Investigation of Renal Cell Carcinoma by Contrast-Enhanced Ultrasound- Predictive Value of Time Intensity Curve Analysis in Establishing Local Tumor Invasion and Stage: A Pilot Study

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Purpose: Contrast-enhanced ultrasound (CEUS) allows for real-time examination of signal intensity changes in a region of interest (ROI) and quantification of contrast agent kinetics. This study assessed the predictive ability of time-intensity curve (TIC) parameters for local tumor invasion and T stage of renal cell carcinoma (RCC).

Materials and Methods: Renal tumors in 41 patients were examined by CEUS. Thirty-two met the inclusion criteria, with a total of 33 tumors (27 clear cell, 4 chromophobe, and 2 papillary type I). Nineteen (57.6%) tumors were included in group A (stages pT1 and pT2) and 14 (42.4%) in group B (stage pT3). ROIs were established as: whole tumor (TuW); tumor area with the highest signal intensity (TuMAX) and renal cortex (Ref). The TIC parameters for each ROI were calculated as below: peak signal intensity, time to peak (TTP), rise time (RT), and mean transit time (MTT). They were analyzed as a whole value for each ROI and as a ratio between the different ROIs.

Results: There were significant differences between the tumors invading and not invading the renal sinus fat for TTP (TuW/Ref) [0.98 (0.67–1.25) vs. 1.18 (1.08–1.3), $P < .05$]. For differentiation between groups A and B, the following ratios were proven as predictors by univariate regression analysis: TTP (TuMAX/TuW); MTT (TuMAX/TuW); RT (TuMAX/TuW) ($P = .03$, $P = .01$ and $P = .02$, respectively). The value derived from the Receiver Operating Characteristic (ROC) curve for RT (TuMAX/TuW) was 0.8 with sensitivity = 78.6%, specificity = 89.5%, and cutoff value of > 0.91.

Conclusion: TIC parameters were predictors of locally noninvasive and invasive RCC.

Keywords: carcinoma; renal cell; radiography; kidney neoplasms; sensitivity and specificity; image interpretation, ultrasonography; contrast media; methods.

INTRODUCTION

Renal cell carcinoma (RCC) accounts for about 2% of malignant tumors in adults, with the clear cell form representing 60–70%.[1] Mortality is directly dependent on the tumor stage and varies between 1.2 and 2.5 per 100,000.[2-4] Staging is based on the TNM classification and can be assessed preoperatively using computed tomography (CT) scan or magnetic resonance imaging (MRI), and after tumor resection by anatomopathological examination (the gold standard). Stages T1 and T2 include tumors limited to the kidney, the key difference between the two being given by the cutoff maximal diameter of 7 cm.[5] Stage T3 comprises tumors infiltrating the perirenal fat, sinus fat and the venous system (in stages T3b and T3c, the vena cava is invaded below and above the diaphragm, respectively). Stage T4 includes exten- sion beyond Gerota's fascia or to the adrenal gland. Accurate differentiation between stages T1/T2 and stage T3 (mainly T3a) aids in the selection of pa- tients in whom nephron-sparing techniques may be performed.[6-11] Staging sensitivity of CT scan and MRI vary between 80–83% and 78–87%,[12] which indicates the disadvantages of ionizing radiation and contraindications of specific contrast agent (CA).[13] Ultrasound (US) is frequently the initial method for detecting renal tumors, providing staging accuracy of 77–85%,[14-16] and 89–100% for venous invasion alone. [17] Gas superpositions and the patient's constitution hamper the grayscale scan, and the angle of the fasci- cle with the vessel axis, together with blood flow ve- locity, alters the Doppler results. In consequence, poor staging performance is encountered in some cases. Contrast-enhanced US (CEUS) consists of intrave- nous administration of second-generation CAs[18-20]

