Effect of Spectral CT on Tumor Microvascular Angiogenesis in Renal Cell Carcinoma

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Research Article

Keywords: angiogenesis, dual-energy CT, renal cell carcinoma, microvessel angiogenesis, quantitative imaging

Posted Date: April 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-398147/v1>

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Version of Record: A version of this preprint was published at BMC Cancer on July 30th, 2021. See the published version at <https://doi.org/10.1186/s12885-021-08586-x>.

Abstract

Background: To examine the value of spectral CT quantitative parameters in renal cell carcinoma (RCC) microvascular angiogenesis.

Methods: The authors evaluated 32 patients with pathologically confirmed RCC who underwent triple-phase contrast-enhanced CT with spectral CT imaging mode from January 2017 to December 2019. Quantitative parameters including the parameters derived from iodine concentration (IC) and water concentration (WC) of 120 keV monochromatic images. The microvascular density (MVD), microvascular area (MVA) and etc. al. of specimens were evaluated. The correlation between IC and WC (including average values and random values) with microvascular parameters were analyzed using Pearson or Spearman rank correlation coefficients.

Results: The MVD of all tumors was 26.00 (15.00-43.75) vessels per field at $\times 400$ magnification. The MVD of RCC correlated positively with the mean IC, mean WC, mean NWC, mean NIC, random IC, random NIC in renal cortical phase, WCD_1 , WCD_2 , $NWCD_2$ and ICD_1 (Spearman rank correlation coefficients, r range, 0.362–0.533; all $p < 0.05$). The MVA of all tumors was (16.16 ± 8.98) % per field at $\times 400$ magnification. The MVA of RCC correlated positively with the mean IC, mean WC, mean NWC, mean NIC, random IC, random NIC in renal cortical, mean WC and mean NWC in renal parenchymal phase, WCD_1 , WCD_2 , WCD_3 , $NWCD_2$, and $NWCD_3$ (Pearson or Spearman rank correlation coefficients, r range, 0.357–0.576; all $p < 0.05$). Microvascular grading correlated positively with the mean NWC, mean NIC and random NIC in renal cortical phase, mean NWC in renal parenchymal phase, $NWCD_2$, WCD_3 , $NWCD_3$, $NICD_2$ and $NICD_3$ (Spearman rank correlation coefficients, r range, 0.367–0.520; all $p < 0.05$). As for tumor diameter (55.19 ± 19.15) , μm , only $NWCD_3$ was associated with it (Spearman rank correlation coefficients, $r = 0.388$; $p < 0.05$).

Conclusions: IC and WC indexes derived from spectral CT, especially the renal cortical phase, difference between renal cortical phase and parenchymal phase were useful indicators for evaluating tumor angiogenesis. Moreover, the difference of the derived parameters of WC in different phases has stronger correlation with tumor angiogenesis than that of IC.

Introduction

Renal cell carcinoma (RCC) is a kind of tumor originated from renal epithelial cells, accounting for more than 90% of renal cell carcinoma, which accounts for 2% of adult malignancies[1, 2]. Clear cell renal cell carcinoma (ccRCC) is the most common subtype[3]. Due to the formation of new microvessels, the structure of microvessels in malignant tumors is different from that in normal tumors. In malignant tumors, microvessels are curved and organized more irregularly. Moreover, their walls are more fragile because of the weak connection between endothelial cells and other pericyte layers, as well as basement membrane[4]. These characteristics are related to the susceptibility of tumor invasion, metastasis and recurrence.

Microvessel density (MVD) and microvessel area (MVA) measured by immunohistochemical technique have been considered as the criteria for evaluating tumor angiogenesis, which reflect the intensity of blood vessels, the area of endothelial lumen and the blood volume for tumor perfusion[5, 6]. However, studies on different types of solid tumors show that MVD and MVA are negatively correlated with survival rate, directly correlated or no significant difference[7]. The reasons for these contradictory findings are not completely clear. CT and MR perfusion imaging, as well as intravoxel incoherent motion (IVIM) model for diffusion weighted, were once considered to be ideal for preoperative noninvasive evaluation of tumor angiogenesis[8]. However, due to the large radiation dose, long examination time and other factors, they are limited in clinical application.

In recent years, spectral imaging has been introduced as a new application of dual energy CT and the advanced CT scanning technology[9]. Spectral CT can provide iodinebased material decomposition image and quantitatively analyze the iodine concentration (IC) of normal tissues and lesions in enhanced images. IC has a certain value in the imaging diagnosis, pathological grading and differential diagnosis of RCC[10, 11]. However, only a few studies focused on the relationship between IC derived from spectral CT and RCC angiogenesis. Therefore, it is of great significance to explore the expression of IC and WC on the angiogenesis of tumor. The purpose of this study was to analyze the relationship between IC and WC with RCC angiogenesis.

