Prediction of recurrence of HCC after TACE using enhanced CT heterogeneity assessment

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Research Article

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Abstract

**Purpose:** To evaluate the value of enhanced computed tomography (CT) heterogeneity in predicting early recurrence of patients with hepatocellular carcinoma (HCC) after transarterial chemoembolization (TACE).

**Methods:** Forty-seven HCC patients (40M/7F) were included in the study. Tumor histogram and texture analysis were performed on contrast-enhanced CT imaging prior to TACE. The clinical diagnosis of HCC at recurrent stages was based on the criteria of the American Association for the Study of Liver Diseases (AASLD) or biopsy. Two parameters of HCC heterogeneity (histogram and texture) were compared in the Early Recurrence (ER) and the Non-Early Recurrence (Non-ER) groups. Analysis was applied to both single-slice ROI and whole-tumor volumetric VOI. Receiver operating characteristic (ROC) was calculated to determine the ability of the parameters to differentiate between ER and non-ER groups.

**Results:** 27 patients with ER and 20 patients with NER. The largest diameters of tumors in ER group were significantly larger than NER group (P < 0.001). Texture results for ROI and VOI analyses were similar. In the histogram analysis, the 50th percentile of pixel intensity predicted early recurrence with a sensitivity (Se) of 92.6%. In texture analysis, entropy, mean, and inhomogeneity were significantly associated with early recurrence (P < 0.05) but not skewness and kurtosis (P > 0.05). Inhomogeneity had the highest diagnostic specificity (95%).

**Conclusions:** This study shows that CT histogram and texture features are significantly different in ER group from Non-ER Group.

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor and the third highest cause of death in the cancer worldwide[1]. Affected by advanced tumor size and/or impaired liver function, most HCC patients are not suitable for surgical resection. Transarterial chemoembolization (TACE) can deliver lipiodol and chemotherapeutics to highly vascularized tumors by targeting blood vessels as an effective treatment recommended for intermediate-stage HCC[2]. However, some HCC patients do not respond well, possibly because lipiodol cannot be completely preserved. Disease progression or relapse after two to three cycles of TACE therapy is often an indicator of discontinuation of the therapy[3]. Accurate early assessment of the effectiveness of TACE is critical for treatment planning and can facilitate the decision to repeat therapy early in patients who respond well to eliminate residual small tumor patterns. Through changes in apparent diffusion coefficient (ADC), Vandecaveye et al[4] found that HCC patients treated with TACE for 1 month had a median progression-free survival (PFS) of only 5 months. Patients with early relapse do not respond well to repeat TACE. Therefore, it is recommended to consider other treatments and it is important to identify recurrence within six months. This study aimed to evaluate the capability of heterogeneity assessment in predicting early recurrence of HCC after TACE treatment. Our study hopes that the assessment of tumor heterogeneity by voxel-wise histogram and texture analysis of CT enhanced imaging data can help predict early recurrence better than CT values.
Materials And Methods

**Patient Population**

This is a retrospective study that included patients from January 2016 and March 2021. Inclusion criteria were those patients with AASLD criteria or biopsy who underwent TACE for unresectable HCC. All patients underwent CT examinations before and after TACE. Those with severe complications or extensive distant metastases were excluded. At the first month after treatment, contrast-enhanced CT scans and liver biochemical and tumor markers (primarily serum alpha-fetoprotein (AFP)) were examined to confirm tumor clearance. Tumor surveillance was repeated every 3 months. According to AASLD guidelines, new lesions with arterial wash-in and portal venous wash-out are defined as intrahepatic recurrence. Those who relapsed within six months were ER, and the rest were NER. All clinical and imaging follow-up results were obtained through electronic medical records and the Picture Archiving and Communication System (PACS). When recurrence occurred, time was recorded in detail. Patients who underwent contrast-enhanced CT exams within the week prior to TACE were selected for analysis. 47 consecutive patients with HCC from pretreatment contrast-enhanced CT studies (40M/7F, median age 55 years) were finally enrolled in our study.