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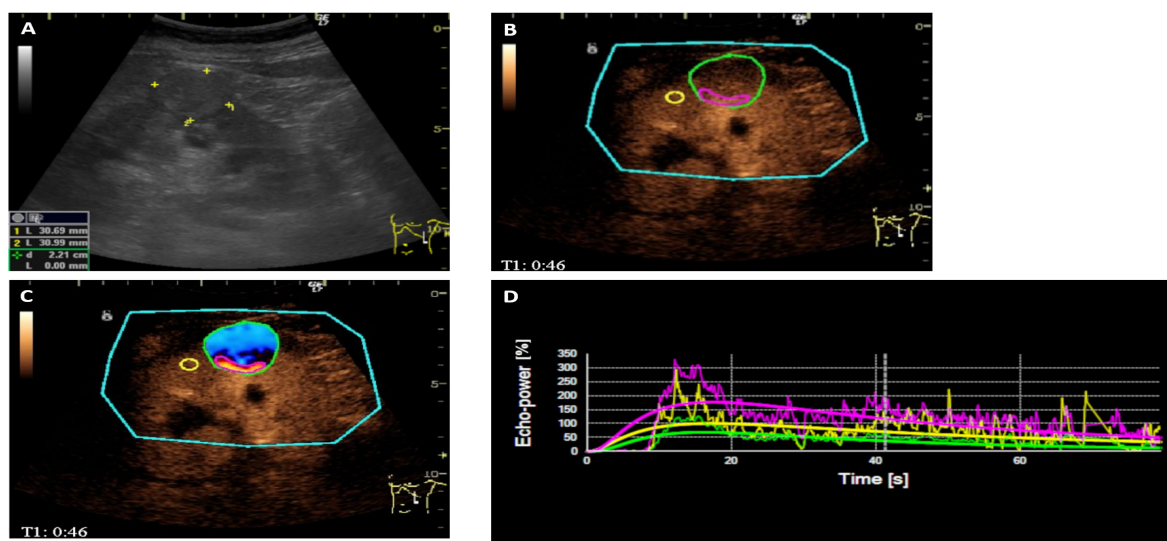Tel: +40 745 512680. Fax: +40 264 534241. E-mail: rbadea@umfcluj.ro.

Figure 1. Clear cell renal cell carcinoma of the left kidney, stage pT1b, in a -63year-old woman.
B-mode sagittal sonogram **(A)** identifying the tumor between the calipers. Selection of regions of interest **(B)** using the signal intensity map provided by the software **(C)**. Signal intensity and time intensity curve graphs **(D)**, normal renal cortex (Ref, yellow), whole tumor parenchyma (TuW, green) and tumor parenchyma with highest signal intensity (TuMAX, purple).

and allows for continuous, real-time visualization of the signal intensity changes in the blood column passing through a region of interest (ROI). CA kinetics can be quantified and displayed as time–intensity curves (TICs), from which, quantitative parameters are obtained - associated with the ROI hemodynamics. The method can be applied in patients in whom iodine- and gadolinium-based CAs are contraindicated, and provide perfusion estimation from small vessels for which Doppler measurements are unavailable. Several studies have been published on the value of CEUS in assessing RCC,[21-24] and with respect to tumor staging, most have been focused on qualitative data evaluation for identifying venous invasion, rather than predicting the final T stage. Although it provides insight into tumor vascularization (which correlates with proliferation, invasiveness and dissemination), TIC has not been analyzed as a possible predictor of invasion and stage.[25] The present pilot study aimed to: 1) obtain quantitative parameters reflecting the perfusion kinetics of RCC using CEUS and TIC; 2) identify the parameters that can act as predictors of specific local invasion (of the sinus fat, intrarenal collecting system, and perirenal fat and venous system); 3) establish the parameters that can act as predictors of T stage, and 4) evaluate the diagnostic performance of the established predictors.

MATERIALS AND METHODS

Study Patients
Approval from the "Iuliu Hatieganu'' University of Medicina and Pharmacy Ethics Committee was obtained and all the patients gave their written informed consent before enrollment. The study was performed in a single center, between 1 January 2012 and 1 May 2014, and prospectively enrolled 41 consecutive patients (27 men, 14 women, aged 30–84 years old). The inclusion criteria were: age > 18 years, no previous history of RCC, diagnosis of solid renal tumor, and being a candidate for tumor resection. The participants were included without any restriction related to the stage of the dis-

ease. Exclusion criteria were: advanced cardiopulmonary disease, pregnancy, breast feeding, and RCC not confirmed in the resected specimens by the pathological examination report.[26] We further excluded cases in which TIC curve fitting was < 60% and in which unusable frames were obtained during motion compensation. A priori sample size estimation was not achievable; therefore, the study protocol managed to maximize the sample size given the research budget available, in an effort to overpower the study as much as possible.

