Preliminary Results of Contrast-Enhanced Sonography in the Evaluation of the Response of Uveal Melanoma to Gamma-Knife Radiosurgery

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ABSTRACT: *Purpose.* Our aim was to prospectively analyze the use of contrast-enhanced ultrasound (CEUS) in the quantitative assessment of the response of uveal melanoma (UM) to gamma-knife radiosurgery (GKR), investigating whether changes in tumor vascularization precede thickness reduction, which on average occurs at 12 months after GKR.

Methods. Ten patients with UM treated with GKR underwent sonography (US) and CEUS at baseline and at 3, 6, and 12 months after GKR. The transverse diameter, thickness, and quantitative parameters of the UM (ie, area under the curve in the wash-in phase, wash-in perfusion index, peak enhancement, and wash-in rate) were calculated by using dedicated software and compared by using Wilcoxon's signed-rank test.

Results. The mean tumor thickness on US was significantly less at both 6 (6.6 mm) and 12 months after GKR (5.8 mm) than it was at baseline (8.3 mm; $p < 0.05$, both comparisons). Compared with baseline data, the median flow quantitative parameters on CEUS were significantly changed as follows: the peak enhancement (in arbitrary units [au]) at baseline was 5×10^6; 6 months after GKR, it was 2×10^1 ($p < 0.05$), and 12 months after GKR, it was 4×10^1 ($p < 0.05$). The wash-in rate (in au) at baseline was 1×10^6; 6 months after GKR, it was 2.1 ($p < 0.05$), and 12 months after GKR, it was 9.3 ($p < 0.05$). The wash-in perfusion index (in au) at baseline was 2×10^7; 6 months after GKR, it was 7×10^1 ($p < 0.05$), and 12 months after GKR, it was 1×10^2 ($p < 0.05$). The area under the curve during the wash-in phase (in au) at baseline was 1×10^8; 12 months after GKR, it was reduced to 6×10^2 ($p < 0.05$).

Conclusions. At 6 months after GKR, a reduction of tumor thickness, as detected on US, occurred in 6 of the 10 patients, whereas a reduction in all the quantitative parameters measured on CEUS occurred in all 10 patients. However, a larger population is needed to investigate whether CEUS could become the first-choice technique for monitoring the response of UM to GKR. *J Clin Ultrasound* **43**:421–430, 2015; Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jcu.22262

Keywords: contrast-enhanced ultrasonography; uveal melanoma; gamma-knife radiosurgery; ultrasonography

INTRODUCTION

Uveal melanoma (UM) is the most common primary intraocular malignancy,[1] but it has an incidence of only 4.9 cases per million population per year in the United States.[2] It is extremely aggressive, and approximately 40% of

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patients develop liver metastases within 10 years of the initial diagnosis.[3]

Irradiation is currently the first choice as conservative treatment of UM, yielding the same survival results as achieved with enucleation.[4–6] Among irradiation modalities, gamma-knife radiosurgery (GKR) has proved to be a valid alternative to enucleation for the treatment of UM. The long-term results of stereotactic irradiation of UM by using GKR are tumor-control rates of around 90%, a reduction in volume over time, and a 5-year survival rate of more than 80%.[5,7,8] Assessment of the response of UM to GKR is mainly based on change in tumor thickness on sonography (US).[7,9] Previous studies[5,10,11] showed that the first sign of tumor regression was not generally detectable on US earlier than 6 months after treatment and that a statistically significant decrease in tumor thickness occurred only 12 months after GKR.

Because UM is a hypervascular tumor,[12,13] a decrease in vascularization[14] after GKR is probably an important parameter for assessing the actual tumor response, which influences the overall survival rate. Previous studies have shown that in hypervascular lesions, changes in vascularization after treatment precede a reduction in tumor diameter.[15]

Contrast-enhanced US (CEUS) uses intravenous administration of small gas-filled microbubbles, which do not extravasate into the interstitial fluid and can increase the signal intensity of a target organ in a specific region of interest; commercially available quantification software packages can calculate time-related intensity values of the contrast enhancement from stored video clips. Therefore, CEUS can be used to prospectively assess changes in vascularization of treated lesions.[16–18] Recently, Yang et al[19] used CEUS quantification to cross-sectionally differentiate the vascular patterns of UM from those of choroidal hemangioma, but to our knowledge, no longitudinal studies with CEUS on treated UM have been performed.

The aims of this preliminary study were to prospectively analyze the use of CEUS as a tool for quantitatively assessing the response of UM to GKR and to investigate whether changes in quantitative parameters of tumor vascularization precede variation in tumor thickness, the conventional response parameter on US, which on average occurs 12 months after GKR.

MATERIALS AND METHODS

Patients and Inclusion and Exclusion Criteria

This prospective, single-center, open-label, single-arm pilot study was approved by the ethics committee of our institution, and informed written consent was obtained from all patients before their enrollment.

