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ABDOMINAL IMAGING

ORIGINAL ARTICLE

Comparison of quantitative volumetric analysis and linear measurement for predicting the survival of Barcelona Clinic Liver Cancer 0- and A stage hepatocellular carcinoma after radiofrequency ablation

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PURPOSE

The prognostic role of the tumor volume in patients with hepatocellular carcinoma (HCC) at the Barcelona Clinic Liver Cancer (BCLC) 0 and A stages remains unclear. This study aims to compare the volumetric measurement with linear measurement in early HCC burden profile and clarify the optimal cut-off value of the tumor volume.

METHODS

The consecutive patients diagnosed with HCC who underwent initial and curative-intent radiofrequency ablation (RFA) were included retrospectively. The segmentation was performed semi-automatically, and enhanced tumor volume (ETV) as well as total tumor volume (TTV) were obtained. The patients were categorized into high- and low-tumor burden groups according to various cut-off values derived from commonly used diameter values, X-tile software, and decision-tree analysis. The inter- and intra-reviewer agreements were measured using the intra-class correlation coefficient. Univariate and multivariate time-to-event Cox regression analyses were performed to identify the prognostic factors of overall survival.

RESULTS

A total of 73 patients with 81 lesions were analyzed in the whole cohort with a median follow-up of 31.0 (interquartile range: 16.0–36.3). In tumor segmentation, excellent consistency was observed in intra- and inter-reviewer assessments. There was a strong correlation between diameter-derived spherical volume and ETV as well as ETV and TTV. As opposed to all linear candidates and 4,188 mm^3 (sphere equivalent to 2 cm in diameter), ETV >14,137 mm^3 (sphere equivalent to 3 cm in diameter) or 23,000 mm^3 (sphere equivalent to 3.5 cm in diameter) was identified as an independent risk factor of survival. Considering the value of hazard ratio and convenience to use, when ETV was at 23,000 mm^3, it was regarded as the optimal volumetric cut-off value in differentiating survival risk.

CONCLUSION

The volumetric measurement outperforms linear measurement on tumor burden evaluation for survival stratification in patients at BCLC 0 and A stages HCC after RFA.

KEYWORDS

Hepatocellular carcinoma, tumor burden, quantitative volumetric analysis, radiofrequency ablation, prognosis

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Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and ranks third in terms of mortality.[1] Barcelona Clinic Liver Cancer staging system (BCLC) is widely used to guide clinical decision-making and survival risk stratification for patients with HCC. In practice, patient allocation for curative-intent therapies is a multifactorial deci-

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sion. For patients with early-stage HCC, BCLC 0 and A stages, the tumor burden evaluation is considered the uppermost aspect; it combines the tumor number and the maximum diameter to stratify individual survival risk optimally.[2] However, the maximum diameter and diameter-based sphere alongside ellipsoid volume are difficult to represent the actual tumor burden due to the irregular 3D geometry of the tumor, which is largely derived from heterogeneity and non-rotational symmetry of tumor growth.[3,4]

In the past decade, owing to the progress of semi-automatic tumor segmentation tools, published studies have validated the feasibility of volumetric segmentation and shown potential perspectives for radiological tumor response assessment.[5,6] Compared with the established anatomic response criteria, such as Response Evaluation Criteria in Solid Tumors (RECIST), modified RECIST, or Milan criteria, the quantitative volumetric assessment has appeared to have better performance for prognostic stratification in intermediate-stage or advanced HCC patients.[7-13] This is possibly because the changes in tumor diameter tend to lag behind changes in tumor volume and functional parameters as well as enhancement under locoregional therapies.[14] As the minimal user interaction and quantitative steps in the semi-automatic segmentation, volumetric analysis of tumor burden has considerable superiority in accuracy, reproducibility, and interobserver or radiologic-pathologic agreement.[15,16] Therefore, this technique should be applied in broader clinic settings.

Evidence on semi-automatic volumetry concerning the tumor burden of BCLC 0 or A stage HCC remains scarce. Such patients, especially with a maximum diameter of less than 3 cm, have relatively low tumor burden. Considering that necrosis is seldom presented, most relevant studies assessing the prognostic role of tumor volume mainly adopt the mathematical, simulating formulae and manual contouring,[17-24] which is not accurate. Recently, the prognostic role of magnetic resonance imaging (MRI)-based tumor volume using semi-automatic segmentation was assessed,[25] but the predictive value is limited due to the absence of multifocal patients. Hence, this study hypothesized that tumor volume may amplify the subtle difference in diameter to reflect the actual tumor burden. The aim of this pilot study is to evaluate the potential capacity of volume analysis for survival stratification compared with linear measurement in the early HCC burden profile after radiofrequency ablation (RFA) and explore the optimal volumetric cut-off value.

Main points

- Tumor volume outperforms diameter for predicting survival in early patients with hepatocellular carcinoma (HCC).
- Volumetric tumor burden improves performance for high tumor burden case-finding in early HCC.
- A seed-growing algorithm in open-source software provides a feasible tumor segmentation.
- An optimal cut-point of tumor volume, namely 23,000 mm^3, was given in Barcelona Clinic Liver Cancer 0 and A stages HCC patients.

Methods

Study cohort

The patients diagnosed with HCC who underwent initial and curative-intent RFA were analyzed retrospectively during 2016–2021. The inclusion criteria were as follows: 1) 18–75 years old; 2) the preoperative multi-phasic MRI was screened within two weeks; 3) BCLC 0 or A stage; 4) all targeted HCC lesions were ablated completely. The exclusion criteria were as follows: 1) previous HCC treatment history; 2) failure of radiological data retrieval; 3) loss of follow-up; 4) presence of secondary carcinoma. The flowchart of patient selection is illustrated in Figure 1. Finally, a total of 73 patients with HCC were analyzed. In twelve patients, HCC was biopsy-proven, and diagnoses in the remaining patients were established in concordance with Liver Imaging Reporting and Data System criteria.[26] Complete ablation evaluation of all targeted tumors was confirmed by contrast-enhanced MRI one month after RFA[27] combined with the eradication of serum level of alpha-fetoprotein (AFP). Demographic, clinical, and laboratory data of the cohort were reviewed and recorded from the electronic medical system. The study procedures conformed to the ethical guidelines of the Declaration of Helsinki, and the Bioethics Committee of Beijing Friendship Hospital, Capital Medical University approved this retrospective study (registration number: 2022-P2-290-01). The requirement for written informed consent for recruitment was waived.