Ultrasound Examination
Investigations were performed by two examiners (R.B. and A.T.) using a General Electric Logiq 7 system (New York, USA) equipped with a convex wide-band transducer (2–5.5 MHz), with the patients in the dorsal or lateral decubitus position. The initial examination of the kidney and tumor consisted of B-mode, color and power Doppler US with settings such as gain, depth and focus adapted to each case. Consequently, the scanning window for CEUS was determined and consisted of a coronal or sagittal plane that encompassed a section of the entire tumor and adjacent cortex. The two planes were chosen based on the ability to maintain the same section of the above-mentioned structures in the US window, even if there were small excursions of the structures (due to breathing). During the examination, the patients were asked to breathe shallowly and not move. As recommended by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines for CEUS, the focus was positioned under the ROI, the mechanical index was set at a low value (0.09–0.11) and the time gain compensation keys were centered.[27,28] After CA injection, dynamic data were captured continuously on movie sequences of 30 seconds over a time span of 90 seconds and stored as Raw Data in the device storage unit. The CA used was SonoVue (Bracco, Milan, Italy), which consists of sulfur–hexafluoride microbubbles encased in a phospholipid shell.[29,30] It was prepared on the spot and a dose of 1.6 mL was administered into the cubital vein

Table 1. Anatomopathological characteristics of the study population.

Tumor Type	No. (%)	Presence of (no.):			
		Sinus fat invasion	Collecting system invasion	Venous system invasion	Perirenal fat invasion
Clear cell	27 (75.8)	5	4	7	4
Chromophobe	4 (9.1)	2	2	1	1
Papillary type 1	2 (6.1)	0	0	0	0

(using a 20 gauge catheter), followed by a 10-mL saline (0.9%) bolus. For each tumor, a single contrast study was performed and considered adequate if the following quality criteria were met: visualization of the entire tumor and adjacent renal cortex; and the same section of the tumor and adjacent cortex were permanently kept in the scanning window throughout the examination.

CEUS Data Analysis
For TIC computations, the data were exported from the device (in DICOM format) on a workstation provided with the Image Arena and SonoLiver software (TomTec, Unterschleissheim, Germany). Before the perfusion analysis, the clips were concatenated into a single 90-second sequence. First, two ROIs were manually traced in consensus by the examiners, as follows: whole tumor (TuW), and renal cortex (Ref). The automatic compensation of motion was applied in order to maintain the correspondence between the traced ROIs and the encompassed structures. Cases in which movement compensation resulted in unusable frames were excluded. Afterwards, using the intensity color map displayed by the software, the tumor area with the highest signal intensity (TuMAX) was also traced with the above method, and the software automatically integrated the new ROI into the motion compensated clip. The depth of the Ref, TuW and TuMAX in relation to the transducer was kept as much as possible at similar values. The ROI area for TuMAX was set between 0.5 and 1 cm^2, and for Ref, it was ≥ 1 cm^2. For each ROI, the software automatically generated a TIC (in direct proportion with CA concentration), with the point of origin corresponding to the moment of contrast injection (the zero second) and calculated the following parameters: (a) peak signal intensity (IMAX) (quantified as %; related to Ref for which the value was always considered 100%); (b) rise time (RT) (the ascending slope of the curve, measured in

seconds, independent of the time of origin); (c) time to peak (TTP) (measured in seconds, representing the time necessary for the signal to reach its peak intensity in the region of interest), and (d) mean transit time (MTT) (measured in seconds, corresponding to the gravity center of the perfusion model) (**Figure 1**). The cases in which the TICs had a poor quality of fit < 60% (as indicated automatically by the software) were excluded.

Histopathological Examination
All the surgical resection specimens were sent to the Anatomopathology Department, sectioned, and analyzed by the same specialist (C.B.). Macroscopic and microscopic examination was performed for each specimen. The longest diameter of the tumor was assessed, together with the tumor cell type and Fuhrman grade (for clear cell carcinoma). Tumor invasion of the following anatomical structures was recorded: sinus fat, collecting system, perirenal fat, pararenal fat, renal and caval venous systems, and ipsilateral adrenal gland. The revised version of the TNM staging as proposed by the American Joint Committee on Cancer in 2010[5] was used.