Between July 2011 and September 2012, 10 consecutive patients (seven men and three women, mean [± SD] age, 66 ± 12 years [range, 45–82 years]) affected by primary UM and who were referred to the ophthalmology department of our institution were enrolled. Inclusion criteria were age older than 18 years, a diagnosis of previously untreated UM with tumor thickness more than 3 mm, eligibility for GKR, adequate organ function, life expectancy of at least 6 months, and absence of metastasis; in addition, women of childbearing age must have been using an adequate method of contraception.

Patients were excluded if they had previously undergone local treatment of their UM, had tumor thickness of less than 3 mm (such thin tumors are not eligible for GKR), were ineligible for MRI of the brain before GKR because of anatomic or functional impairment, had metastases, were simultaneously participating in another clinical trial, or had any physical or psychological impediment that could lead the investigator to anticipate poor compliance with the study. Patients with ischemic cardiac disease or other severe cardiovascular disorders requiring medical intervention or those who had had a myocardial infarction within the last 2 years were also excluded, as recommended by the European Agency for the Evaluation of Medicinal Products.

The diagnosis of UM was established on the basis of clinical findings on indirect ophthalmoscopy (Figure 1) and was confirmed on A- and B-mode US scanning. In no case was fine-needle aspiration biopsy necessary to establish the diagnosis, which was evident on imaging.[20,21] All 10 patients underwent a complete ophthalmologic evaluation as well as B-mode and color/power Doppler scanning and CEUS at baseline and at 3, 6, and 12 months after GKR. Because UM metastasizes primarily to the liver in 60–80% of patients, liver US and liver-function analyses were carried out in all patients at baseline and 3, 6, and 12 months after GKR to exclude the presence of liver metastases during follow-up. Chest radiograph to exclude lung metastasis was also performed at baseline and 12 months after GKR.[3]

Ophthalmologic Evaluation

All patients underwent a complete ophthalmologic examination of both eyes, including measurement of visual acuity with Snellen charts

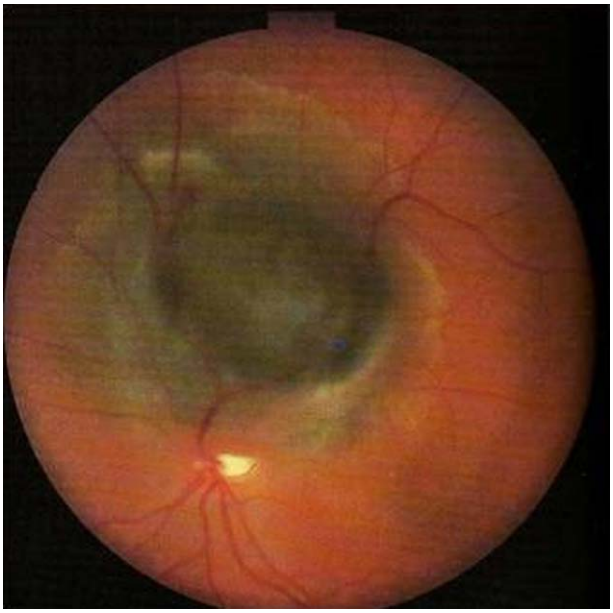

FIGURE 1. Photograph of the fundus of a large juxtapapillary choroidal uveal melanoma illustrates the highly pigmented lesion with its "collar-stud" appearance.

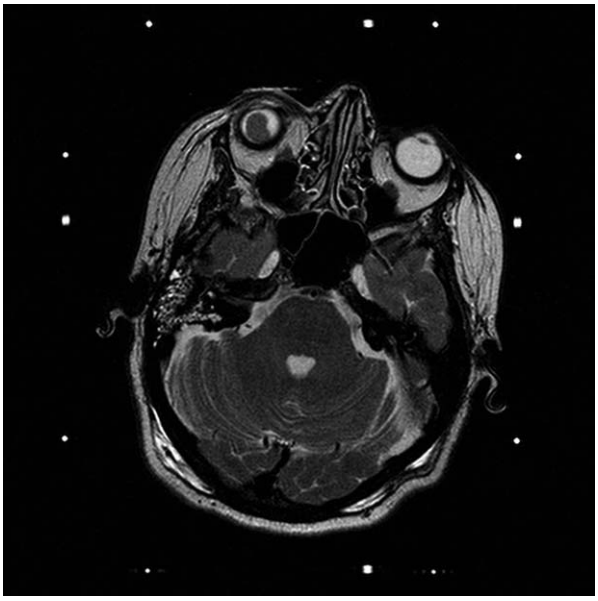

FIGURE 2. This stereotactic MRI was used in planning for gamma-knife radiosurgery in a 59-year-old woman. The T2-weighted sequence shows a hypointense lesion in the right ocular globe that is suggestive of uveal melanoma.

and measurement of intraocular pressure with Goldmann applanation tonometry (normal range, 10–21 mm Hg) at baseline and at 3, 6, and 12 months after GKR. Indirect ophthalmoscopy was used for the detection of the lesion in the affected eye and for determining tumor location (ie, choroid, iris, or ciliary body) and position (ie, temporal, nasal, superior, inferior). B-scan US was performed to determine the lesion's dimensions and to detect any scleral infiltration. Symptoms present before GKR and any treatment-related complications present 3, 6, and 12 months after GKR were recorded. All ophthalmologic examinations were performed by the same ophthalmologist (G.M.).