Radiofrequency ablation

All RFA procedures, guided by computed tomography scan with a percutaneous approach, were performed under local anesthesia combined with procedural sedation and analgesia in all patients. Vital signs were monitored throughout the procedure. Two RFA systems, Rita Starburst Flex/talon electrode (RITA Medical Systems, Mountain View, Calif., USA) and CELON ProSurge (Olympus Winter & Ibe GmbH, Hamburg, Germany), with a 2–5 cm deployment, were determined by the type of generator model. They were equipped with internal liquid circulation (saline solution) to maintain surface temperature. The generator model selection and electrode shaft distribution depended on the size, location, and adjacent structure of the tumor. Multiplanar reformation ensured

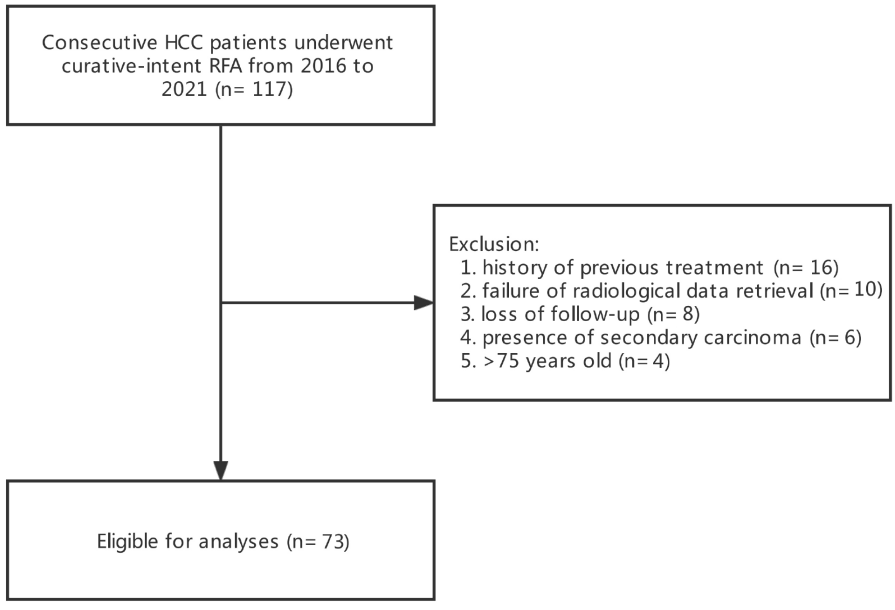

Figure 1. The flowchart of patient selection. HCC, hepatocellular carcinoma; RFA, radiofrequency ablation.

that the tip of the electrode shaft was inside or at the center of the tumor and covered in expandable needles with at least a 5–10 mm safety margin. Ablation-related parameters were set as per the manufacturer's instructions regarding tumor-related characteristics.

Magnetic resonance imaging image acquisition

A standardized MRI protocol with 3.0T MRI scanners (GE, GE Healthcare, Boston, USA) was performed for the routine liver imaging in the institution. Multiphasic contrast-enhanced T1-weighted imaging (T1WI) was obtained in the arterial phase (25–35 sec after injection), portal venous phase (60–70 sec after injection), and delayed phase (3–5 min after injection). The contrast agent, an extracellular contrast agent, gadobenate dimeglumine (Magnevist, Bayer Schering Pharma, Berlin, Germany), was intravenously injected with a dose of 0.1 mmol/kg (maximum dose, 20 mL) at a rate of 2 mL/s followed by a 50 mL saline flush (2 mL/s).

Tumor measurement and segmentation

All images were exported in digital imaging and communication in medicine files from the workstation and segmented in cubic millimeters (mm^3) using the open-source software ITK-SNAP (www.itksnap.org/pmwiki/pmwiki.php). The axial linear and volumetric measurements of all HCC lesions at the late arterial phase (20 sec) of baseline MRI were reviewed repeatedly with an interval of two weeks by two radiologists (SW.Y. and QY.C.) who were blinded to the patient's survival outcomes and who had five years of experience in abdominal imaging. Enhanced tumor volume (ETV) and total tumor volume (TTV) values were recorded in tumor volumetric analyses. The axial linear measurement and seed-setting evaluation were re-checked by another board-certified radiologist (TH.S) who had more than ten years of experience in radiology. The individual measurements were determined by the average of the two reviewers. Inconsistency between the two reviewers was resolved by consensus.

The segmentation was performed based on the volumetric mask on the MRI images at the late arterial phase (20 sec) with a seed-growing algorithm, namely a region-growth algorithm, as depicted in previous literature.[25,28,29] Four quadrate regions of interest (ROIs) were prescribed in the liver parenchyma of the ipsilateral lobe, being adjacent to tumor boundaries and away from blood vessels, liver boundaries, and other structures.[30] The mean intensity value (MIV) was derived from the average value of four ROIs. When placing bubbles within the tumor, the necrotic areas, cysts, and vessels close to the index lesion at the certain slice were also avoided, as identified on T2WI or arterial early-phase images. Furthermore, the pre-enhanced T1W images were scrutinized to distinguish high signal intensity, like hemorrhage, to avoid overestimating ETV value. The exterior delineated bubbles were placed inside the edge of the enhanced part of the lesion, tangent to the inner margin in principle, and the interior delineated bubbles were placed randomly. Cross-referencing with coronal and sagittal reconstructed MRI images was used to supplement the bubble.