**Transarterial Chemoembolization**

TACE was performed by two interventional radiologists using Seldinger puncture. Superselective cannulation of tumor-feed hepatic artery was performed if possible. First, portal circulation and tumor location were evaluated through abdominal angiography using 5-French RH (COOK, Bloomington, IN, United States). The feeding artery of HCC was found utilizing a coaxial microcatheter, and the catheter was advanced to the distal area. Subsequently, an emulsion of oxaliplatin (Jiangsu HengRui Medicine co., LTD, China) and iodized oil (Yantai LuYin pharmaceutical co., LTD, China) at a volume ratio of 1:1 was injected into the tumor-feed hepatic artery. The injected volume depended on the tumor size. Thereafter, embolization was performed using gelatin sponge particles or polyvinyl alcohol. At the end of treatment, the catheter was removed and the site of puncture was pressed to prevent hemorrhage. TACE was repeatedly every 4–6 weeks according to medical guidelines.

**CT Enhancement Imaging Technique and Analysis**

All scans were performed on a 256-slice MDCT scanner (Philips iCT, Best, The Netherlands). Two-phase liver CT imaging consisting of arterial and portal phase images were performed and portal venous phase exams were used for all measurements. The abdominal position was the first image from the diaphragm to the lower border of the liver. Subsequently, 100 ml of iodinated contrast (Iohexol 350 mg/ml, GE pharmaceutical (Shanghai) co., LTD) was administrated through the antecubital vein at a rate of 4.0ml/s. Images were acquired using bolus tracking. FOV and slice thickness were fixed at 350mm and 5mm respectively. Lamellar images with a slice thickness of 3mm were also reconstructed for follow-up studies.
One abdominal radiologist (with 10 years of clinical experience in abdominal imaging) and one CT technician (a medical student) analyzed the contrast-enhanced CT images. They were familiar with the tumor pattern but not the clinical information and prognosis. All axial images were exported in DICOM format and image analysis was performed using commercially available Firevoxel software (https://wp.nyu.edu/ firevoxel). CT images were reviewed with PACS to confirm the margin of the tumor, and the single slice with the largest diameter of the HCC was selected for ROI analysis. Subsequently, the CT technologist, under the supervision of an abdominal radiologist, manually drew a free-hand ROI along the margin of the HCC in the largest cross-sectional area. VOI analysis consists of two stages. First, the lesion margin was drawn manually slice by slice on the two-dimensional axial image. Consequently, after the software generated a 3-dimensional VOI, the contour of the VOI was edited manually if necessary to assure that the adjacent vessel was not included and the whole tumor volume was covered (Fig.1).

**Histogram and texture parameters**

Histogram (mean, standard deviation (SD), 10th, 50th, and 90th) and texture (kurtosis, skewness, entropy, and inhomogeneity) parameters of the target lesion were automatically calculated and extracted.

- Skewness, a measure of asymmetry of data points, which indicates the majority of data points on the right or left side of the histogram in comparison to the normal distribution.
- Kurtosis, a measure of the peakedness of data points, which was used to describe the sharpness of the data distribution.
- Entropy and inhomogeneity, as the two most important texture parameters, they represent the irregularity and intensity of pixel values within the ROI or VOI.

\[
\text{Entropy} = \sum_{i=0}^{255} p_i \log p_i
\]

\[
\text{Inhomogeneity}=\text{sd.} /\text{mean}
\]

Inhomogeneity=sd. /mean  \( (2) \)

\( i \) indicates the gray value of the pixel \( (0 \leq i \leq 255) \), while \( j \) represents the neighborhood gray mean \( 0 \leq j \leq 255 \). \( \text{sd.} \) is the standard deviation, and \( \text{mean} \) is the average intensity.

The 10th, 50th, and 90th percentiles of the histogram represent pixel values below which 10, 50, and 90% of the voxels in the corresponding ROI or VOI.