Materials And Methods

Participants

The study was approved by the Institutional Ethics Committee of the First Hospital of Jilin University, Changchun, China. Written informed consent was obtained from the patients by email or letter. We retrospectively reviewed the Picture Archiving and Communication System for renal cell carcinoma patients who had undergone renal CT plain scan plus three-phase enhancement before surgery (January 2017 to December 2019). The inclusion criteria were as follows: 1) patients aged between 18 and 80 years; 2) renal CT plain scan plus three-phase enhancement before surgery; 3) patients who had undergone CT performed according to our standard MM protocol; 4) patients who had received standard nephrectomy; 5) There is an interval between CT scan and surgery and the interval was no more than 2 weeks.

The exclusion criteria were as follows: 1) patients with renal cell carcinoma complicated with hemorrhage were excluded; 2) Imaging data with poor quality due to body movements or other artifacts.

Energy Spectrum CT Imaging

Renal three-phase enhancement CT were performed on a Revolution GSI CT scanner (GE Healthcare). Scanning range was conducted from “diaphragmatic apex to lower pole of kidney” with supine position. The CT scan was conducted with the following parameters: tube voltage, automatically selected by the machine according to the patient’s weight; tube current, 485 mA; pitch, 0.984; field of view (FOV), medium; image matrix, 512×512 ; rotation speed, 0.6; slice thickness/gap, 5/5mm. Renal three-phase enhancement scan: high pressure syringe was used to inject non-ionic contrast agent iohexol or iopatul through

elbow vein, with the dosage of 1.0-1.5ml/kg and the speed of 3.5ml/s. The cortical phase scanning was performed at 25s after injection, renal parenchymal phase scanning was performed at (60 ± 5) s, and excretion phase scanning was performed at (180 ± 30) s after injection. During the scanning, the patient was asked to hold his breath after deep inspiration to avoid motion artifacts affecting the image quality.

Acquisition of iodine and water concentration

The iodine and water concentration of renal three-phase enhancement CT were measured in Picture Archiving and Communication Systems (Neusoft, Version 5.5, China). The IC of each tumor needs to take the average of six values on the decomposition images for iodine (120kev). These six values include four values in the axial maximum plane and two values in the coronal maximum plane. The four measurement values of the maximum axial plane include the measurement values of the front, back, left and right directions, and the region of interest (ROI) of these four directions should not cross each other as far as possible, and avoid obvious necrosis. And keep the area of ROI consistent. The WC of each tumor was obtained on the water equivalent images in the same way as above. The iodine concentration in the aorta (IC_{ao}) at the same slice of the lesion was obtained. Normalized iodine concentration (NIC) was normalized to the IC_{ao} using the formula $NIC = IC/IC_{ao}$. In the same way, the normalized WC (NWC) was obtained using the formula $NWC = WC/WC_{ao}$. WCD_1 = The difference of WC between renal cortical phase and parenchymal phase. $NWCD_1$ = The difference of NWC between renal cortical phase and parenchymal phase. WCD_2 = The difference of WC between renal parenchymal phase and excretory phase. $NWCD_2$ = The difference of NWC between renal parenchymal phase and excretory phase. WCD_3 = The difference of WC between renal cortical phase and excretory phase. $NWCD_3$ = The difference of NWC between renal cortical phase and excretory phase. ICD_1 = The difference of IC between renal cortical phase and parenchymal phase. $NICD_1$ = The difference of NIC between renal cortical phase and parenchymal phase. ICD_2 = The difference of IC between renal parenchymal phase and excretory phase. $NICD_2$ = The difference of NIC between renal parenchymal phase and excretory phase. ICD_3 = The difference of IC between renal cortical phase and excretory phase. $NICD_3$ = The difference of NIC between renal cortical phase and excretory phase.

Interpretation of clinicopathological results

According to the electronic medical records, the pathologic findings such as tumor types, tumor classifications, and stage of tumor were assessed. Distant metastasis was defined by imaging examinations and clinical follow-up[12].

Immunohistochemical analysis

Immunohistochemistry was used to stain tumor microvessels. The first antibody was CD34. The thickness of paraffin fixed sections was 5 μ m, which were dewaxed and hydrated. Microwave antigen repair, 2% citrate buffer, PH 6.0, 15min. After the first antibody was dripped, the slices were incubated in a wet box at 4 °C overnight. After the universal second antibody was dripped, the slices were placed in a wet box and incubated at 37 °C for 30 min. The slices were washed with TBS (0.01 mol / L, pH7.4) for 3 times. After DAB staining, the nuclei were stained with hematoxylin. Finally, seal the slide.