Statistical Analysis
The tumors were grouped according to the histopathological examination of invasion into the sinus fat, perirenal fat, collecting system and venous system (any of the following: renal vein, inferior vena cava below the diaphragm or above). Also, the tumors were distributed into group A, which included stages T1 and T2, and Group B, which included stage T3. The D'Agostino and Pearson omnibus normality test was first applied and the Mann–Whitney test *U* was used for comparison of medians (*P* < .05). The analysis was done with the help of GraphPad Prism (La Jolla, CA, USA), Medcalc (Ostend, Belgium) and Microsoft Excel 2010 (Redmond, WA, USA). The parameters obtained following TIC generation (IMAX, TTP,

Table 2. Time-intensity curve parameters for the 33 tumors, non-discriminant for invasion and groups.*

ROI	IMAX (%)	TTP (s)	MTT (s)	RT (s)
Ref	100***	20.49 (18.97-23.48)	36.8 (30.9-43.04)	15.23 (12.27-17.18)
TuW	71.95 (36.75-91.98)	24.45 (21.31-28.14)	52.4 (37.14-66.18)	20.44 (13.98-23.52)
TuMAX	128.23 (92.96-196.35)	22.52 (18.41-25.14)	38.67 (27.46-47.74)	14.5 (11.21-17.88)
TuW/Ref**	0.72 (0.36-0.91)	1.08 (1.02-1.21)	1.26 (1.06-1.55)	1.16 (1.03-1.32)
TuMAX/Ref**	1.28 (0.92-1.96)	1.04 (0.94-1.14)	1 (0.83-1.23)	0.96 (0.83-1.13)
TuMAX/TuW**	1.98 (1.66-2.45)	0.98 (0.9-0.99)	0.81 (0.7-0.96)	0.84 (0.69-0.98)

Abbreviations: ROI, region of interest; Ref, region of reference; TuW, whole tumor; TuMAX, area in the tumor with the highest signal intensity; IMAX, peak signal intensity; TTP, time to peak (of signal intensity); RT, rise time; MTT, mean transit time.
* Data are presented as medians (95% confidence intervals).
** Without units of measurement, being ratios.
*** For Ref the IMAX value was always considered 100%.

Table 3. Statistical analysis of medians for the invasion of sinus fat and for group.*

Parameters	Values	Mann-Whitney *U* Test	Univariate Regression	Multivariate Regression
		P Value	*P* Value	*P* Value
Invasion of sinus fat versus no invasion				
TTP (TuW/Ref)	0.98 (0.67-1.25) vs. 1.18 (1.08-1.3)	.020	.067	-----
MTT (TuMAX/TuW)	1.02 (0.7-1.68) vs. 0.76 (0.59-0.88)	.045	.136	-----
Group A versus group B				
MTT (TuMAX/Ref)	0.84 (0.65-1.05) vs. 1.29 (0.82-2.29)	.034	.068	-----
TTP (TuMAX/TuW)	0.91 (0.81-0.98) vs. 0.99 (0.91-1.06)	.025	.036	.963
MTT (TuMAX/TuW)	0.67 (0.53-0.77) vs. 1(0.86-1.1)	.001	.017	.157
RT (TuMAX/TuW)	0.76 (0.6-0.83) vs. 1 (0.89-1.05)	.003	.021	.637

Abbreviations: TTP, time to peak (of signal intensity); TuW, whole tumor; Ref, region of reference; MTT, mean transit time; TuMAX, area in the tumor with the highest signal intensity; IMAX, peak signal intensity; RT, rise time.

* The univariate and multivariate analyses for the determining of single and combined predictors for: invasion of sinus fat, localized, and locally invasive tumors. Values are presented as medians (95% confidence intervals). Group A, T1 and T2 anatomopathological stages; group B, T3 anatomopathological stage.