Any voxel inside or peripheral of the seed was clustered if the intensity was located in the interval threshold between the MIV+2 standard deviations (SD) and abdominal aorta signal intensity. In this step, the corresponding voxels were combined and considered as the viable tumor part, then ETV was obtained. The low or delayed enhancement part as well as the non-enhanced part within the tumor would then be supplemented to get TTV value. The detailed descriptions are shown in Supplementary Material 1. The color map reflecting tumor enhancement heterogeneity is presented in Figure 2.

Definitions

The study endpoint was overall survival (OS), which was calculated by subtracting the RFA date from the date of death or the last follow-up visit date (May 31, 2022). Volumetric and linear cut-off values were used to distinguish the high and low tumor burden groups. The ETV and TTV refer to the volume of all targeted lesions in the presence of multiple tumors. Index tumor was the dominant HCC lesion, and it refers to the one with the largest diameter in multifocal patients. The cut-off value of elevated AFP levels was defined as 400 ng/mL in reference to the initial assessment in the HCC guideline.[31]

Cut-off values selection

According to the cut-off value in defining BCLC 0 and A stages as well as another cut-off value of small HCC, a maximum diameter of 2 and 3 cm were chosen as the linear cut-off values, respectively. The corresponding maximum diameter-derived respective spherical volumes, 4,188 mm^3 and 14,137 mm^3 (equation), were used as volumetric cut-off values as suggested by the previous publications.[8,10] In addition, another volumetric value, obtained from binary classification in the endpoint-related decision-tree model, was used as the third cut-off value. The software X-tile was used to determine the fourth volumetric cut-off value based on the maximum log-rank statistic.[32]

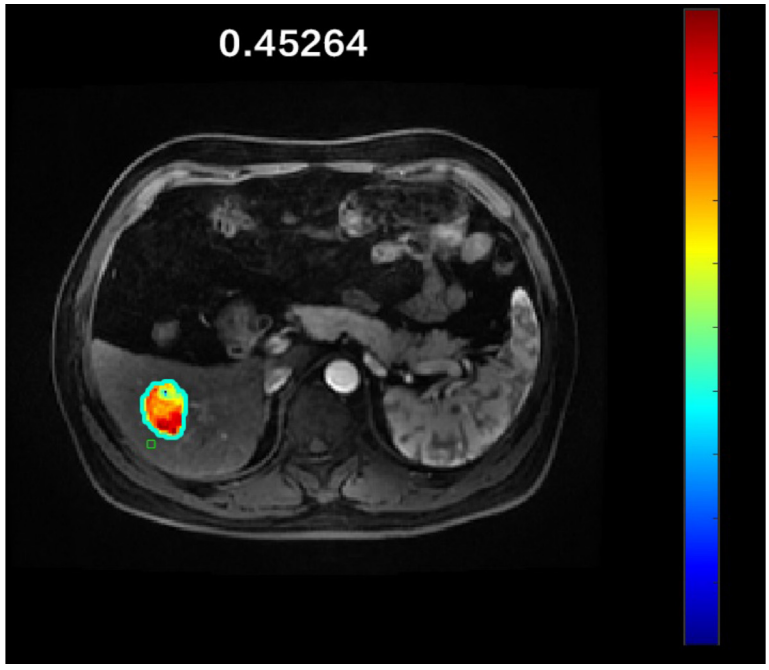

Figure 2. The color map. The number value on the image is the average signal intensity within the normalized regions of interest. The color on the spectrum column represents the signal intensity. Compared with the liver parenchyma signal intensity, the closer the color is to red, the higher the signal intensity, and the closer the color is to blue, the lower the signal intensity. Visual display of signal intensity inside the tumor is provided.

Statistical analysis

Continuous variables expressed as the mean ± SD or median (interquartile range) depending on the data distribution type were compared using two-sample t-tests or Mann–Whitney U tests, and categorical variables expressed as number (percentage) were compared using the χ^2 test or Fisher's exact tests. The comparison between diameter-derived sphere volume and tumor volume was performed using the Paired-Samples test. The intraclass correlation coefficient (ICC) was calculated for inter-reviewer agreement. Generally, ICC ≥0.90 indicates excellent consistency, and ≥0.75 indicates good consistency between both reviewers.[33] The Pearson correlation and Bland–Altman analysis were used to evaluate the correlation and consistency between the linear measurement and volume of the tumor. Risk factors for live status were identified in univariate and multivariate binary logistic regression, and the cut-off values for continuous risk factors were determined in decision-tree analysis using the statistical package R version (version 4.2.1, www.r-project.org).

The Kaplan–Meier method using the log-rank test was applied to compare the survival curves of patients with different tumor burdens. After testing the proportional hazards assumption using Schoenfeld residuals, univariate time-to-event Cox regression was performed to identify the prognostic factors of OS. The variables with $P < 0.1$ were selected in the adjusted multivariate analysis (forward step-wise). Regarding the tumor burden value, either linear or volumetric measurement as dichotomization according to the corresponding cut-off value was incorporated into the model. The two-tailed $P < 0.05$ was indicative of a significant difference. All statistical analyses were performed in the SPSS (Version 26.0; IBM Corp., Armonk, NY) and R software.

Results

Baseline characteristics of the cohort

The demographics and clinical characteristics of the cohort are summarized in Table 1. The mean age of the participants was 59.3 ± 10.5 years, and 65.8% of them were men. Out of 73 participants, 58 (79.5%) had HBV-related liver disease.

Solitary tumor was the predominant subtype (90.4%), and the mean tumor maximum diameter was 2.2 cm ± 1.1 cm, with 37 (50.7%) being more than 2 cm and 16 (21.9%) being more than 3 cm in diameter. A total of 81 lesions were analyzed in the cohort. ETV and TTV were 4,748.00 mm³ (2,076.68, 10,845.50) mm³ and 47,48.00 (2,076.68, 11,981.00) mm³, respectively. In addition, Child–Pugh class A (79.5%) was mostly observed in the liver function reserve evaluation.