All above parameters were calculated on the two-dimensional and three-dimensional images.

**Statistical Analysis**

Statistical analysis was performed using commercially available statistics software SPSS Statistics (release 16.0, SPSS Inc, Chicago, IL) and the Medcalc version 16.1 statistical software (MedCalc Software bvba, Ostend, Belgium). Obtained continuous variables were summarized with descriptive statistics and data were presented as means ± SD. To assess the association between different texture parameters,
matrix scatter was utilized in ROI and VOI. Bland–Altman 95% limits of agreement and bias were computed to assess the agreement between ROI and VOI measurements. All variables of the histogram and texture parameters were tested for normal distribution with the Kolmogorov–Smirnov test. According to the results of the Kolmogorov-Smirnov test, an unpaired t-test or nonparametric test was performed for the comparison of tumor size (mainly the largest diameters) and computed parameters between the ER and the Non-ER groups. Receiver operating characteristic curves (ROC) with calculation of the area-under-curve (AUC) were constructed to determine the best predictive parameter and cut-off value for identifying patients responding to TACE. The level of significance was set at a two-tailed P value < 0.05.

Results

1. General data analysis

Demographic data and tumor characteristics of the patients were listed in Table 1.

In total, 27 ER patients (57.4%) and 20 NER patients (42.6%) were compared. The maximum long and short diameters of the ER group were (88.2±36.3) mm and (66.9±30.2) mm, respectively, and the maximum and short diameters of the Non-ER group were (41.4±21.4) mm and (29.3±19.8) mm. Notably, the maximum diameter of the ER group was higher than that of the Non-ER group, and the difference between the two groups was statistically significant (t=4.87, P<0.001 and t=4.61, P<0.001).
Table 1. Patient and tumor characteristics

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER group</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>23/4</td>
</tr>
<tr>
<td>Age, (range, years)</td>
<td>33-75</td>
</tr>
<tr>
<td>Clinical symptom</td>
<td></td>
</tr>
<tr>
<td>Hepatitis history</td>
<td>4</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>13</td>
</tr>
<tr>
<td>Chance discovery</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
</tr>
<tr>
<td>Number of tumor</td>
<td></td>
</tr>
<tr>
<td>Single lesion</td>
<td>19</td>
</tr>
<tr>
<td>Multiple lesions</td>
<td>8</td>
</tr>
<tr>
<td>Location of tumor</td>
<td></td>
</tr>
<tr>
<td>Left liver lobe</td>
<td>3</td>
</tr>
<tr>
<td>Right liver lobe</td>
<td>18</td>
</tr>
<tr>
<td>Whole liver in two lobes</td>
<td>6</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td></td>
</tr>
<tr>
<td>With/Without</td>
<td>9/18</td>
</tr>
</tbody>
</table>

Comparison between ROI and VOI

Association between different parameters was assessed using matrix scatter (Fig.2). When comparing ROI vs. VOI measurements of lesions, the overall results were fairly similar in Bland–Altman analysis. For instance, the limits of agreement were -0.2, 0.11, and bias -0.04 for entropy (Fig.3). Subsequently, to prevent selection bias, all subsequent analyses were based on VOI data.

Histogram and Texture Analysis

For SD (10th and 50th percentile of pixel intensity), histogram analysis showed significantly lower values for the ER group (p = 0.001, 0.001, and 0.013, Table 2). In contrast, no significant difference was found in the 90th percentile (p=0.06). Entropy, mean, and inhomogeneity of texture analysis all showed significant differences between the two groups (Table 2, Fig.4), but not skewness and kurtosis (Table 2).
Table 2 CT histogram and texture analysis of HCC before TACE according to recurrence time

