during the first days after drug application \cite{3,4}.

Recently, we observed elevated serum levels of the cell death marker nucleosomes in HCC patients as compared with healthy controls and these levels further increased already 24 hours after TACE therapy. In addition, the levels of circulating nucleosomes 24 hours after TACE indicated that later treatment response with high values is associated with poor therapy outcome \cite{5}. As the enzyme DNase catalyses hydrolyzation of chromatin to shorter nucleosomal fragments during apoptotic cell death and because it possibly plays a relevant role in clearance of nucleosomes from the blood \cite{6}, it seemed to be a promising marker that should be investigated in this setting. Additionally, we analyzed the levels of the DNA-binding nuclear protein HMGB1. HMGB1 is released separately or as complexes with nucleosomes – actively upon cytokine stimulation or passively during cell death – and can elicit an immune response via interaction with macrophages, dendritic and other antigen presenting cells \cite{7}. In earlier studies, elevated HMGB1 expressions levels were reported in colon, prostate, pancreatic, breast cancer and melanoma. Furthermore, HMGB1 levels were associated with invasion, metastasis and prognosis in diverse cancers (reviewed in \cite{8}). Here, we investigated serum DNase activity and HMGB1 levels concerning their prognostic impact in HCC patients undergoing TACE therapy.

Methods

Patients

50 HCC patients (42 males and 8 females, mean age 66.7 years), diagnosed and treated at the University Hospital Munich-Grosshadern between 2006 and 2008, were consecutively included in this prospective study (Table 1). Diagnosis of HCC was confirmed according to the Barcelona EASL Conference 2000 criteria. Staging investigations including CT and MRI were performed before start of the TACE therapy.
For evaluation of prognosis only 38 patients were considered as 6 patients were additionally treated with radiofrequency ablation (RFA) 1 day after TACE and another 6 patients were treated with bridging intention prior to liver transplantation. As these additional treatments influence patient outcome, only the remaining 38 patients who were solely treated with TACE were included in this study.

The treatment procedures were carried out by staff of the Institute of Radiology at the University Hospital Munich as described earlier [5]. The embolizing agent was typically mixed with 3 – 5 ml lipiodol, microparticles of 150 – 500 μm (e.g., Contour SE®, Boston Scientific, Ratingen, Germany) and farnorubicin (1 mg/kg b.w.) [5].

The study was approved by the local ethics committee and written informed consent was obtained from each patient prior to study entry.

### Sample collection and assays

Blood samples of all participating patients were prospectively collected before (0 h) and 24 hours after TACE. Following centrifugation at 3,000 × g for 15 min within 1 – 2 hours after venipuncture, aliquots of serum samples were stored at –80 °C. The quantification of serum DNase activity was performed by a solid phase enzyme immunoassay (ELISA) of Orgentec Diagnostika GmbH (Mainz, Germany), which measured the reduction of activity of deoxyribonuclease. Pathologic samples are supposed to exhibit a higher activity reduction (%AR), which means lower DNase activities. HMGB1 concentration was measured by use of a Sandwich ELISA from SHINO-Test/IBL (Tokio, Japan/Hamburg, Germany).

### Statistics

Concentrations of all measured markers before and 24 hours after TACE as well as their percentage changes were considered for statistical evaluation. For the analysis of overall survival, defined as time from the first evaluated TACE treatment to death or last follow-up, Kaplan-Meier curves and the log-rank test were used. Medians and quartiles of the marker levels were used as cut-offs in Kaplan-Meier analyses. To identify independent predictors of survival we used the Cox-regression model. Akaike information criterion (AIC) was calculated to compare the prognostic strength with different recent models. In this multivariate analysis, we additionally used markers of a prior study on other laboratory markers, measured in the same patient setting [9], to check whether the new markers could improve the former prognostic model. All markers were used as logarithms, medians or quartiles.

A p-value of < 0.05 was considered statistically significant. All calculations were performed by software of SAS (version 9.2, SAS Institute Inc., Cary, N.C., USA).

### Results

For all of the 38 patients included in this study, follow-up was more than one year.
Within the first year after the first observed TACE treatment, 16 patients died due to tumor progression. No correlation between therapy response according to staging investigations with overall survival was found in our setting (p = 0.384). With regards to the biomarkers, DNase activity reduction revealed a prognostic impact. Taking the median as cut-off, low DNase activity levels (high AR%) were significantly associated with more favorable outcome when measured before (AR cutoff 17.2%; p = 0.0016) and 24 hours after TACE (AR cutoff 20.5%; p = 0.0016). HMGB1 levels were not associated with prognosis (Figure 1).