Analysis of tumor microvessels

Five sections per patient, and the average value of microvessel density, area and grading was taken from the five sections. When no less than one slice has characteristic blood vessels, the characteristic blood vessels can be defined as positive.

1) Microvessel density (MVD): After staining, the high vascular density area was found under low power microscope. The top five regions with the higher vascular density in each slice were selected. The number of microvessels in each field of vision was counted. The average value of the five regions is calculated as MVD. When counting, only a single endothelial cell or cell cluster is counted, otherwise the vascular lumen is not counted as microvessel.

2) Microvascular area (MVA): Percentage of microvessel area in tumor tissue area.

3) Microvascular classification: Punctiform microvessels showed that the microvessels were divided by tumor cells and distributed in punctiform shape, and there was no communication between the microvessels. The linear microvessel showed that there were punctate microvessels on both sides of the microvessel, and the middle was long and narrow, which was linear. The annular microvessels are characterized by thick microvessels, which encircle the tumor cells in an island shape and communicate with each other. Strip type microvessels showed thick microvessels, parallel to tumor trabeculae, communicating with each other, showing a thick network. The tumor microvascular classification was semi quantified: the punctiform type was grade I, the linear type was grade II, and the annular or strip type was grade III. The microvascular grade of the tumor was based on the majority of the grading results in any 10 high-power fields.

4) Analysis of special vascular structure: Record whether there are vascular lakes, vascular trees (One main vessel accompanied by branch vessels), thick muscular vessels and vessels with diameter more than 50 microns appearing.

Statistical analysis

Pearson's or Spearman's correlation test was used to test the correlation between WC and IC values (mean WC, mean IC, random WC, random IC, mean NWC and mean NIC in renal cortical phase, parenchymal phase and excretory phase; WCD, NWCD, DIC and NICD parameters) with microvessel parameters (MVD, MVA, specific microvascular, microvascular grading, vessel diameter and tumor diameter). All statistical analyses were performed using SPSS 21.0 (SPSS). $P < 0.05$ was considered statistically significant.

Results

Demographic and Relevant Characteristics

A total of 32 patients with RCC were included in the study. Table 1 shows their demographic, pathological microvascular data and values of iodine and water concentration in three phase enhancement. RCC in this study affected men more often than women. Most of the measurement data accord with normal distribution, only a few (MVD, NMWC in renal cortical phase, NMIC in renal excretory phase, RIC in renal cortical phase, ICD₁, ICD₂, NICD₂, NICD₃, NWCD₂ and NWCD₃) are skew distribution.

Table 1
Demographic and clinical characteristics

Variable	Patients (n = 32)
Age (years)	53.53 ± 8.15
Male/Female	25 (78.1%)/ 7(21.9 %)
MWC/MNWC (mg/ml)	
Renal cortical phase	95.61 ± 33.89/ 0.42(0.31–0.47)
Renal parenchymal phase	82.74 ± 20.54/ 0.39 ± 0.15
Renal excretory phase	65.37 ± 12.21/ 0.78 ± 0.17
RWC/RNWC (mg/ml)	
Renal cortical phase	95.53 ± 44.48/ 0.39 ± 0.23
Renal parenchymal phase	85.06 ± 23.07/ 0.73 ± 0.21
Renal excretory phase	65.26 ± 14.14/ 0.75 ± 0.18
MIC/MNIC (mg/ml)	
Renal cortical phase	28.99 ± 14.52/ 0.33 ± 0.14
Renal parenchymal phase	24.65 ± 8.37/ 0.71 ± 0.23
Renal excretory phase	17.09 ± 6.60/ 0.91(0.78–1.05)
RIC/RNIC (mg/ml)	
Renal cortical phase	27.00(19.50-34.25)/ 0.32 ± 0.16
Renal parenchymal phase	24.16 ± 8.86/ 0.70 ± 0.27
Renal excretory phase	16.95 ± 5.49/ 0.79 ± 0.27

Data are expressed as mean ± standard deviation, median (interquartile range) or n (%). MVD = microvessel density, MVA = microvessel area. WCD₁ = The difference of mean WC in renal cortical phase and parenchymal phase, NWCD₁ = The difference of mean NWC in renal cortical phase and parenchymal phase, WCD₂ = The difference of mean WC in renal cortical and parenchymal phase, NWCD₂ = The difference of mean NWC in renal cortical and parenchymal phase, WCD₃ = The difference of mean WC in renal parenchymal phase and excretory phase, NWCD₃ = The difference of mean NWC in renal parenchymal phase and excretory phase. ICD₁ = The difference of mean IC in renal cortical phase and parenchymal phase, NICD₁ = The difference of mean NIC in renal cortical phase and parenchymal phase, ICD₂ = The difference of mean WC in renal cortical and parenchymal phase, NICD₂ = The difference of mean NIC in renal cortical and parenchymal phase, ICD₃ = The difference of mean WC in renal parenchymal phase and excretory phase, NICD₃ = The difference of mean NIC in renal parenchymal phase and excretory phase.