RT and MTT) were included in the statistical analysis either as primary values or as ratios. Primary values: for each ROI (TuW, TuMAX and Ref) of all the above groups were calculated and the medians of the parameters were compared according to the model: IMAX (TuW) of group A versus IMAX (TuW) of group B. Ratios: the medians were calculated from the ratios obtained by dividing the parameters of TuW to Ref, TuMAX to Ref and TuMAX to TuW, according to the model: IMAX (TuW/Ref) = IMAX (TuW) / IMAX-(Ref) and TTP (TuW/Ref) = TTP (TuW) / TTP (Ref). The resultant medians were compared between the groups, as follows (e.g.): IMAX (TuW/Ref) of group A versus IMAX (TuW/Ref) of Group B. The statistically significant parameters were included in the univariate logistic regression analysis for establishing the predictive value. The predictors of invasion and group were included in the multivariate logistic regression analysis in order to identify the combined predictive value. For the identified predictors the receiver operating characteristic (ROC) curve analysis was used and sensitivity (Se), specificity (Sp), area under the curve (AUROC) and cutoff values were calculated. Post-hoc power analysis was performed with the help of Gpower software (Universitat Kiel, Kiel, Germany) by applying the post hoc analysis module to calculate the achieved power of the Mann–Whitney *U* test (two-tailed, α error probability set at 0.05).

RESULTS

Of the 41 patients initially examined by CEUS, nine were excluded. Three had benign tumors (2 angiomyolipomas and 1 oncocytoma); two did not undergo surgery; two had TIC fitting quality < 60%, and in two cases the whole tumor and adjacent renal cortex could not be maintained in the same scanning plane during CEUS and thus resulted in unusable frames after motion compensation. In the remaining 32 patients (20 male and 12 female; age ± standard deviation [SD] 60.9 ± 10.43 years), 33 solid tumors were analyzed (1 case with bilateral RCC). Partial nephrectomy was performed in five (15.15%) cases and 28 (84.84%) underwent radical surgery. The average time between CEUS examination and surgery was 20.67 days (range 7–29 days). The median and 95% confidence interval (CI) for the maximal diameter (as measured by the pathologist) was 56 mm (32–76 mm). The histopathological features are presented in **Table 1**.

Table 4. Achieved power of the Mann–Whitney *U* test as obtained by post-hoc power analysis (two-tailed, α error probability set at 0.05).

Parameters	Sample Size of Each Group	Effect Size	Resultant Power (1-β)
Invasion of sinus fat versus no invasion			
TTP (TuW/Ref)	7 and 26	0.76	0.39
MTT (TuMAX/TuW)	7 and 26	0.68	0.32
Group A versus group B*			
MTT (TuMAX/Ref)	19 and 14	0.79	0.56
TTP (TuMAX/TuW)	19 and 14	0.94	0.71
MTT (TuMAX/TuW)	19 and 14	1.05	0.80
RT (TuMAX/TuW)	19 and 14	1.01	0.77

Abbreviations: TTP, time to peak (of signal intensity); TuW, whole tumor; Ref, region of reference; MTT, mean transit time; TuMAX, area in the tumor with the highest signal intensity; IMAX, peak signal intensity; RT, rise time.