Gamma-Knife Radiosurgery

A Leksell stereotactic gamma knife (model G; Elekta Instruments, Stockholm, Sweden) with a mechanical precision of ±0.3 mm was used for treatment. Contrast-enhanced MRI of the brain was performed with a Philips Achieva 1.5-T scanner (Eindhoven, The Netherlands) for tumor visualization (Figure 2), and the images were transferred to the gamma knife's three-dimensional treatment-planning system (Gamma Plan 9.0) after delineation of the lesion's margins.

The patient was positioned prone or supine in the stereotactic attachment inside the selected collimator helmet with the predetermined tumor coordinates.[7] After inducing retrobulbar anesthesia with long-acting agents (eg, 5 mL of 1% ropivacaine) to obtain complete akinesia, the affected globe was fixed to the frame of the collimator helmet and immobilized during treatment. All GKR procedures were performed by the same neurosurgeon (P.P.).

Sonographic Technique

All CEUS examinations were performed by the same radiologist (M.V., who has more than 20 years of experience in US), who was positioned on the right side of the patient. Examinations were performed with an ATL-Philips IU-22 US unit (Philips Healthcare, Andover, MA) equipped with a 5–9-MHz linear transducer. A standard protocol created for the study of the UM, characterized by the following technical parameters, was used: mechanical index 0.06, 32-dB dynamic range, 87% gain, and focus immediately beneath the level of the lesion. All acquisitions were performed in the same plane, usually the transverse. All 10 patients were studied in the supine position, with closed eyelids before and during contrast administration. The overall duration of the examination was 10–15 minutes.

CEUS was always preceded by a B-scan US morphologic study for measurement of the two main diameters—tumor transverse diameter and tumor thickness—and a color/power Doppler examination to evaluate tumor vascularization. CEUS was performed after intravenous

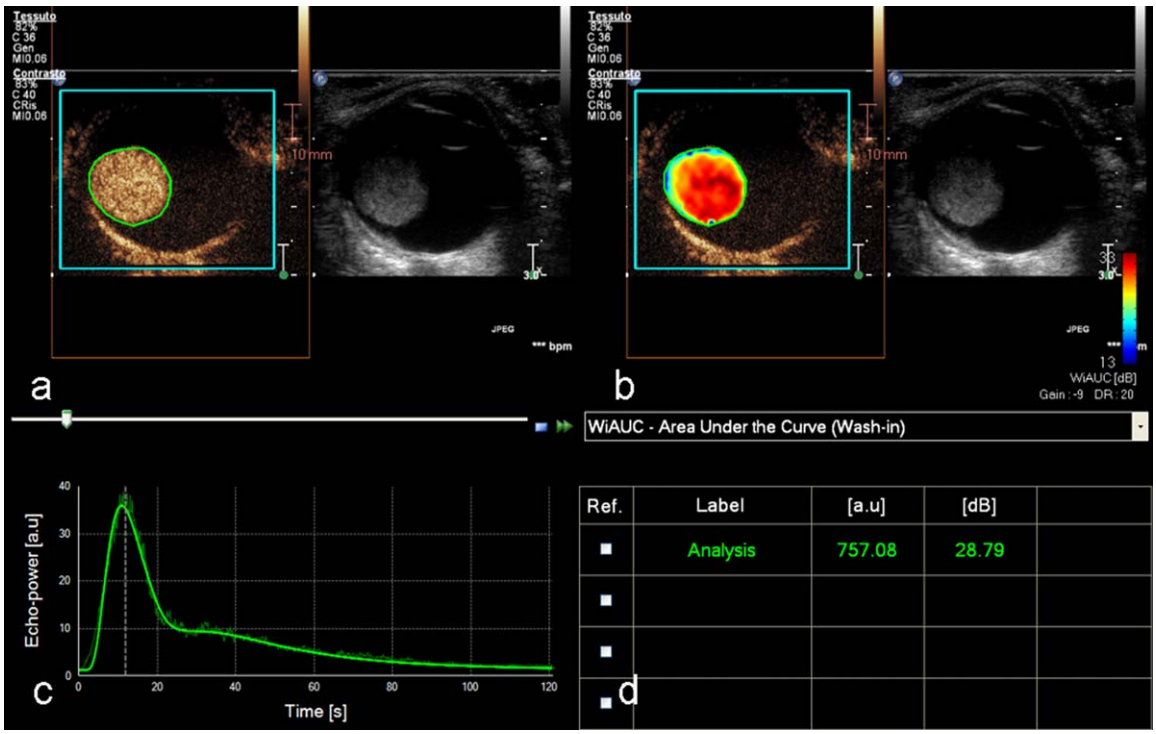

FIGURE 3. Screenshot of the software in four quadrants. **(A)** This side-by-side panel simultaneously shows the contrast enhancement and sonographic appearance of the uveal melanoma; the specific region of interest (green) delimiting the tumor is outlined. **(B)** This side-by-side panel shows the parametric image that represents the blood perfusion kinetics; in this case, the parametric map indicates the spatial variations in this area under the curve of the wash-in phase (WiAUC). The black and blue areas represent necrotic zones, whereas the red areas indicate zones with higher WiAUCs. **(C)** This time-intensity curve is the defined line that results from the fitting process of the raw data (dashed line) in a mathematical model. **(D)** Graph shows the results of the analysis of the WiAUC, expressed in arbitrary units (au) and also in decibels (dB).