<table>
<thead>
<tr>
<th>Histogram parameter</th>
<th>ER group</th>
<th>Non-ER group</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>20553.90±4095.80</td>
<td>23407.20±4634.20</td>
<td>0.031</td>
</tr>
<tr>
<td>SD</td>
<td>5013.81±1131.596</td>
<td>4062.9±666.34</td>
<td>0.01</td>
</tr>
<tr>
<td>10th percentile</td>
<td>13800±5064.216</td>
<td>18800±4816.408</td>
<td>0.01</td>
</tr>
<tr>
<td>50th percentile</td>
<td>20400±4227.11</td>
<td>23900±4686.473</td>
<td>0.013</td>
</tr>
<tr>
<td>90th percentile</td>
<td>26600±3429.622</td>
<td>29000±4530.423</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Texture parameter</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>entropy</td>
<td>4.20±0.20</td>
<td>3.90±0.20</td>
<td>0.001</td>
</tr>
<tr>
<td>skewness</td>
<td>-0.20±0.40</td>
<td>-0.10±0.40</td>
<td>0.168</td>
</tr>
<tr>
<td>kurtosis</td>
<td>0.70±1.30</td>
<td>0.60±0.90</td>
<td>0.819</td>
</tr>
<tr>
<td>inhomogeneity</td>
<td>0.25±0.17</td>
<td>0.18±0.13</td>
<td>0.005</td>
</tr>
</tbody>
</table>

HCC hepatocellular carcinoma, TACE transarterial chemoembolization, SD Standard deviation

<sup>a</sup> Unpaired-sample t test

Table 3 and Fig. 5 showed the results of ROC analysis of histogram and texture measures used to differentiate patients with early local recurrence from those with non-early local recurrence. For the histogram approach, the 50th percentile of pixel intensity had higher diagnostic sensitivity (92.6%) than the SD (62.9%). For the texture approach, inhomogeneity had the highest diagnostic specificity (95%), rather than the mean (55%).
Table 3 Effectiveness of multi-parametric analysis of HCC in differentiating early recurrence from non-early recurrence

<table>
<thead>
<tr>
<th>parameter</th>
<th>AUC</th>
<th>Cut-off value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>entropy</td>
<td>0.783</td>
<td>0.4135</td>
<td>63</td>
<td>85</td>
</tr>
<tr>
<td>inhomogeneity a</td>
<td>0.693</td>
<td>0.2474</td>
<td>48.1</td>
<td>95</td>
</tr>
<tr>
<td>mean</td>
<td>0.759</td>
<td>24817</td>
<td>88.9</td>
<td>55</td>
</tr>
<tr>
<td>10th</td>
<td>0.768</td>
<td>20250</td>
<td>88.8</td>
<td>55</td>
</tr>
<tr>
<td>50th b</td>
<td>0.725</td>
<td>25050</td>
<td>92.6</td>
<td>55</td>
</tr>
<tr>
<td>SD</td>
<td>0.761</td>
<td>4698</td>
<td>62.9</td>
<td>90</td>
</tr>
</tbody>
</table>

a with the highest value in specificity, b with the highest value in sensitivity.

Discussion

HCC was a rapidly growing tumor with more than 500,000 new cases per year[6], and the recurrence rate within the Milan criteria (ie, a single HCC tumor <5 cm or all three tumors >3 cm) was gradually increasing[7]. Rou WS[8] studied 134 patients initially diagnosed with HCC and identified factors affecting early local recurrence after TACE. They found that tumor size greater than 2cm significantly predicted early local recurrence after complete TACE. Similar to our results, there was a significant correlation between local recurrence and tumor size for the entire cohort. However, tumor size alone cannot predict response to treatment in early locally recurrent lesions due to the relative resistance of existing undifferentiated cells in TACE-induced hypoxic condition.

Su X et al[9] discussed that most treatments were not adequately addressed due to tumor heterogeneity, which may be one of the most important factors. Tumor heterogeneity represents hemorrhage, necrosis, and regional differences in cell density[10]. Not only can it be visualized, but also it manifests at the cellular and molecular levels due to the morphological diversity[11]. Intratumor heterogeneity, as the most promising prognostic factor, combined with histopathologic grade can predict survival[12] and therapeutic response[13].