Variable	Patients (n = 32)
WCD ₁ /NWCD ₁ (mg/ml)	12.87 ± 21.26/ -0.30 ± 0.13
WCD ₂ /NWCD ₂ (mg/ml)	30.24 ± 27.82/ -0.40[-0.46-(-0.30)]
WCD ₃ /NWCD ₃ (mg/ml)	17.37 ± 13.38/ -0.12(-0.22-0.02)
ICD ₁ /NICD ₁ (mg/ml)	4.14(-2.91-8.17)/ -0.388 ± 0.15
ICD ₂ /NICD ₂ (mg/ml)	13.17(2.18–16.34)/ -0.56[-0.75-(-0.42)]
ICD ₃ /NICD ₃ (mg/ml)	7.56 ± 4.92/ -0.21[-0.36-(-0.05)]
Microvascular grading	
II	15 (46.9%)
III	17 (53.1%)
MVD	26.00(15.00-43.75)
MVA(%)	16.16 ± 8.98
Microvascular diameter (μm)	20.63 ± 9.73
Tumor diameter (μm)	55.19 ± 19.15
Microvascular diameter/ Tumor diameter (%)	40.98 ± 19.66
Data are expressed as mean ± standard deviation, median (interquartile range) or n (%). MVD = microvessel density, MVA = microvessel area. WCD ₁ = The difference of mean WC in renal cortical phase and parenchymal phase, NWCD ₁ = The difference of mean NWC in renal cortical phase and parenchymal phase, WCD ₂ = The difference of mean WC in renal cortical and parenchymal phase, NWCD ₂ = The difference of mean NWC in renal cortical and parenchymal phase, WCD ₃ = The difference of mean WC in renal parenchymal phase and excretory phase, NWCD ₃ = The difference of mean NWC in renal parenchymal phase and excretory phase. ICD ₁ = The difference of mean IC in renal cortical phase and parenchymal phase, NICD ₁ = The difference of mean NIC in renal cortical phase and parenchymal phase, ICD ₂ = The difference of mean WC in renal cortical and parenchymal phase, NICD ₂ = The difference of mean NIC in renal cortical and parenchymal phase, ICD ₃ = The difference of mean WC in renal parenchymal phase and excretory phase, NICD ₃ = The difference of mean NIC in renal parenchymal phase and excretory phase.	

Imaging Data

Correlation between microvessel parameters and WC/IC in renal cortical phase

The correlations between the WC/IC in renal cortical phase and microvessel parameters were analyzed. The mean WC, mean IC, mean NWC, mean NIC, random IC and random NIC from renal cortical phase scanning correlated positively with the MVD of RCC (Spearman rank correlation coefficients, all $p < 0.05$), as shown in Table 2. In the same scanning phase, the correlations between the mean NIC and mean NWC with MVD were better than those between the mean IC and mean WC with MVD. Moreover, for the same phase, the correlations between the mean IC and mean NIC with MVD were stronger than those between the random IC and random NIC with MVD. The mean WC, mean IC, mean NWC, mean NIC, random IC and random NIC from renal cortical phase scanning correlated positively with the MVA of RCC (Pearson or Spearman rank correlation coefficients, all $p < 0.05$), as shown in Table 2. In the same scanning phase, the correlations between the mean IC and mean NWC with MVA were better than those between the mean NIC and mean WC with MVA. Moreover, for the same phase, the correlations between the mean IC and mean NIC with MVA were stronger than those between the random IC and random NIC with MVA. The mean NWC, mean NIC and random NIC had moderate positive correlation with microvascular grading (Spearman rank correlation coefficients, all $p < 0.05$), and the correlation between the mean NIC and microvascular grading was stronger than that between the mean NWC with microvascular grading.