injection of a 4.8-mL bolus of sulfur hexafluoride–filled microbubbles (SonoVue; Bracco, Geneva, Switzerland) through an 18-gauge cannula located in a peripheral vein of the left arm, followed by flushing with 10 mL of normal saline. Several sweeps covering the whole treated zone in the same plane were continuously recorded from the time of injection until 3 minutes after the administration of the contrast agent and were saved as DICOM multiframe cine clips (15 frames per second, each cine clip of 30 seconds' duration).

Patient compliance (described as tolerance of the ocular globe immobilization) and any systemic allergic reactions or other adverse events occurring within 30 minutes of contrast administration were recorded. At baseline, the CEUS enhancement pattern was defined for each lesion (ie, homogeneous versus centripetal versus centrifugal). Areas with a lack of contrast enhancement, suggestive of intralesional necrosis, were documented.

Image Analysis

Images recorded during the CEUS examination were reviewed by two reference radiologists (C.C. and M.V.) during one consensual reading session and were processed by dedicated, user-independent, off-line software (SonoTumor, version 4.1.2.0; Bracco, Geneva, Switzerland), which enabled the assessment of perfusion by using patented curve fitting. Time-intensity curves describing the contrast enhancement of the lesion were obtained for each patient at four different intervals: before (ie, baseline) and 3, 6, and 12 months after GKR. The time-intensity curves showed perfusion estimates made by a curve-fitting process that adjusts the parameters of a mathematical model function to best fit the raw linearized signals. Specific quantitative parameters from the time-intensity curve were obtained delimiting a specific region of interest that was manually outlined around the entire tumor area, including areas of necrosis (Figure 3).

The software automatically determined the quality of fit (QOF) for each examination. The QOF parameter is a statistical value, defined as a percentage, that measures the difference between the experimental signal and its corresponding fitted signal, according to a given model function (eg, bolus-perfusion model); it is used to describe the adequacy of the

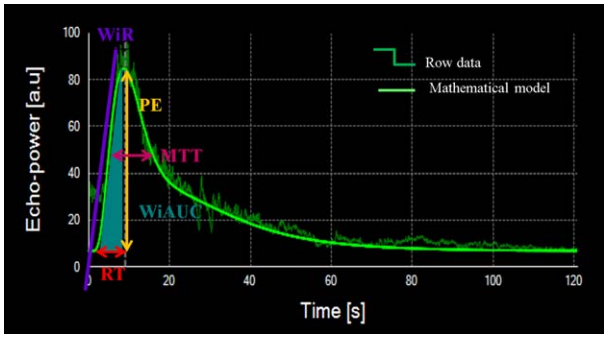

FIGURE 4. This schematic representation of a bolus time-intensity curve shows the quantitative parameters assessed by the SonoTumor software. The parameters related to blood volume and their respective units of measurement are as follows. The area under the curve in the wash-in phase (WiAUC; expressed in arbitrary units [au]) is the area under the curve between the origin and the maximum intensity of the curve fit. The wash-in perfusion index (WiPI; expressed in au) is an estimation of blood flow in the wash-in phase; it can be calculated as the ratio between the WiAUC and the rise time (RT; expressed as mL/second). The peak enhancement (PE; expressed in au) represents the maximum intensity of the curve. Parameters related to blood flow (perfusion dynamic) are the following: the mean transit time (MTT; expressed in seconds) is the time interval during which the signal is greater than 50% of the signal intensity peak; the wash-in rate (WiR; expressed in au) represents the maximum slope of the curve in the wash-in phase; the rise time (RT; expressed in seconds) is the period from the intersection of the maximum slope in the wash-in phase with the *x*-axis (time) to the instance corresponding to the maximum intensity of the fitting curve; and the time to peak (TTP; expressed in seconds) is the time it takes to reach the maximum intensity of the curve.

mathematical model of the time-intensity curve fitting the raw data obtained by the perfusion software. A QOF closer to 100% indicates a better fit. Motion compensation was always applied to correct movements within the image plane because of eye shift during the recording and to consequently improve the QOF.

The following quantitative parameters for each patient were automatically calculated: (1) area under the curve in the wash-in phase (WiAUC, expressed in arbitrary units [au]), (2) wash-in perfusion index (mL/seconds), and (3) peak enhancement (au) as parameters related to blood volume, (4) mean transit time (MTT, seconds), (5) wash-in rate (WiR, au), (6) rise time (seconds), and (7) time to peak (seconds) as parameters related to blood flow (Figure 4). The quantitative parameters were calculated from the time of microbubble visualization in the scanning plane until the first 120 seconds to eliminate the influence of microbubble arrival time variability in different patients, which is related to different physiologic parameters such as cardiac output.