On contrast-enhanced CT imaging, larger HCCs lead to markedly heterogeneous enhancement due to structural inhomogeneity. Traditionally, CT values typically reflect average density within a defined region of interest (ROI) and may not be suitable for assessment of tumor biological behavior before treatment. Fortunately, histogram and texture analysis, currently the most popular techniques, have been applied in medical images to quantify the heterogeneity of the whole tumor. CT-enhanced imaging-based histogram measurements, such as percentile values, minimum, and maximum, can account for potential heterogeneous distributions and thus be used to predict treatment response[14] and correlate with pathological outcomes[15]. Texture analysis provides information about the tumor microenvironment
through a variety of mathematical methods that consider not only the overall pixel intensity, but the location and distribution of pixel pairs in the image\cite{16-17}. It can quantify and characterize heterogeneous distributions by providing better representations and has been successfully applied in several areas, including distinguishing benign and malignant lesions\cite{18} and the prediction of survival time\cite{19}. However, to date, the application of histogram and texture analysis to predict HCC tumor recurrence is not very widespread.

According to recent research, heterogeneity is the dominating factor in cancer recurrence\cite{20-21}. It may be attributed to a heterogeneous tumor cell population composed of cancer stem cells that had been isolated and characterized in many types of cancers\cite{22}. Previous studies have shown that the extracellular matrix (ECM) is an important part of the tumor microenvironment, consisting of a variety of stromal cells, which play an important role in tumor recurrence and progression by interacting with cancer cells or with each other. Intratumoral heterogeneity of HCC exists not only at the morphological but molecular level\cite{7}, including immunohistochemical and genetic intratumor heterogeneity. Through a systematic analysis of 23 HCC without medical pretreatment, Friemel et al\cite{23} found that morphological heterogeneity was detectable in 87% of HCC cases, and immunohistochemical heterogeneity was consistently associated with morphological abnormalities in 39% of tumors. These findings might be responsible for treatment failure in many HCC cases.

Tumor vascularity was assessed by analyzing data from preprocessed functional imaging, supported by a number of previous studies. Histogram and texture characteristics have been shown to associate with clinical outcomes such as response to therapy in a variety of tumors [16]\cite{24}. Reiner CS et al [16] measured arterial perfusion to assess whether tumor heterogeneity helps predict response to transarterial radioembolization (TARE) by histogram analysis of CT perfusion. They found that responders had significantly higher arterial perfusion than non-responders, suggesting that higher vascularization was associated with better TARE responses\cite{25}. Selective catheterization is very important to achieve effective TACE therapy in patients with hypervascular HCC. Similar to Reiner CS's study\cite{16}, according to our histogram results, the non-ER group had significantly higher pixel intensities at the 10th and 50th percentiles.

In fact, in a study by Chang Y et al\cite{26}, the lifetime of HCC patients with vascularity was significantly longer than that of oligovascular patients (P<0.01) for TACE treatment. This was similar to our results, which showed a strong association between tumor heterogeneity using histogram and texture analysis and response to TACE in HCC patients. Contrast-enhanced CT has been used in a number of clinical trials to assess and monitor the effect of therapy. However, influenced by tumor microenvironment\cite{27}, the average CT value drawn on a single slice could not favorably reflect the heterogeneity in the whole volume tumor. Retrospective analysis of histograms and textures using standard portal-phase liver CT scans not only did not affect survival prediction\cite{28}, but eliminated concerns that data from these studies could confound extreme results. Furthermore, the choice of interest is another important influencing factor, considering intratumoral heterogeneity. Our texture evaluation results were very similar when
comparing ROI with VOI mode. Previous studies described heterogeneous disease characterization based on whole lesions rather than single slice analysis, which could reduce selection bias\textsuperscript{[14]}\textsuperscript{[29]}. Consistently, Ng F et al\textsuperscript{[30]} found that whole-tumor analysis was more representative of tumor heterogeneity than maximum cross-sectional area analysis. Given the above, our research analysis was conducted within the VOI.