Table 2
Linear regression results evaluating the correlation of microvessel parameters and WC/IC in renal cortical phase

Microvessel Parameters	Mean WC		Mean IC		Mean NWC		Mean NIC		Random WC		Random IC		Random NWC		Random
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	
MVD	0.362	0.042*	0.421	0.016*	0.524	0.002*	0.484	0.005*	0.151	0.409	0.413	0.019*	0.287	0.112	0.3
MVA	0.494	0.004*	0.477	0.006*	0.520	0.002*	0.469	0.007*	0.236	0.149	0.370	0.037*	0.293	0.104	0.3
Specific microvascular	0.121	0.509	0.139	0.450	0.259	0.153	0.259	0.157	0.032	0.863	0.160	0.381	0.246	0.175	0.1
Microvascular grading	0.214	0.240	0.281	0.119	0.448	0.010*	0.479	0.006*	-0.031	0.868	0.285	0.114	0.156	0.393	0.3
Vessel diameter	0.158	0.338	0.127	0.487	0.259	0.152	0.265	0.201	0.046	0.802	0.192	0.292	0.103	0.576	0.0
Tumor diameter	-0.242	0.182	-0.242	0.181	-0.013	0.943	-0.127	0.490	-0.190	0.298	-0.307	0.087	-0.075	0.684	-0.
Vessel diameter/ Tumor diameter	0.161	0.380	0.156	0.393	0.142	0.439	0.107	0.560	0.037	0.840	0.321	0.073	0.063	0.731	0.0

*means that $P \leq 0.05$, with statistical significance.

Correlation between microvascular parameters and WC/IC in renal parenchymal phase

The relationships between microvascular parameters and WC/IC of renal parenchymal phase among the 32 patients enrolled in the study are presented in Table 3. The mean WC and mean NWC correlated positively with the MVA of RCC (Pearson rank correlation coefficients, both $p < 0.05$). And even more the correlation between the mean NWC and MVA was slightly stronger than that between the mean WC with MVA. At the same time, the mean NWC was also found to be positively correlated with microvascular grading (Pearson rank correlation coefficients, $p < 0.05$).

Table 3
Multiple linear regression results evaluating the correlation of microvessel parameters and WC/IC in renal parenchymal phase

Microvessel Parameters	Mean WC		Mean IC		Mean NWC		Mean NIC		Random WC		Random IC		Random NWC		Random
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	
MVD	0.158	0.387	0.221	0.225	0.284	0.115	0.227	0.211	0.120	0.513	0.118	0.520	0.179	0.326	0.130
MVA	0.355	0.046*	0.336	0.060	0.363	0.041*	0.279	0.122	0.274	0.130	0.306	0.088	0.289	0.108	0.247
Specific microvascular	0.014	0.938	-0.023	0.900	0.055	0.765	-0.072	0.695	0.064	0.730	-0.169	0.355	0.061	0.742	-0.091
Microvascular grading	0.200	0.272	0.187	0.307	0.390	0.027*	0.200	0.272	0.268	0.138	0.163	0.373	0.326	0.069	0.234
Vessel diameter	-0.022	0.906	-0.034	0.853	0.016	0.932	-0.037	0.839	-0.150	0.413	-0.158	0.388	-0.086	0.638	-0.141
Tumor diameter	-0.122	0.508	-0.175	0.339	0.041	0.822	-0.117	0.522	-0.083	0.650	-0.204	0.263	0.056	0.761	-0.104
Vessel diameter/ Tumor diameter	-0.046	0.801	-0.008	0.967	-0.060	0.745	0.007	0.972	-0.167	0.360	-0.074	0.686	-0.143	0.433	-0.084

*means that $P \leq 0.05$, with statistical significance.

Correlation between microvascular parameters and WC/IC in renal excretory phase

The correlations between the WC/IC indexes in renal excretory phase and microvascular parameters of RCC were shown in Table 4. There was no significant correlation between all WC / IC derived indicators and microvascular parameters (Pearson or Spearman rank correlation coefficients, all $p \geq 0.05$).

Table 4
Multiple linear regression results evaluating the correlation of microvessel parameters and WC/IC in renal excretory phase

Microvessel Parameters	Mean WC		Mesn IC		Mean NWC		Mean NIC		Random WC		Random IC		Random NWC		Randor
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value	
MVD	-0.095	0.606	0.215	0.237	-0.035	0.850	0.137	0.455	-0.042	0.819	0.272	0.132	-0.026	0.889	0.137
MVA	0.059	0.749	0.186	0.308	0.019	0.917	-0.007	0.971	0.092	0.618	0.333	0.162	0.053	0.779	-0.045
Specific microvascular	-0.156	0.394	-0.032	0.863	-0.298	0.098	-0.159	0.385	0.001	0.994	0.023	0.900	-0.084	0.648	0.159
Microvascular grading	-0.112	0.542	0.132	0.471	-0.261	0.148	-0.193	0.285	0.024	0.897	0.156	0.393	-0.170	0.353	-0.193
Vessel diameter	-0.303	0.092	-0.062	0.735	-0.246	0.175	0.137	0.455	-0.245	0.177	0.068	0.710	-0.207	0.255	0.151
Tumor diameter	-0.308	0.086	-0.164	0.369	-0.213	0.242	-0.095	0.603	-0.292	0.105	-0.287	0.111	-0.223	0.220	-0.122
Vessel diameter/ Tumor diameter	-0.135	0.462	-0.006	0.975	-0.054	0.767	0.236	0.194	-0.124	0.500	0.177	0.332	-0.046	0.803	0.312
*means that P≤0.05, with statistical significance.															