The quantitative analysis for each CEUS examination was performed by the same radiologist (C.C.).

Statistical Methods

The analyses of our results are only exploratory owing to the preliminary nature of the study (only 10 patients were included). Statistical analyses were performed by using SPSS version 15.0 software (IBM, Armonk, NY). Means and SDs were assessed for tumor diameters, and medians and interquartile ranges were used for values from the quantitative analysis. Statistical analysis of data at the four different test intervals was performed with Wilcoxon's signed-rank test. A two-tailed p value less than 0.05 was considered statistically significant.

RESULTS

Ophthalmologic Evaluations

All 10 melanoma lesions were located in the choroid; seven of them were located in the right eye. The position of the lesions was inferotemporal in five patients, superotemporal in three, and inferonasal in two. Scleral infiltration was present in no patient.

At baseline, six patients reported decreased visual acuity and three patients reported scotoma; one patient was asymptomatic. Visual acuity 3 months after GKR was stable in three patients and decreased in seven patients; at 6 and 12 months after GKR, visual acuity remained stable in only one patient, decreasing in the remaining nine patients.

Six patients experienced the following complications during follow-up: one patient had high intraocular pressure (24 mm Hg) at 6 months and localized retinal detachment caused by subretinal exudation at 12 months; one patient had high intraocular pressure (28 mm Hg) at 3 months and neovascular glaucoma at 6 and 12 months; three patients developed cataracts at 6 months, and one of them also developed neovascular glaucoma at 12 months; finally, one patient developed cataract at 12 months.

Image Analysis

Tumor thickness reduction was noted in 6 of the 10 patients 6 months after GKR and in all 10 patients 12 months after GKR. Reductions in the perfusion parameters PE, WiR, and WiPI were obtained in all 10 patients at both 6 and 12 months after GKR. No patients developed liver metastases (according to US examinations), alterations in liver-function test results, or lung metastases (according to chest radiographs) during the 12-month follow-up.

TABLE 1
Comparison of of Uveal Melanoma Dimensions on Sonography

Test Interval	Tumor Dimension	Mean (± SD), mm	Range, mm	*p* Value*
Before GKR	Transverse diameter	10.7 ± 3.28	7–15	—
	Thickness	8.3 ± 2.50	4–12	—
3 months after GKR	Transverse diameter	8.8 ± 1.39	7–11	0.21
	Thickness	7.4 ± 2.69	4–11	0.17
6 months after GKR	Transverse diameter	9.4 ± 1.69	7–12	0.22
	Thickness	6.9 ± 2.45	3–10	<0.05
12 months after GKR	Transverse diameter	9.3 ± 1.21	7–10	0.47
	Thickness	5.8 ± 1.72	3–8	<0.05

Abbreviation: GKR, gamma-knife radiosurgery.
*p values refer to the comparisons between the tumor dimensions on sonography before GKR and those at 3, 6, and 12 months after GKR.

The means, SDs, and ranges of the two main diameters recorded on US for each time point are shown in Table 1. Tumor thickness did not differ significantly from the baseline measurement at 3 months after GKR ($p = 0.17$), although significant reductions of 20.5% and 24.2% were obtained at 6 and 12 months after GKR ($p < 0.05$ for both comparisons).

No significant differences were found between the tumors' transverse diameters at baseline and those obtained at 3 ($p = 0.21$), 6 ($p = 0.22$), and 12 months after GKR ($p = 0.47$).

The color/power Doppler US examinations revealed a relatively hypervascular pattern in all the examined lesions that was less evident at 6 and 12 months after GKR than it was at baseline and at 3 months after treatment.

All patients complied well with the CEUS examination. No systemic allergic reactions or other adverse events occurred after administration of the contrast agent.

Concerning the qualitative tumor assessment with CEUS, all UMs showed a centripetal contrast-filling pattern with fast wash-in and wash-out phases. Areas of intralesional necrosis increased over time after treatment in each patient.

The mean QOF value of the time-intensity curves, used to describe the accuracy of the CEUS examination, was 92 ± 7.1% at baseline; those at 3, 6, and 12 months after GKR were acceptable, even if statistically significantly lower than the baseline value: 3 months after, 89 ± 33.2%, $p < 0.05$; 6 months after, 87 ± 33.8%, $p < 0.05$; and 12 months after, 91 ± 20.5%, $p < 0.05$.

No statistically significant differences were found between each quantitative perfusion parameter at baseline and 3 months after GKR. Comparing the PE, WiR, and WiPI values before and 6 months after GKR revealed significant differences in all 10 patients ($p < 0.05$ for all comparisons). The difference between the WiAUC at baseline and 6 months after treatment was not statistically significant ($p = 0.06$). At 12 months after GKR, the PE, WiR, WiPI, and WiAUC results differed significantly from those of the respective parameters at baseline ($p < 0.05$ for all comparisons). The complete descriptive statistics of the CEUS quantitative parameters are reported in Table 2, and the changes in PE, WiPI, WiR, and WiAUC over time are shown in Figure 5. Figure 6 summarizes the behavior of the UM on CEUS in one patient at the four different test intervals. No significant differences were found between each perfusion parameter at 6 and 12 months after GKR ($p > 0.05$).