Table 1. Demographics and clinical characteristics of the cohort

Variable	Cohort (n = 73)
Sex (male, %)	50 (65.8)
Age (year)	59.32 ± 10.5
Etiology (HBV/HCV/alcoholic/others, %)	58 (79.5)/1(1.4)/7(9.6)/7(9.6)
BCLC stage (0/A, %)	34 (46.6)/39 (53.4)
Tumor number (single/multiple, %)	66 (90.4)/7 (9.6)
Child–Pugh class (A/B, %)	58 (79.5)/15 (20.5)
MELD-Na score	8.55 ± 2.7
ALB (g/L)	36.06 ± 5.6
TBIL	16.23 (12.03,22.03)
ALT	25.07 ± 10.3
PLT	111 (75.5, 175.5)
GGT	42 (27.5,68.0)
AFP	5.66 (3.17, 22.91)
Tumor maximum diameter (cm)	2.2 ± 1.1
≤2 cm/>2 cm, % ≤3 cm/>3 cm, %	36 (49.3)/37 (50.7) 57 (78.1)/16 (21.9)
ETV (mm³)	4748.00 (2076.68, 10845.50)
TTV (mm³)	4748.00 (2076.68, 11981.00)

Continuous variables with non-normal distribution were expressed as median (interquartile range), otherwise expressed as mean ± standard deviation. BCLC, Barcelona Clinic Liver Cancer staging system; MELD-Na, model for end-stage liver disease incorporating sodium; ALB, albumin; TBIL, total bilirubin; ALT, alanine aminotransferase; PLT, platelet; GGT, glutamyl transferase; AFP, alpha-fetoprotein; ETV, enhancing tumor volume; TTV, total tumor volume.

Additional volumetric cut-off values

After univariate and multivariate binary logistic regressions, BCLC A stage and ETV were identified as risk factors associated with survival; therefore, two variables were selected in the decision-tree analysis. The cut-off value of ETV (>23,000 mm³) in the decision-tree model is shown in Figure 3.

Additionally, X-tile software showed that a plateau of ETV values ranging from 12,424.00 mm³ (sphere equivalent to 2.87 cm in diameter) to 13,560.32 mm³ (sphere equivalent to 2.94 cm in diameter) enables significant stratifications in survival analysis. As the above-mentioned values were close to 14,137 mm³, a total of three volumetric cut-off values were analyzed in Cox regression models, including 4,188 mm³ (sphere equivalent to 2 cm in diameter), 14,137 mm³ (sphere equivalent to 3 cm in diameter), and 23,000 mm³ (sphere equivalent to 3.5 cm in diameter). The analytical process in X-title is exhibited in Supplementary Material 1.

Intra- and inter-reviewer agreement assessment and correlation between linear and volumetric measurement

Table 2 illustrates that there was excellent consistency in intra- and inter-reviewer assessments. Considering the presence of multiple tumors, the correlation between linear and volumetric measurement were analyzed for index tumor. Figure 4 shows that the maximum tumor diameter was robustly positively correlated with ETV ($R = 0.846$, $P < 0.001$), and good consistency was observed between diameter-derived spherical volume and ETV, especially for individuals with a volume <30,000 mm³ (Figure 5). It is noted that diameter-derived spherical volume was overestimated in comparison with ETV ($P = 0.003$). Similarly, there was a strong correlation between ETV and TTV ($R = 0.966$, $P < 0.001$).

Tumor burden measurements associated with survival

After a median follow-up of 31.0 (16.0, 36.3) months, 14 patients died. The cumulative OS at 1, 3, and 5 years was 97.0%, 82.1%, and 72.2%, respectively. As for survival stratification regarding the tumor burden, patients could be divided into two groups according to 3 cm in diameter or all volumetric cut-off values, with significant differences in

survival analysis between high and low tumor burden groups and a markedly worse prognostic status in the high tumor burden group (all $P < 0.05$). The corresponding survival curves are displayed in Figure 6.

Comparison among different multivariate Cox models incorporating tumor burden measurements

After the univariate Cox regression (Table 3), each eligible tumor burden cut-off value was analyzed in the multivariate Cox model as a covariate. A total of four models were constructed, as shown in Table 4.

At multivariate analysis, ETV cut-off values, 14,137 mm^3 [hazard ratio, 3.896; 95% confidence interval (CI): 1.012–14.993] or 23,000 mm^3 (hazard ratio: 4.343; 95% CI: 1.176–16.034), were associated with impaired long-term survival. Considering hazard odd and ease of use, 23,000 mm^3 was regarded as the optimal tumor burden cut-off value. Except for tumor volume, BCLC stage and elevated serum AFP level were also associated with a reduced survival rate in other models.

Discussion

In this study, the comparison of prognostic performance between linear and volumetric measurements in differentiating survival was presented. ETV was a better parameter than the diameter for assessing tumor burden in patients with HCC at BCLC 0 and A stages after RFA. For those patients, a volumetric value of 23,000 mm^3 was the optimal cut-off value in terms of maximum statistical power and convenience of use.

An important finding from this study is the recognition that HCC patients at BCLC 0 and A stages who achieved longer survival had lower ETV, which aligns with prior results.[8,34] The linear measurement is still the mainstay of tumor burden marker. Regarding tumor volumetry at BCLC 0 and A stages, most prior studies adopted diameter-derived formulas to assess tumor volume. However, compared with semi-automatic segmentation, the formula estimation appears to be idealistic and ignores the discrepancies in tumor growth among different planes.[35]

In clinical practice, HCC lesions at early stages are characterized by small size and little avascular necrosis, thus rendering diameter-based assessment feasible. However, tumor diameter could not thoroughly reflect the actual tumor burden compared with tumor volume.[6] In this study, tumor volume appeared to be more sensitive and accurate than the diameter in predicting survival. It is surmised that tumor volume could amplify the subtle difference in diameter.

In addition, it is noted that HCC lesions at BCLC A stage were comparable to each other in most cases, possibly because of multiple-center carcinogenesis. Even though some studies indicated that there was no additional predicting value when comparing single index lesion with all targeted ones,[34,36] their conclusions were drawn from intermediate-stage or advanced patients with HCC cohort. Therefore, for multifocal HCCs, the total volumetric tumor burden, consisting of all radiologic measurable lesions, was calculated and included in survival analysis rather than the maximum diameter or volume of the index lesion.