* Group A, T1 and T2 anatomopathological stages; group B, T3 anatomopathological stage.
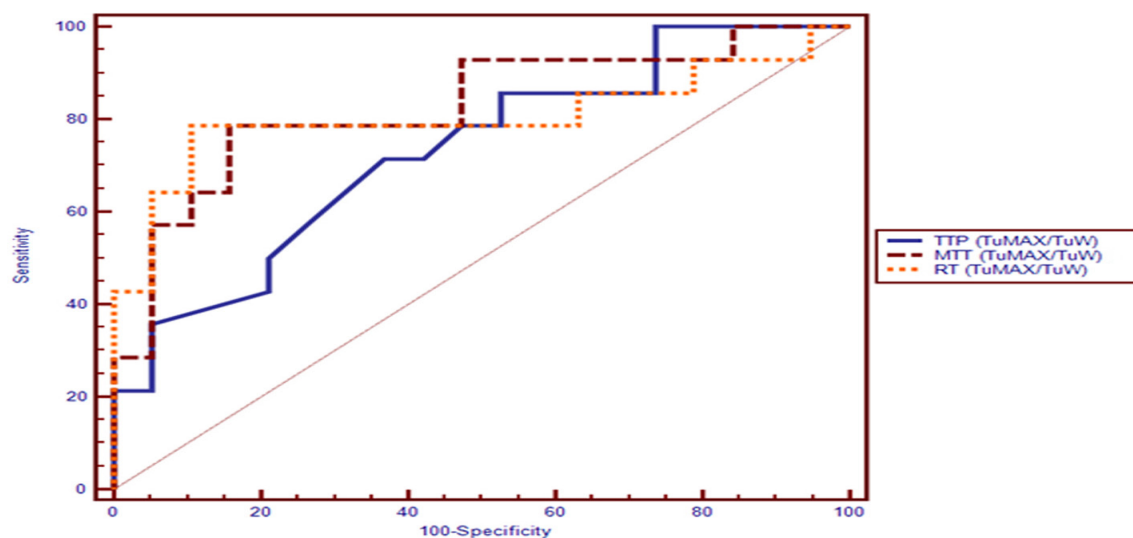
Figure 2. Comparison of Receiver Operating Characteristic (ROC) curves. ROC curves plotted for each of the group (A and B) predictors, TTP (TuMAX/TuW) in blue, MTT (TuMAX/TuW) in red, and RT (TuMAX/TuW) in yellow.
Abbreviations: ROC, receiver operating characteristic; TTP, time to peak; TuMAX, tumor area with the highest signal intensity; TuW, whole tumor; MTT, mean transit time; RT, rise time.

Nineteen (57.6%) tumors were included in group A (stages T1 and T2) and 14 (42.4%) in group B (stage T3). The TIC parameters for all 33 RCCs are presented in **Table 2**. There were significant differences for IMAX (Ref) versus IMAX (TuW) ($P = .001$), IMAX (Ref) versus IMAX (TuMAX) ($P = .04$) and IMAX (TuW) versus IMAX (TuMAX) ($P = .003$). No significant differences were found between TTP, MTT and RT of Ref versus TuW, Ref versus TuMAX, and TuW versus TuMAX. Analysis of the cases with invasion of the adjacent structures and the non-invading ones and the comparative analysis of groups A and B are presented in **Table 3** (for cases with $P < .05$). The table also contains the results of the univariate logistic regression analysis that identified the predictive factors of the sinus fat invasion and of the group (between Groups A and B). The parameters that preserved their predictive value in the univariate regression were included in the multivariate logistic regression. For the parameters presented in **Table 3** that were significant as predictors of the groups (A and B), the ROC curves were plotted (**Figure 2**). The ROC curve characteristics were: TTP (TuMAX/TuW): AUROC = 0.73, Se = 71.4%, Sp = 63.2%, and cutoff value > 0.94; MTT (TuMAX/TuW): AUROC = 0.82, Se = 78.6%, Sp = 84.2%, and cutoff value > 0.87; RT (TuMAX/TuW): AUROC = 0.8, Se = 78.6%, Sp = 89.5%, and cutoff value ≥ 0.91. The post hoc power analysis is presented in **Table 4**.

DISCUSSION

Grayscale and Doppler US represent useful techniques for the detection and characterization of RCC.[31] The accuracy in staging varies among studies, from 20%[32] to 77–85%.[14-16] The sensitivity for visualizing renal vein invasion is 100% and 89–100% for the inferior vena cava.[15,17,33,34] However, the method remains operator-dependent and may be ineffective in excessively obese patients. The current guidelines recommend the routine use of CT/MRI, for which overall RCC staging accuracy is 80–83% (CT) and 78–87% (MRI).[12]