DISCUSSION

CEUS is a highly sensitive, noninvasive technique for the evaluation of organ and tumor vascularization, particularly in the abdominal organs such as the liver, kidney, and bowel,[22] and its use has recently been proposed for the characterization of hypervascular ocular lesions like UM.[19] An ocular lesion surrounded by the vitreous, the intraocular liquid, which represents a natural acoustic window, is easily detectable on US. Moreover, CEUS of the eye, differently from the liver, is not influenced by respiratory kinetics, and therefore, allows us to obtain accurate quantitative measurements in cooperative patients.

Local tumor control is a key point in determining the efficacy of a conservative treatment like GKR. GKR works to treat lesions by using beams of highly focused gamma rays, which provide a very intense dose of radiation without a surgical incision or other opening. The histopathologic changes that result in tumor cells are related to the distortion and destruction of DNA, causing the cells to be unable to

TABLE 2
Statistics of Uveal Melanoma Contrast-Enhanced Sonographic Quantitative Analysis*

Parameter	b-GKR	3-GKR	*p*	6-GKR	*p*	12-GKR	*p*
RT	7.6 (3.15)	9.7 (4.62)	0.61	12.6 (7.97)	0.18	9.9 (2.64)	0.59
WiAUC	1×10^8 (1×10^9)	2×10^3 (3×10^8)	0.50	1×10^3 (1×10^3)	0.06	6×10^2 (1×10^3)	**<0.05**
PE	5×10^6 (2×10^7)	8×10^1 (7×10^7)	0.87	2×10^1 (2×10^1)	**<0.05**	4×10^1 (4×10^1)	**<0.05**
MTT	3×10^1 (2×10^1)	3×10^1 (6×10^1)	0.17	2×10^1 (2×10^2)	0.40	2×10^1 (7.42)	0.53
WiPI	2×10^7 (9×10^7)	2×10^2 (2×10^8)	0.61	7×10^1 (2×10^2)	**<0.05**	1×10^2 (1×10^2)	**<0.05**
WiR	1×10^6 (4×10^6)	1×10^1 (9×10^6)	0.61	2.1 (2×10^1)	**<0.05**	9.3 (6.8)	**<0.05**
TTP	9.8 (2.72)	7.7 (6.28)	0.50	1×10^1 (7.20)	0.47	$1 \times \times 10^1$ (1.86)	0.11

Abbreviations: b-GKR, before gamma-knife radiosurgery (GKR); 3-GKR, 3 months after GKR; 6-GKR, 6 months after GKR; 12-GKR, 12 months after GKR; RT, rise time (expressed in seconds); WiAUC, area under the curve during the wash-in phase (expressed in arbitrary units [au]); PE, peak enhancement (au); MTT, mean transit time (seconds); WiPI, wash-in perfusion index (au); WiR, wash-in rate (au); TTP, time to peak (seconds).

*All values are expressed as the median (interquartile range). *p* values refer to the comparisons between contrast-enhanced sonographic quantitative parameters of uveal melanoma at baseline and at 3, 6, and 12 months after GKR; values in bold type represent statistical significance.

reproduce and grow, so the tumor shrinks over time. Moreover, GKR induces progressive narrowing and obliteration of the lumen of the blood vessels within the tumors secondary to damage to the endothelial cells, followed by progressive thickening of the intimal layer caused by proliferation of smooth muscle cells. Finally, cellular degeneration and hyaline transformation occur.[23]

Changes in tumor thickness detected on US examination have been reported as the most important parameter for evaluating the response of UM to GKR,[7,9] which on average occurs 12 months after treatment.[7–11] Monitoring the diameter of solid tumors during treatment follow-up is accepted worldwide according to the Response Evaluation Criteria in Solid Tumors (RECIST), but changes in tumor size can be misleading when applied to hypervascular lesions treated with antiangiogenic drugs or local treatments.[24] On the basis of these reports, modified RECIST criteria were introduced to take into account tumor necrosis induced by treatment, using contrast-enhanced radiologic imaging.[25] Some authors[26] have stated that changes in intralesional vascularization precede volume reduction and that enhancement models, such as modified response evaluation criteria in solid tumors, more accurately predict long-term survival,[27] giving us new prognostic factors.