This study also aimed to optimize the volumetric cut-point for patients with HCC at BCLC 0 and A stages. With respect to intermediate-stage HCC, mounting studies have adopted cut-off values derived from sphere volume equivalent to 3 or 5 cm in diameter and proved that volumetric cut-points derived from the Milan or RECIST criteria were effective to identify a survival benefit or a more positive tumor response compared with current linear criteria.[6,8,10,12,13] As such, the cut-off values, 4,188 mm^3 and 14,137 mm^3 (sphere volume equivalent to 2 or 3 cm in diameter), in reference to BCLC 0 or A stage, were selected. Moreover, with the purpose of maximizing the statistical power and avoiding redundancy from other endpoint-related variables,

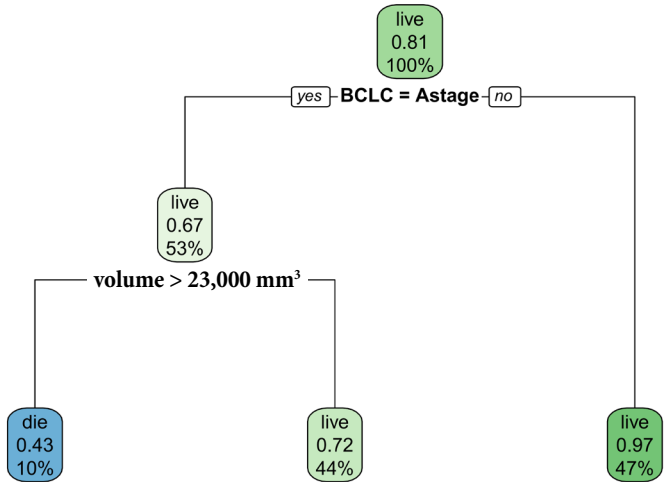

Figure 3. The decision-tree analysis for selecting a cut-off value. The cut-off value of tumor volume, 23,000 mm^3, and Barcelona Clinic Liver Cancer stage were selected in the decision-tree model. BCLC, Barcelona Clinic Liver Cancer staging system.

Table 2. The evaluations of intra- and inter-reviewer agreements in various tumor burden parameters and correlations between diameter and volume as well as ETV and TTV

Parameters	Intra-reviewer agreement		Inter-reviewer agreement	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Maximum tumor diameter	0.986 (0.968–0.992)	<0.001	0.964 (0.944–0.978)	<0.001
Index tumor volume	0.930 (0.878, 0.955)	<0.001	0.942 (0.880, 0.956)	<0.001
ETV	0.912 (0.863, 0.950)	<0.001	0.846 (0.790, 0.880)	<0.001
TTV	0.914 (0.866, 0.958)	<0.001	0.840 (0.774, 0.868)	<0.001
	Correlation (r)		*P* value	
Diameter vs. ETV	0.846		<0.001	
ETV vs. TTV	0.966		<0.001	

HR, hazard ratio; CI, confidence interval; ETV, enhancing tumor volume; TTV, total tumor volume.

X-tile and decision-tree analysis were used to select additional volumetric cut-off values. In the decision-tree analysis, as one of two bifurcate variables, larger tumor volume was associated with higher mortality, being a prerequisite of the BCLC A stage. Furthermore, tumor volume is expected to grow in importance as a powerful marker in the context of HCC at BCLC A stage, possibly because the difference in individual tumor volume widens substantially with the increase in tumor diameter and number.

In the selection of tumor burden parameter, ETV as a volumetric candidate was used in prognostic evaluation rather than TTV, thereby minimizing the user interaction in the segmentation process. There was a strong agreement between ETV and TTV, likely owing to the fact that early HCC lesions were seldom prone to tumor necrosis and signal heterogeneity, especially for those less than 3 cm in diameter. This study's results showed that the diameter-derived sphere volume was larger than the tumor volume on the basis of the assumption of rotational symmetry, which was consistent with prior findings in experimental and clinical animal studies.[37-39] Consequently, the spherical cut-points may facilitate the re-identification in patient subgroups that possibly benefit from locoregional or salvage therapies.[34,38]

This study's analyses showed a high intra- or inter-reviewer consistency in the results of semi-automated tumor segmentation of ETV and TTV. The algorithm adopted a combination of quantitative steps (signal in-

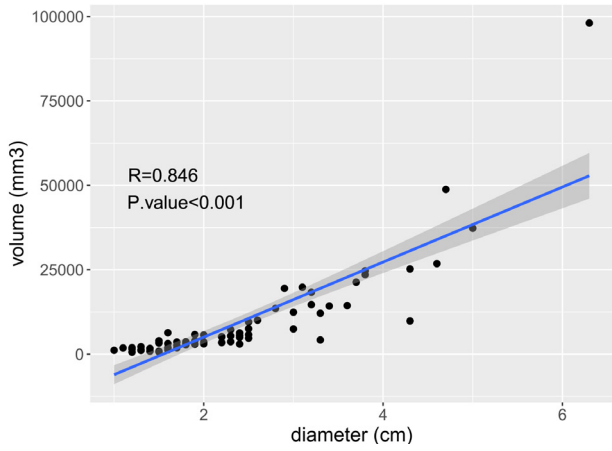

Figure 4. The maximum tumor diameter was robustly positively correlated with tumor volume.