With the advent of new-generation US equipment, CEUS has repositioned US imaging in renal oncology, and thus a re-evaluation of its role in RCC staging is needed. The advantages of CEUS are represented by the good temporal resolution, which is superior to contrast-enhanced CT (CECT)/MRI and the angio-specific character (CA remains strictly intravascular). The capacity of CEUS to detect vascularization, even with low velocity is higher than that of CECT.[35] Also, there are general studies confirming that, when it comes to tumoral perfusion quantification, the findings of CEUS are consistent with those obtained by dynamic contrast-enhanced MRI, CECT and fluorodeoxyglucose positron emission tomography.[36,37] It has been shown that the late-phase washout is suggestive for RCC (Se = 77% and Sp = 96%).[38] The performance of CEUS in diagnosing RCC is still debated. Zhou and colleagues mentioned Se = 86% and Sp = 93%, while Ignee and colleagues concluded in a study of 137 renal tumors concluded that the differentiation between RCC and benign tumors is difficult.[22,39] The EFSUMB guidelines currently recommend the use of CEUS in kidney neoplasms for differentiation between solid and cystic tumors; between pseudotumors and tumors; for follow-up of tumors during/after US-guided ablations, and for differentiation of complex cystic masses into benign/undetermined/malignant.[27] Recent studies, however, refer to the value of CEUS in the staging of renal cancer.[28] The majority of the above-mentioned articles have focused on the qualitative assessment of the kinetics of CAs. TIC studies regarding renal malignancies are scarce. One of them mentions that a lower signal intensity is associated with a good response in the case of advanced/metastatic RCC treated by kinase inhibitors.[40,41] We could find only one study that described the usage of TIC parameters for the diagnosis of RCC.[42] The authors investigated the most vascularized area of the tumor and assessed the differences between TTP and signal intensity in the RCC and Ref. Although the TIC has the advantages of objec-