Subsequently, DNase levels before (0 h) and 24 hours after TACE were included into a multivariate Cox regression analysis – together with clinical and other biochemical parameters that had proven to be of prognostic relevance in an earlier study in the same patient cohort. These markers included nucleosomes, cytokeratin-19-fragments (CYFRA 21-1), α-fetoprotein (AFP), several liver enzymes and inflammatory biomarkers [9]. Of all possible models including a maximum of three parameters, the combination of DNase (24 h), choline esterase (CHE; 24 h) and CYFRA 21-1 (24 h) yielded the best prognostic model with an AIC of 88.1. This was in fact slightly better than the model obtained in the previous study comprising CHE (24 h) and AFP (24 h) with an AIC of 90.3 (Table 2) [9].

The observation that higher DNase activity reduction was associated with longer overall survival was surprising as from earlier studies reporting high serum nucleosome levels in cancer disease (reviewed in 3) it was assumed that patients with advanced cancers and with poor prognosis would have lower DNase activity (higher AR%) resulting in reduced elimination of nucleosomes from the blood circulation. One possible explanation of our findings may be that the dramatically increased release of nucleosomes in patients with poor survival causes a maximum upregulation of DNase activity especially in these patients. Indeed, high nucleosome levels 24 hours after TACE treatment were observed in non-responsive patients and in patients with poor prognosis, possibly despite upregulated DNase activity [5].

Apart from DNase activity, all univariate-ly relevant prognostic variables of an earlier evaluation on the same patient cohort were included into the multivariate analysis [9]. To avoid overfitting of the model to the present sample, biomarkers were used as logarithmic variables or only quartiles were tested as potential cutoffs. As many biomarker combinations yielded similar prognostic strength and

### Table 2. Multivariate prognostic models for overall survival.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate</th>
<th>χ²-Square</th>
<th>p-value</th>
<th>Hazard-ratio</th>
<th>95%-Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNase (24 h; median)</td>
<td>−2.019</td>
<td>8.0</td>
<td>0.0047</td>
<td>0.1</td>
<td>&lt; 0.1 – 0.5</td>
</tr>
<tr>
<td>CHE (24 h; log)</td>
<td>−2.229</td>
<td>7.2</td>
<td>0.0074</td>
<td>0.1</td>
<td>&lt; 0.1 – 0.6</td>
</tr>
<tr>
<td>CYFRA 21-1 (24 h; median)</td>
<td>1.415</td>
<td>4.3</td>
<td>0.0378</td>
<td>4.1</td>
<td>1.1 – 15.6</td>
</tr>
</tbody>
</table>

Figure 1. Kaplan Meier curves showing the overall survival of patients according to A) pre-therapeutic (0 h) and B) post-therapeutic (24 h) DNase activity reduction (p-values for both comparisons are p = 0.0016).
DNase and HMGB1 levels for prognosis in HCC patients on TACE

b biomarkers would in part be exchangeable, we tested all possible two and three-marker combinations and compared them using the Akaike information criterion that indicates the strength of a prognostic model. The thus identified best model of pre- and post-therapeutic independent prognostic biomarkers comprised the combination of DNAse, CHE and CYFRA 21-1 (all 24 h) which in fact showed a slightly stronger prognostic impact than the model described earlier consisting of AFP and CHE (both at 24 h) [9].

The control of subsequent treatment effects is a well-known, considerable problem of every predictive and prognostic evaluation. In order to avoid any influence of additional treatments, we only focused on patients treated with palliative intention and excluded patients who were additionally treated by RFA or who were scheduled for liver transplantation later on. Obviously, the remaining number of patients in the present study is limited. However, it has to be emphasized that all patients were included prospectively into the study and with no other selection criteria as indicated, and all pre-analytical and analytical steps were consequently controlled to guarantee quality and completeness of the data presented.

Conclusions

With the aid of uni- and multivariate analyses, the present study has identified serum DNase activity as a prognostic parameter in patients with liver cancer undergoing treatment using TACE. Further validation in prospective studies is recommended.

References