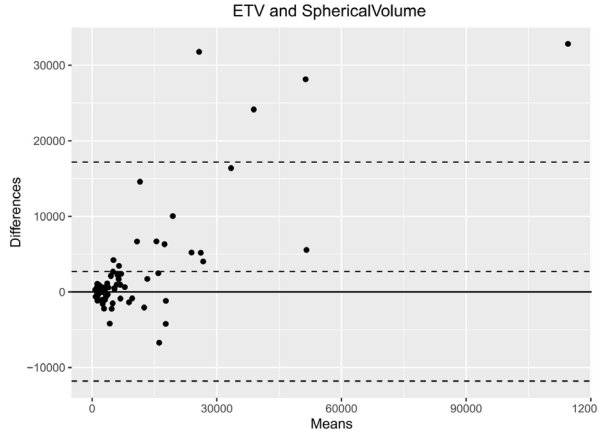

Figure 5. The Bland–Altman analysis. A good consistency was observed between diameter-derived spherical volume and the enhanced tumor volume, especially for individuals with volume <30,000 mm³. ETV, enhancing tumor volume.

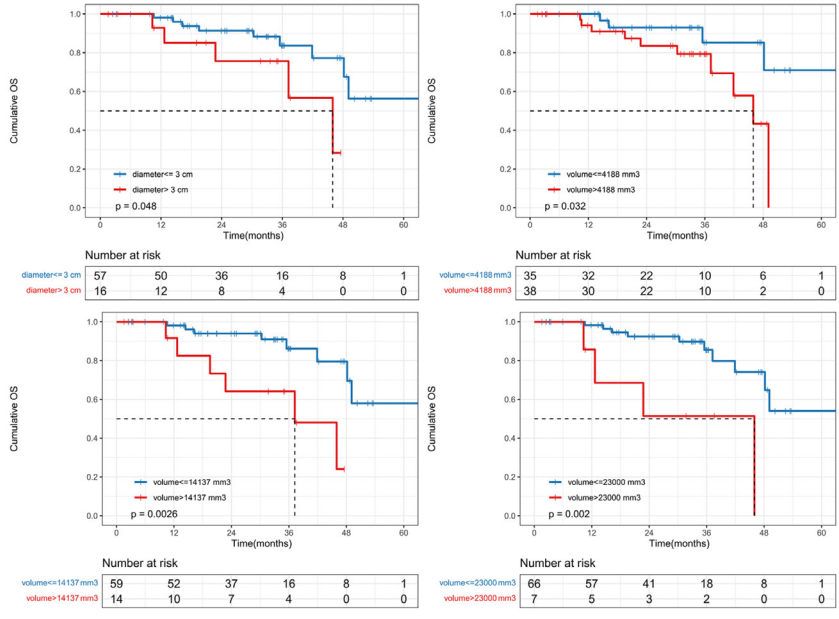

Figure 6. Four survival curves stratified by different tumor burden cut-off values. The 3 cm diameter and 3 volumetric cut-off values achieved good separation of the survival curves.

Table 3. Univariate Cox regression analyses of risk factors associated with overall survival

Variable	Univariate	
	HR (95% CI)	*P* value
Sex (male)	3.919 (0.866, 17.727)	0.176
Age (>60 years)	0.860 (0.300, 2.463)	0.779
Etiology (HBV)	0.244 (0.083, 0.715)	0.150
BCLC (A)	12.595 (1.638, 96.849)	<0.001
Tumor number (multiple)	3.314 (0.886, 12.394)	0.112
Child–Pugh (B)	1.932 (0.658, 5.668)	0.231
MELD-Na score	1.089 (0.950, 1.247)	0.221
ALB (<35 g/L)	1.548 (0.510, 4.703)	0.440
GGT (>40 U/L)	2.522 (0.873, 7.281)	0.077
PLT	1.804 (0.588, 5.537)	0.302
AFP (>400 ng/mL)	4.369 (1.151, 16.590)	0.024
Diameter (>2 cm)	2.071 (0.689, 6.225)	0.195
Diameter (>3 cm)	3.032 (0.956, 9.568)	0.048
ETV (>4,188 mm^3)	3.462 (1.046, 11.458)	0.032
ETV (>14,137 mm^3)	4.832 (1.553, 15.035)	0.003
ETV (>23,000 mm^3)	5.439 (1.631, 18.139)	0.002

HR, hazard ratio; CI, confidence interval; BCLC, Barcelona Clinic Liver Cancer staging system; MELD-Na, model for end-stage liver disease incorporating sodium; ALB, albumin; TBIL, total bilirubin; ALT, alanine aminotransferase; PLT, platelet; GGT, glutamyl transferase; AFP, alpha-fetoprotein; ETV, enhancing tumor volume; TTV, total tumor volume.

Table 4. Multivariate Cox regression models incorporating different tumor burden parameters

Variable HR (95% CI) *P* value	Model 1	Model 2	Model 3	Model 4
BCLC (A)	12.032 (1.560, 92.794) 0.017	12.032 (1.560, 92.794) 0.017		
AFP (>400 ng/mL)			6.886 (1.459, 32.511) 0.015	9.257 (1.801, 47, 591) 0.008
GGT (>40 U/L)				
Diameter (>3 cm)				
ETV (>4,188 mm^3)				
ETV (>14,137 mm^3)			3.896 (1.012, 14.993) 0.048	
ETV (>23,000 mm^3)				4.343 (1.176, 16.034) 0.028

Tumor burden measurements incorporated in model 1: diameter >3 cm; model 2: ETV >4,188 mm^3; model 3: ETV >14,137 mm^3; model 4: ETV >23,000 mm^3. HR, hazard ratio; CI, confidence interval; BCLC, Barcelona Clinic Liver Cancer staging system; GGT, glutamyl transferase; AFP, alpha-fetoprotein; ETV, enhancing tumor volume; TTV, total tumor volume.

tensity determination, region-growing) and visual identification (seed-point), exhibiting a strong correlation on radiologic-pathologic assessment.[40,41] Compared with full-automated segmentation, the accuracy and agreement as well as necessary subjective adjustment were balanced in semi-automated segmentation.[40,42,43]

Collectively, this study presented a potential algorithm for case-finding of higher tumor burden in patients at BCLC 0 and A stages to improve predicting performance. However, several limitations persisted. First, the limited number of multifocal patients necessitates further validation for volumetric cut-off value. Second, the tumor volume assessment required sophisticated processing using patent commercial software in prior publications. An algorithm of tumor segmentation in the open-source software was provided in this study, yet the individual segmentation process would still take about 3–5 minutes, which inevitably poses a time-consuming challenge for practitioners. Third, the single-center and retrospective nature of this study as well as a limited sample size could lead to a cautious interpretation of the results.