Correlation between microvascular parameters and DWC

The correlations between the WCD indicators and microvessel parameters were exhibited in Table 5 and Fig. 1–3. The WCD₁, WCD₂, and NWCD₂ correlated positively with the MVD of RCC (Spearman rank correlation coefficients, all p < 0.05). And the correlation between the WCD₁ and with MVD was higher than that between WCD₂ with MVD. Moreover, the correlation between the NWCD₂ was lower than that between WCD₂ with MVD.

Table 5
Multiple linear regression results evaluating the correlation of microvessel parameters and DWC

Microvessel Parameters	WCD ₁		NWCD ₁		WCD ₂		NWCD ₂		WCD ₃		NWCD ₃	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
MVD	0.533	0.002*	0.197	0.281	0.509	0.003*	0.366	0.040*	0.333	0.063	0.184	0.313
MVA	0.445	0.011*	0.091	0.619	0.576	0.001*	0.401	0.023*	0.491	0.004*	0.413	0.019*
Specific microvascular	0.216	0.234	0.233	0.200	0.216	0.234	0.441	0.012	0.266	0.142	0.202	0.266
Microvascular grading	0.220	0.225	-0.027	0.883	0.268	0.138	0.520	0.002*	0.397	0.025*	0.476	0.006*
Vessel diameter	0.273	0.130	0.249	0.169	0.326	0.069	0.432	0.013	0.243	0.180	0.271	0.134
Tumor diameter	-0.268	0.138	-0.158	0.386	-0.159	0.383	0.177	0.332	0.095	0.606	0.388	0.028*
Vessel diameter/ Tumor diameter	0.301	0.094	0.309	0.085	0.255	0.159	0.126	0.490	0.052	0.779	-0.067	0.714
*means that P≤0.05, with statistical significance.												

For MVA, the WCD₁, WCD₂, NWCD₂, WCD₃, and NWCD₃ correlated positively with the MVA of RCC (Pearson or Spearman rank correlation coefficients, all p < 0.05). According to the strength of association, the order is as follows: WCD₂, WCD₃ and WCD₁. The correlations between the WCD₂ and WCD₃ with MVA were stronger than those between the NWCD₂ and NWCD₃ with MVA.

The WCD₃, NWCD₂, and NWCD₃ correlated positively with the microvascular grading of RCC (Spearman rank correlation coefficients, all p < 0.05). The correlation between the WCD₃ with microvascular grading was weaker than that between the NWCD₃ with microvascular grading. What's more, DNWC₃ was moderately correlated with the vessel diameter (Spearman rank correlation coefficients, p < 0.05).

Correlation between microvascular parameters and DIC

The correlations between the DIC indicators and microvessel parameters were exhibited in Table 6 and Fig. 1–3. The DIC₁ correlated positively with the MVD of RCC (Spearman rank correlation coefficients, p < 0.05). NICD₂ and NICD₃ correlated positively with the MVD of RCC (Spearman rank correlation coefficients,

both $p < 0.05$). The correlation between the NICD₂ with microvascular grading ($r = 0.397$) was stronger than that between the NICD₃ with microvascular grading ($r = 0.383$).

Table 6
Multiple linear regression results evaluating the correlation of microvessel parameters and DIC

Microvessel Parameters	ICD ₁		NICD ₁		DICD ₂		NICD ₂		ICD ₃		NICD ₃	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
MVD	0.473	0.006*	0.172	0.347	0.408	0.020	0.111	0.546	0.060	0.746	0.067	0.714
MVA	0.398	0.024	0.017	0.925	0.540	0.001	0.225	0.215	0.427	0.015	0.243	0.180
Specific microvascular	0.225	0.215	0.317	0.077	0.153	0.403	0.199	0.274	-0.019	0.919	0.094	0.609
Microvascular grading	0.217	0.233	0.187	0.305	0.234	0.197	0.397	0.024*	0.190	0.298	0.383	0.030*
Vessel diameter	0.211	0.246	0.162	0.375	0.227	0.212	-0.035	0.849	0.025	0.890	-0.084	0.648
Tumor diameter	-0.210	0.248	0.068	0.710	-0.149	0.415	-0.052	0.777	-0.077	0.675	-0.034	0.853
Vessel diameter/ Tumor diameter	0.300	0.096	0.077	0.677	0.256	0.157	-0.122	0.505	-0.005	0.976	-0.178	0.330
*means that $P \leq 0.05$, with statistical significance.												