To date, some results of the quantitative assessment of tumor perfusion with different CEUS quantitative software have been published. Most of these studies have a cross-sectional design,[28] and the majority of the prospective studies have been preclinical.[17] SonoTumor is a package of relatively new specific quantifications that allows quantitative and reproducible assessment of tumor perfusion and

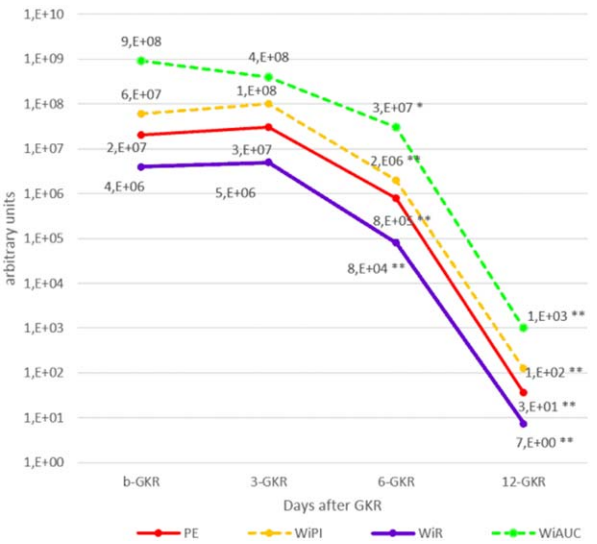

FIGURE 5. Graph shows the mean peak enhancement (PE), wash-in perfusion index (WiPI), wash-in-rate (WiR), and area under the curve in the wash-in phase (WiAUC) values expressed in arbitrary units for each time point (at baseline: b-GKR; 3 months after gamma-knife radiosurgery [GKR]: 3-GKR; 6 months after GKR: 6-GKR; 12 months after GKR: 12-GKR). The asterisks indicate statistically significant differences between the groups (*$p = 0.063$; **$p < 0.05$).

that may be able to accurately indicate changes in enhancement after a local treatment.

For all of these reasons, we prospectively investigated CEUS in the quantitative assessment of the response of UM to GKR in a clinical setting. According to the cross-sectional study by Yang et al,[19] our CEUS analysis showed a centripetal enhancement with fast wash-in and wash-out phases, typical of UM at baseline, confirmed by the time-intensity curves with high PE and WiR and low rise time, time to peak, and MTT values.

The mean QOF was acceptable at each time point, indicating good reliability of the CEUS examination. The raw data were adequately