In conclusion, the volumetric measurement outperforms the linear measurement on tumor burden evaluation for survival stratification in patients at BCLC 0 and A stages HCC, and ETV >23,000 mm^3 suggests patients with poorer survival.

Conflict of interest disclosure

The authors declared no conflicts of interest.

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Supplementary Material 1.

1. Tumor segmentation

In most cases, tumor burden analysis is limited to sophisticated processing and patent commercial software, leading to the utilization of linear tumor measurement, like diameter or cross-product on a single axial slice, as a proxy for actual volume. Here,we use a open-source software ITK-SNAP (www.itksnap.org/pmwiki/pmwiki.php) with a region-growth computed algorithm to calculate tumor volume and enhanced volume, with the aim of a cost-effective, standardized, and repeatable measurement in routine clinical settings.

The segmentation processes were based on a seed-growing algorithm, namely region-growth algorithm. This algorithm was based on voxel thresholding, thereby quantifying the signal in a voxel-by-voxel fashion.

1) Four quadrate regions of interest (ROIs), within the ipsilateral lobe of the lesion if possible, were prescribed on the magnetic resonance imaging (MRI) at the late arterial phase (20 sec) in areas adjacent to tumor boundaries, away from blood vessels, liver boundaries, and other structures. And at least 2 ROIs were placed in which the largest tumor area was emerging. The signal intensity of each ROI region is the average value of the signal intensity of the voxels in the corresponding region. The final threshold, namely the mean intensity value (MIV), is the average value of the four ROI regions.

2) When the segment tool was set up, a region including the index tumor was chosen manually using a square frame.

3) The seed points were set in the hyperenhancement part of the tumor visually based on the hepatocellular carcinoma (HCC) enhanced characteristic. The necrotic areas, cysts, and vessels related to each lesion at the certain slice were also avoided, as identified on T2-weighted imaging (T2WI) or arterial early phase images. Meanwhile, the pre-enhanced T1WI image was scrutinized to distinguish high signal intensity, like hemorrhage, so as to avoid overestimate enhanced tumor volume value. Using the "color map editor" function to adjust the signal intensity contrast between the tumor and liver background with aim of easier bubble placement. The exterior delineated bubbles were placed inside the edge of the enhanced part of lesion with being tangent to the inner margin in principle, and the interior delineated bubbles were placed randomly. The bubble radius was adjustableto accommodate tumors of different sizes. In addition, the cross-referencing with coronal and sagittal reconstructed MRI images were used for an accurate supplement of bubble.

4) After placing the bubbles, the number of iterations of the bubble evolution needs to be set, which is determined the tumor size, region competition force and smoothing force. The latter two parameters can be adjusted, with reference to the animation presentation. In this study, the region competition force was set as 0.8, and smoothing force value was set as default value. In regard to the small HCC diameter in this study, the numbers of iterations of the bubble evolution were set as 8-12. The voxel inside or peripheral of the seed was clustered if the signal intensity was distributed within the threshold interval, ranging from the MIV+2 standard deviations to abdominal aorta signal intensity. In this step, tumor enhanced volume was obtained. Considering the presence of low or delayed enhancement components, such as fibrotic scarring within the tumor, the brush tool was used to cover the unmasked components in the tumor. Then, the total tumor volume was calculated.

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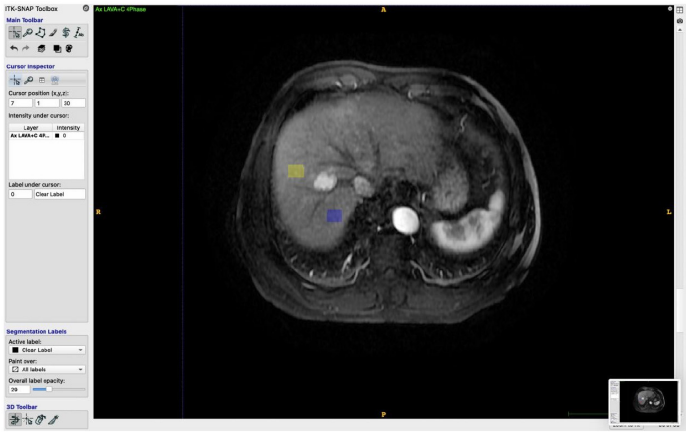

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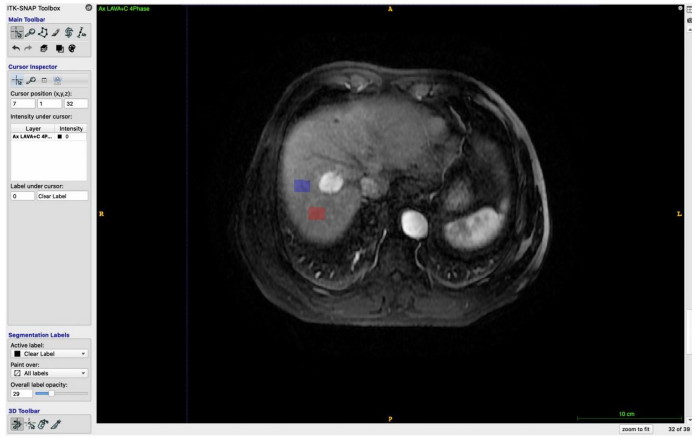

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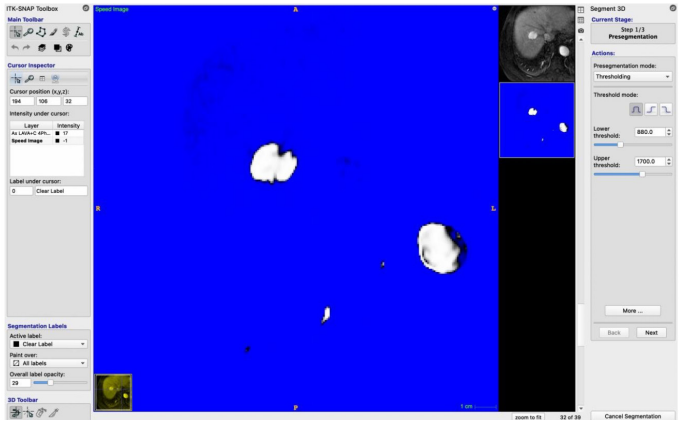