Discussion

The incidence rate of RCC has increased in recent years, ranking seventh in the most frequently diagnosed malignancies[13]. Some renal cell carcinoma is one of the most hyper-vascularized tumors. Some renal cell carcinoma is one of the most vascularized tumors. High levels of HIF-1 and HIF-2 mediate the production of vascular endothelial growth factor (VEGF), which explains the high vascularization of RCC. Metabolic disorders and angiogenesis caused by hypoxia are the microenvironment basis of renal cell carcinoma metastasis. Angiogenesis is crucial for tumor growth and metastasis. The new microvessels in the tumor are the main sites for tumor cells to enter the circulation. The 5-year survival rate of metastatic renal cell carcinoma was less than 10%[14].

CT and MR perfusion to assess tumor angiogenesis. Nevertheless, perfusion parameters are not correlated to microvascular angiogenesis. Perfusion index (PI) reflects the ability of blood perfusion. The high PI of tumor tissue is not only related to the number of microvessels, but also related to that of large vessels. It may be for this reason that microvascular parameters such as MVD sometimes have no obvious correlation with CTP and MTP parameters. Nevertheless, due to the limitation of exam methods, the above opinions need to be supplemented by new means. The minimum voltage (80kvp) and the maximum voltage (140kvp) are usually used to achieve the maximum energy separation to distinguish different substances. Spectral CT scan quantitatively analyze the iodine and water density of RCC by generating decomposition image Spectral CT can be combined with iodine contrast to calculate iodine concentration in lesions. It can be used to evaluate the microvascular parameters including MVD, MVA and etc. al in various types of solid tumors.

In our study, iodine and water were used as the basis material pair in material decomposition and to measure IC and WC in kidney. The density of iodine and water in tumor can be quantitatively analyzed by spectral CT. In our study, the MVD, MVA and other microvascular parameters were evaluated on the basis of macroscopic imaging[15]. Only the mean IC, mean WC, mean NWC and mean NIC of renal cortex phase were positively correlated with MVD, suggesting that tumor cells were metabolized vigorously and blood supply was abundant, while there were no significant correlations between the above parameters and MVD in the other two phases. In the other two phases, the correlation between IC value and microvascular parameters is lower than that of cortical phase, which may be related to the blood flow characteristics of the kidney itself. In addition, RCC is rich in blood supply, so the IC of cortical phase can best reflect the real situation of microvessels. Some studies have convinced that the NIC value of enhanced CT scan was positively correlated with MVD[16]. A retrospective study of 60 patients with lung cancer showed that MVD was positively correlated with IC, ICD and NIC (range 0.581-0.800, all < 0.001). Similar to our study, the correlation between IC and ICD and MVD is better than that between NIC and MVD. There was a certain amount of iodine in tumor tissue without injection of CT contrast agent. In addition, there is a mixture of different substances in the tissue, and the mixture of the two basic materials can be quantitatively measured by spectral CT. ICD can better eliminate these confounding factors. NIC usually refers to the ratio of IC in tumor to IC in thoracic aorta at the same level. It can be seen that NIC may also be affected by the degree of aortic enhancement when the blood supply of the subjects is different, which may also make NIC deviate from the actual IC of the lesion[17, 18]. It is believed that although angiogenesis is related to microvascular density, it does not necessarily lead to high blood flow, because the increased interstitial fluid pressure of immature and leaky new vessels may reduce blood flow and lead to tissue hypoxia[19].

IC mainly reflects the blood supply of microvascular RCC in the renal cortical phase, while IC may indicate the flow and retention of blood supply in the renal medullary phase. It is worth noting that cortical phase enhancement is more prominent in RCC, suggesting that cortical phase IC is more useful for the detection of angiogenesis in RCC. The IC of renal cortical phase image reflects the density of tumor capillaries, and the difference of renal cortical phase and medullary phase image reflects the clearance of tumor capillaries and tumor matrix[20]. In renal parenchymal phase, mean IC, mean NIC, random IC and random NIC had no significant correlation with microvascular parameters. Iodine is the main component of CT contrast agent, so IC can effectively evaluate the blood supply of tumor. Given renal medulla is located in the deep layer of renal cortex, accounting for the vast majority of renal parenchyma, with few blood vessels. It is reasonable to explain that the indicators derived from IC are not related to microvascular conditions.