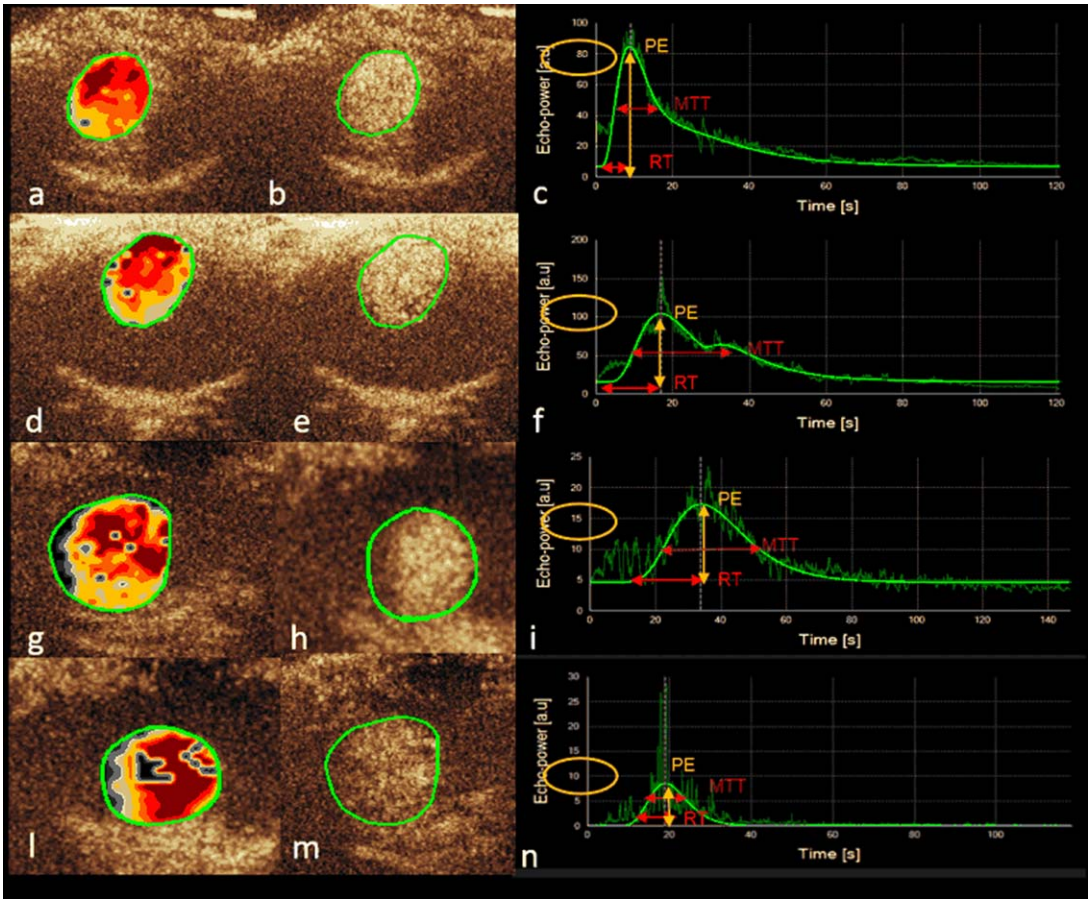

FIGURE 6. Screenshot shows uveal melanoma in follow-up. Parametric map, contrast-enhanced ultrasound (CEUS) evaluation, and time-intensity curves are shown at baseline **(A–C)** and at 3 months **(D–F)**, 6 months **(G–I)**, and 12 months **(L–N)** after gamma-knife radiosurgery (GKR). The parametric maps depict the variation in peak enhancement (PE) in different areas of the region of interest: an increase in the dark-blue areas inside the lesion occurs over time as the result of an extension of necrosis. Reduction in vascularization is also illustrated in the CEUS images: at 6 and 12 months after GKR, one can detect a lower concentration of microbubbles than visualized at baseline. Time-intensity curves at 6 and 12 months after GKR show a decrease in PE (circled in yellow) and an increase in mean transit time (MTT) and rise time (RT).

fitted by the mathematical model into the time-intensity curves by using the motion-compensation correction, which is a minor adjustment, compared with the major adjustments required to correct for respiratory motion in the assessment of hepatic lesions. In the assessment of the eye in cooperative patients, no adjustment of the image plane is required to keep the target region in plane.[29]

In our study, the mean PE, WiR, and WiPI values obtained 3 months after GKR were slightly higher than the baseline mean values, but no statistically significant differences were found. The small increase in these parameters, even if not significant, could be due to an inflammatory response of the lesion after GKR, which could lead to an increase in vascularization. Other studies investigating the response of UM to radiotherapy on US examination have revealed similar behavior of the lesions.[5,8,30]

In our experience, no significant difference was found between the two main diameters before and 3 months after GKR. For this reason, it is possible to hypothesize that the biometric parameters of the lesion are not always reliable earlier than 3 months after radiation treatment.

Concerning the CEUS quantitative parameters, a statistically significant reduction in PE, WiPI, and WiR was found at 6 months after GKR in all 10 patients. Because these three parameters describe the characteristics of intralesional perfusion, a statistically significant reduction represents depletion of tumor blood vessels that could be secondary to necrosis induced by tumor irradiation after GKR.

On the basis of these preliminary findings, we suggest that the evaluation of the response of UM to treatment may not be useful 3 months after GKR, but that instead, one should wait until at least 6 months after treatment.

In contrast with the results of other studies,[5,7–11] we found a statistically significant reduction in tumor thickness at 6 months after GKR, even if this reduction was noted on US in only 6 of the 10 patients, whereas a significant reduction in PE, WiR, and WiPI at 6 months after GKR was found at CEUS in all 10 patients. According to our preliminary results, the changes in the parameters assessed on CEUS that occurred in all patients by 6 months after GKR seemed to precede tumor thickness reduction, which occurred in all patients at 12 months after GKR, when we also found a persistent reduction in PE, WiR, and WiPI and a statistically significant reduction in WiAUC. These data indicate a constant decrease in UM vascularization by 12 months after GKR. Furthermore, the lesion thickness was statistically significantly reduced 12 months after GKR in all 10 patients. Because tumor thickness reduction is currently considered the gold standard for UM response to GKR, the reduction in lesion perfusion at 6 and 12 months after GKR can be interpreted as a good response of the lesion to GKR in all patients.

The MTT describes the contrast enhancement of the lesion, giving information about the wash-in and wash-out phases: an elongation of the MTT was found with slower wash-in and wash-out times over 6 months, but no statistically significant difference was found between the baseline MTT and that at 6 and 12 months after treatment.

Furthermore, according to these preliminary findings, quantification of tumor perfusion with CEUS may be a reliable tool for the evaluation of tumor response after radiation treatment, because it can quantitatively describe the vascular pattern of the lesion, not only assessing the shrinkage of the entire tumor but also considering the ischemic necrosis induced by GKR. The need for quantitative perfusion parameters seems to be mandatory for small tumors, such as UM, for which measurement of the lesion diameters may not be accurate enough in case of minimal changes.

Moreover, CEUS can be considered a minimally invasive, well-tolerated technique of proven safety that does not involve the use of ionizing radiation.

We believe that the results of our study, even though only preliminary, may lead to new future prospects because the evaluation of tumor response by CEUS later than 12 months after GKR could provide new information about the biologic behavior of UM after treatment and may even predict its metastatic potential.[31] One could investigate whether there is a correlation between patients' overall survival and UM vascularization before treatment to eventually identify the most aggressive lesions and to consequently adapt the overall management of patients to the biologic characteristics of their tumors.

The main limitations of this study are the relatively short follow-up and the small cohort of patients enrolled, even if UM is a very rare tumor, so our analyses are only exploratory in nature.

However, from the results of this study, we conclude that CEUS can be a reliable tool in the quantitative assessment of UM vascularization and in perfusion monitoring of the lesion after GKR. Our findings revealed reduced tumor vascularization 6 months after GKR, which is earlier than the reduction in tumor diameter that occurred on average at 12 months. Further studies are necessary to give more statistical strength to our findings and consequently to determine the role of CEUS either as a complementary diagnostic modality to conventional US or as the first-choice technique for monitoring the response of UM to GKR.

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