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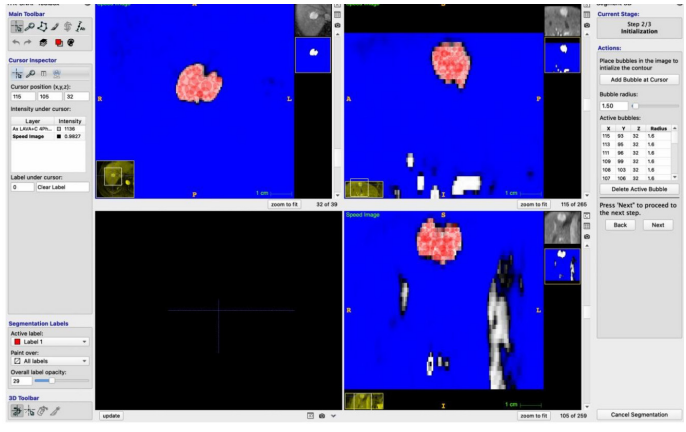

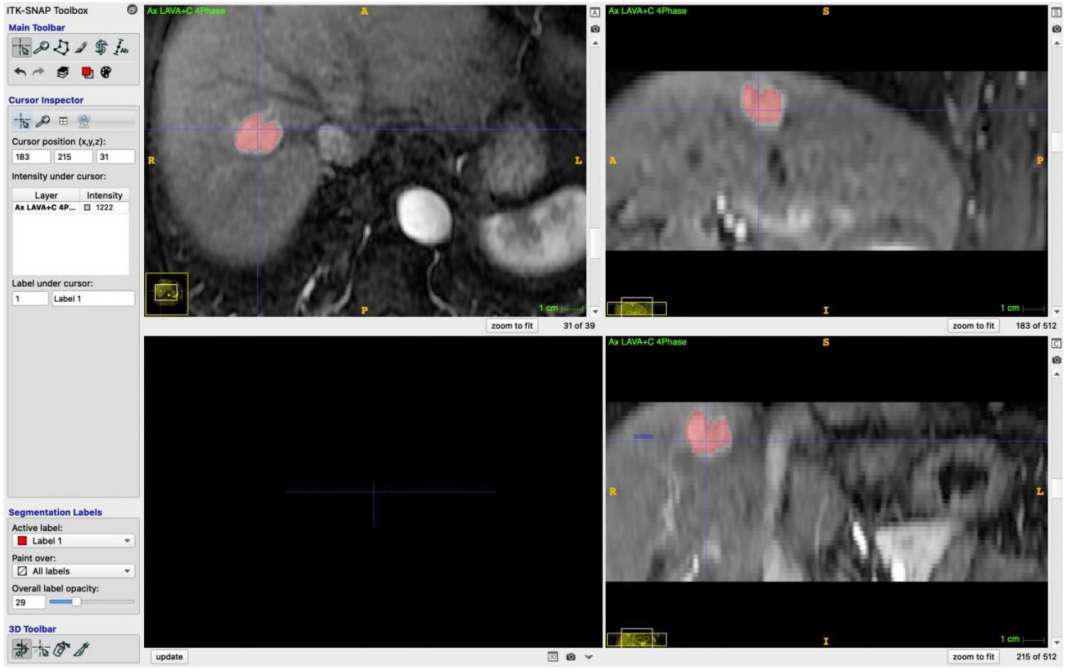
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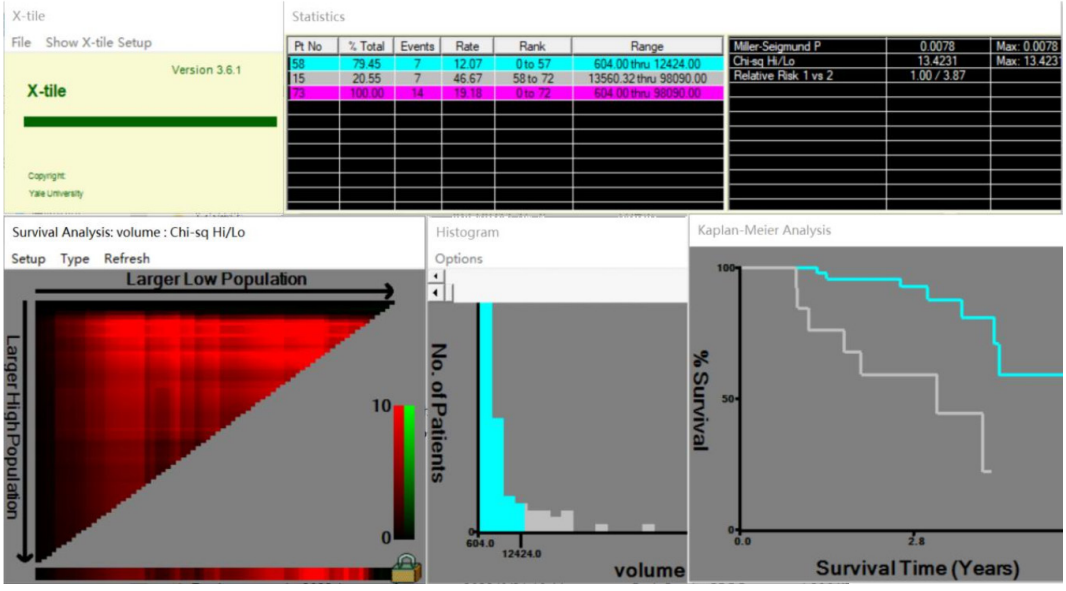
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