In order to normalize the differences in individual circulation between patients, NIC and NWC were calculated. We found that the correlation between NIC and NWC with microvascular parameters was not significantly better than that of IC and WC. NIC and NWC did not show more stable as an index, which was slightly inconsistent with previous studies[21]. Some studies also suggest that NIC depends on the extent of lesion and aortic enhancement, and may make NIC deviate from the actual IC of lesion. This conclusion is similar to our result[17]. Therefore, in view of our findings in this study, IC may be a better indicator of tumor angiogenesis than NIC. When the RCC microvessels are relatively mature and the blood flow is fast, the contrast medium is easy to enter. In tumor tissue, when the differentiation degree of tumor is low, RCC grows fast, and the neovascularization structure is incomplete and unevenly distributed. These lesions show abnormal hemodynamics, resulting in uneven distribution of blood supply[22]. Therefore, in cortical and medullary phase, especially in cortical phase, NIC and NWC correlated with microvascular grading.

It has been shown that the average iodine density of larger tumors is significantly lower than that of the smaller ones[23], which is inconsistent with the results of our study. There was no significant correlation between derived parameters of IC and WC with tumor diameter except $NWCD_3$. That is to say, in this study, the larger the tumor, the faster the water excretion, from the cortical phase to the renal pelvis secretory phase. The present study showed that in the difference between cortical phase and medullary phase as well as medullary phase, the correlation between WC derived indexes and angiogenesis seems to be more significant than that of IC derived indexes. This could be because with the increase of microvascular area and the complexity of microvascular structure, the water outflow from tumor tissue is more easily affected by the changes of microvascular structure[24]. In addition, the molecular weight of water is smaller than that of iodine compound, and the flow rate is faster. The change of WC in different phases can better represent the situation of microvessels.

Several limiting factors must be considered in this study. Firstly, the study included a relatively small number of RCC patients. We believe that our research may encourage future research, a large multicenter study will be desirable. Secondly, the relationship between the prognosis of these RCC patients and IC and WC is worth exploring. Moreover, although pathologists and radiologists are particularly careful in sampling, it is difficult to accurately match histopathological sections with imaging sections. RCCs may show different characteristics in different fields. In clinical practice, the ROI is delineated at the maximum level of axial and coronal tumor, and the average value of each measurement is calculated as the final result to minimize the systemic errors. Although strict inclusion and exclusion criteria were established, selection bias still existed due to the retrospective study. Further studies will be carried out with expanded samples. Finally, the correlation between WC and IC parameters on microvascular status is still in the research stage, and it needs to be further verified in the follow-up study before it can be applied in clinical practice.

Conclusion

In conclusion, Due to the objectivity and accuracy of spectral CT imaging, this study provides more objective and detailed information from a new perspective, thus contributing to the diagnosis and management of RCC.

Abbreviations

ccRCC = Clear cell renal cell carcinoma

IC = Iodine concentration

ICD = Iodine concentration difference

IVIM = Intravoxel incoherent motion

MVA = Microvascular area

MVD = Microvascular density

MIC = Mean iodine concentration

MNIC = Mean normalized iodine concentration

MNWC = Mean normalized water concentration

MWC = Mean water concentration

PI = Perfusion index

RCC = Renal cell carcinoma

RIC = Random iodine concentration

ROI = Region of interest

RWC = Random water concentration

NRIC = Normalized random iodine concentration

NRWC = Normalized random water concentration

WC = Water concentration

WCD = Water concentration difference

Declarations

Authors' contributions

HXH and ZB designed the study idea. WQ and QX performed data mining. DXB and WJ performed data statistical analyses. LJ prepared figures and tables. ZB drafted the initial manuscript. SPF participated in the coordination of the study and reviewed the manuscript. HXH had primary responsibility for the final content. All authors have approved the final version of the manuscript.

Funding information

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the Personal Information Protection Act, but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

We confirm that all methods were carried out in accordance with relevant guidelines and regulations. This study was approved by the Institutional Ethics Committee of the First Hospital of Jilin University, Changchun, China. Written informed consent was obtained from the patients by email or letter.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest or potential conflicts of interest relevant to this article to disclose.

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Figures

Figure 1

The relationship between WCD1, ICD1 and MVD in thirty-two RCC patients was set out. The relationship between NWCD1, NICD1 and MVD was also shown for comparison.

Figure 2

The relationship between WCD2, ICD2 and MVD in thirty-two RCC patients was set out. The relationship between NWCD2, NICD2 and MVD was also shown for comparison.

Figure 3

The relationship between WCD3, ICD3 and MVD in thirty-two RCC patients was set out. The relationship between NWCD3, NICD3 and MVD was also shown for comparison.