tive, reproducible measurements of the contrast kinetics,[42-44] no studies have investigated their possible value as a predictor of tumor invasion and stage. We studied extensively the parameters obtained from the TIC and identified predictive factors for the low or high T stages (groups A and B) in relation to the gold standard of histopathological analysis. The assessment was performed for three different ROIs (Ref, TuW and TuMAX). We considered it necessary to explore TuMAX because most RCCs in our group presented with non-enhancing areas or inhomogeneous enhancement. Thus, we attempted to limit the influence on the results of those regions with reduced or absent perfusion (indicative for necrosis, hemorrhage, or fibrosis). Evaluation of the IMAX was significant for all the ROIs; the scale of the IMAX being TuMAX > Ref > TuW, without being associated with a certain type of invasion or a specific group. The assessment was quantitative and included the signal intensity data over an interval of 90 seconds. The IMAX is considered to correspond to the circulating blood volume in the tumor.[45] There is no unanimous acceptance regarding the signal intensity characteristics of RCC. Jianga and colleagues have demonstrated a marked enhancement in over 80% of the renal cancers investigated.[46] Xu and colleagues have found that most renal cancers are hyper- or iso-enhancing in the cortical phase (93%), becoming hypo-enhanced in the late phase (82%).[47] These variations and the differences we found between the most enhancing tumoral area and the entire tumor were the reasons why we also used the ratios between the TIC parameters of the different ROIs. Another factor was the attempt to minimize the variability among patients, caused by the examination environment, the device settings, post-processing and individual hemodynamic status.[48] We consider that the ratios between TuMAX and TuW could also prove beneficial in the case of large tumors in which concomitant viewing of the entire tumor and adjacent parenchyma during CEUS may be difficult. This was the case in two of our patients initially examined and afterwards excluded from the study. Assessment of the other parameters (TTP, MTT and RT) between the TuW and TuMAX of all 33 tumors and the Ref did not show significant differences. When looking at tumors that presented with pathologically proven invasion of the venous system in comparison with the non-invading ones, we found no consistent difference among the TIC parameters (neither as absolute values nor as ratios). From this point of view, it has been described by others that qualitative interpretation of CEUS data has a similar accuracy as CECT for showing renal vein invasion.[27] Regarding the targeted assessment of invasion into the collecting system, sinus and perirenal fat, the studies using CEUS were limited and need completion. Cokkinos and colleagues concluded that the presence of an enhancing structure in the caliceal system is a good criterion for differentiation between neoplastic tissue and debris/pus.[23] Ignee and colleagues found that accurate CEUS staging of RCC was obtained in 88% of cases (91% with CECT).[22] In our study, the median values of TTP (TuW/Ref) and MTT (TuMAX/TuW) were significantly different between the tumors invading and not invading the sinus fat, but none were significant as predictors. These results may be due to the small number of patients and tumors that invaded the sinus fat (7 cases). None of the parameters presented a statistically significant value in relation to invasion of the collecting system or perirenal fat. Although these results were negative, this is believed to be the first time that TIC parameters have been used for predicting local invasion of RCC. The comparative analysis of groups A and B demonstrated that several parameters differed significantly: MTT (TuMAX/Ref), TTP (TuMAX/TuW), MTT (TuMAX/TuW) and RT (TuMAX/TuW). The highest power was demonstrated for MTT (TuMAX/TuW) and RT (TuMAX/TuW) (0.8 and 0.77, respectively). According to the univariate logistic regression analysis, only TTP (TuMAX/TuW), MTT (TuMAX/TuW) and RT (TuMAX/TuW) were predictors of the group. It is important to emphasize that all these parameters included TuMAX in the ratio. This suggests that inclusion in the analysis of the highest signal intensity area leads to consistent results (in the context of the analysis related to TuW). In contrast, multivariate logistic regression analysis did not validate any of the three predictors. This does not necessarily nullify the predictors, because it could be explained by the correlations among these parameters; all are related to the same perfusion model equation.[49] When we introduced the predictors of groups A and B (achieved by univariate analysis) into the analysis of the ROC curves, the best diagnostic performances were obtained for MTT (TuMAX/TuW) (Se = 78.6%, Sp = 84.2%, AUROC = 0.82, and cut-off value > 0.87) and RT (TuMAX/TuW) (Se = 78.6%, Sp = 89.5%, AUROC = 0.8, and cutoff value > 0.91). These two parameters are of interest also because there were no super-positions between the median values of the two groups and this contributed to the predictive capacity. Given these, the two seem to constitute as factors that are worthwhile investigating in future research. Furthermore, owing to the fact that the abovementioned parameters did not use the adjacent renal cortex as a factor in the ratio, exclusion of patients related to the inability to maintain the Ref in the scanning plane could be limited in forthcoming studies. Our study did not assess the variations of the TIC parameters owing to the settings of the equipment. Regarding this, Gauthier and colleagues proved that the RT is more constant compared with the MTT (variation coefficient 0.7–6.9% vs. 0.8–19%).[48] Also, Ignee and colleagues stated that RT and TTP do not vary significantly in relation to the depth and lateral shift.[50] To validate the diagnostic value of the parameters used in the prediction of localized and advanced RCC, it would have been ideal to compare larger patients groups and a control group. It was one of the limitations of the present study. Other limitations were as follows. We did not quantify the inter-observer agreement for establishment of the ROIs; they were selected in consensus. No cases presented with invasion of the pararenal fat or adrenal gland, and a limited number presented with invasion of the venous system. Some patients were excluded owing to benign pathology, TIC poor fitting and inability to maintain both the Ref and TuMAX in the scanning plane. In future studies, investigation of the following TIC parameters might also prove useful: area under the entire curve, and area under the wash-in and wash-out curves (could not be calculated with the provided SonoLiver software version).[45] Despite its limitations, we believe that our study creates strong prerequisites for further research regarding the TIC parameters and their potential role in RCC staging. Using TIC in predicting tumor stage could prove especially beneficial for those patients with contraindica-

tions for CECT or MRI (due to renal insufficiency, allergic reactions, or risk of nephrogenic systemic fibrosis). To date, we have not yet seen a one-stop imaging tool capable of overcoming all the challenges posed by RCC diagnosis and staging, but rather a combination of US, CEUS, CT, MRI and nuclear imaging. All these methods provide complementary data. CEUS with its analysis of perfusion is an interesting and rapidly evolving technique. It provides an exciting new spectrum of information in addition to that of the above-mentioned techniques. In order to fulfill its potential considerable efforts are still needed.[51]

CONCLUSION

The signal intensity differed significantly among the areas investigated; the scale being TuMAX > Ref > TuW. Quantitative assessment of the CA kinetic parameters extracted from the automatic analysis of the TIC identified TTP (TuMAX/TuW), MTT (TuMAX/TuW), and RT (TuMAX/TuW) as predictors of locally advanced tumors. Among them, MTT and RT had the highest predictive performance. CEUS is a non-invasive method, with minimal limitations, that may be valuable in the preoperative protocol of RCC staging.

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CONFLICT OF INTEREST

None declared.

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