Texture analysis, an image processing algorithm, can be used to assess the pixel distribution within the tumor to quantify texture. Entropy is an important parameter for statistical measurement of gray intensity irregularity, and the entropy tends to be higher when there are more variable values in the region of interest. Entropy and inhomogeneity strongly reflect heterogeneity in tumor characterization\textsuperscript{[31]} related to tissue density, angiogenesis and fibrosis\textsuperscript{[32]}. Our study found that entropy and inhomogeneity of the ER group were positively correlated with early recurrence, suggesting that tumor heterogeneity in HCC correlated negatively with response to TACE. The lower pixel value of ER group supported the argument. Skewness and kurtosis describe the asymmetry and the sharpness of the distribution of data points compared to the normal Gaussian distribution. They are more sensitive to outliers. In Lubner MG`s study\textsuperscript{[33]}, skewness was negatively associated with KRAS mutation, but there was not a significant predictive value in our analysis and prior study\textsuperscript{[24]}. Similar to Hounsfield unit (HU), the mean mainly reflects the average vascular permeability measured on contrast-enhanced CT imaging.

There were several limitations in our study. First, our research analyzed the whole tumor volume heterogeneity of the target lesion, instead of taking into account the spatial distribution within the tumor. Firstly, intraintraleonal heterogeneity in HCC has important implications for targeted therapy, suggesting that a single tumor biopsy is not representative of the entire tumor\textsuperscript{[23]}, which may result in an incomplete therapy response due to the sensitivity of only one tumor subclone. Secondly, Although our results suggested that histogram and texture analysis were able to predict response to treatment, manual drawing of lesion boundaries might be biased. Improving the algorithm for edge detection and developing a fully automated method will be the next task to improve reproducibility. Thirdly, TACE is considered a selective method used in the terminal stage of HCC. Many patients in poor health could not undergo long-term follow-up imaging. This excluded many patients originally and became the handicap to increase the patients` database. Tumor heterogeneity indicates differences in spatial distribution, including hypoxia, necrosis, tumor solid area, and peritumoral edema, which may further illustrate the reactivating lesion and lucubrately need more research.

**Conclusion**

Our results suggest that certain histogram and texture features of pre-treatment portal phase CT reflect the tumor heterogeneity of HCC and can be used to predict recurrence in patients treated with TACE. They may become an effective tool for decision-making in selecting patients for TACE and predicting patient response to TACE.
Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lina Dou. The first draft of the manuscript was written by Ru Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Biomedical Research Ethics Review Committee of Xuzhou Central Hospital (approval number:XZXY-LK-20220629-057 ). The requirement for individual informed consent was waived due to the retrospective nature of the study.

References


Figures
Figure 1

HCC patients did (a and b) and did not recur within (d and e) half a year after receiving TACE; Contrast-enhanced CT images in the portal venous phase before TACE (a and d) with manually drawn margin around the lesion (b and e). c and f were the output results corresponding to the targeted tumor.
Figure 2

Matrix Scatter Plots illustrating the association between each of the overall texture parameters within ROI and VOI and comparing measurements between ROI and VOI analysis.
Figure 3

Bland Altman plot comparing ROI and VOI measurements for the entropy measurement, demonstrating good agreement with the limits of agreement -0.2, 0.11, and bias -0.04.
Figure 4

Box-and-whisker plots showing the apparent histogram and texture features in this study. (a), Three apparent histogram parameters including SD, 10th, and 50th percentiles. Three apparent texture parameters (b) entropy, (c) mean, and (d) inhomogeneity. They were compared between patients in the ER group and those in the Non-ER group after receiving TACE.
comparison of diagnostic ability for discriminating early recurrence from non-early recurrence of HCC after TACE between histogram and texture analysis. Histogram-based 50th percentile and texture-based inhomogeneity show distinctly diagnostic sensitivity and specificity respectively, suggesting better individual features in the differentiation of early local recurrence from non-early local recurrence of HCC.