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Validation of Dynamic Contrast-Enhanced Ultrasound in Predicting Outcomes of Antiangiogenic Therapy for Solid Tumors
The French Multicenter Support for Innovative and Expensive Techniques Study

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Objectives: Dynamic contrast-enhanced ultrasound (DCE-US) has been used in single-center studies to evaluate tumor response to antiangiogenic treatments: the change of area under the perfusion curve (AUC), a criterion linked to blood volume, was consistently correlated with the Response Evaluation Criteria in Solid Tumors response. The main objective here was to do a multicentric validation of the use of DCE-US to evaluate tumor response in different solid tumor types treated by several antiangiogenic agents. A secondary objective was to evaluate the costs of the procedure.

Materials and Methods: This prospective study included patients from 2007 to 2010 in 19 centers (8 teaching hospitals and 11 comprehensive cancer centers). All patients treated with antiangiogenic therapy were eligible. Dynamic contrast-enhanced ultrasound examinations were performed at baseline as well as on days 7, 15, 30, and 60. For each examination, a perfusion curve was recorded during 3 minutes after injection of a contrast agent. Change from baseline at each time point was estimated for each of 7 fitted criteria. The main end point was freedom from progression (FFP). Criterion/time-point combinations with the strongest correlation with FFP were analyzed further to estimate an optimal cutoff point.

Results: A total of 1968 DCE-US examinations in 539 patients were analyzed. The median follow-up was 1.65 years. Variations from baseline were significant at day 30 for several criteria, with AUC having the most significant association with FFP ($P = 0.00002$). Patients with a greater than 40% decrease in AUC at day 30 had better FFP ($P = 0.005$) and overall survival ($P = 0.05$). The mean cost of each DCE-US was 180€, which corresponds to $250 using the current exchange rate.

Conclusions: Dynamic contrast-enhanced ultrasound is a new functional imaging technique that provides a validated criterion, namely, the change of AUC from baseline to day 30, which is predictive of tumor progression in a large multicenter cohort. Because of its low cost, it should be considered in the routine evaluation of solid tumors treated with antiangiogenic therapy.

Key Words: solid tumors, antiangiogenic treatments, functional imaging, DCE-US

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In recent years, targeted antitumor agents have significantly improved outcomes across a wide range of solid tumors.[1,2] Progression-free survival and overall survival are the preferred end points for assessing response to treatment with these agents. However, because of the high variability in postprogression survival and the possibility of multiple subsequent treatment lines, the impact of treatment is increasingly difficult to evaluate on the basis of overall survival. In addition, tumor response measures such as Response Evaluation Criteria in Solid Tumors (RECIST)[3] have proved to be of limited value in assessing response to antiangiogenic agents because early necrosis is often observed before reduction in tumor size.[4]

Dynamic contrast-enhanced ultrasound (DCE-US) is a new functional technique enabling a quantitative assessment of solid tumor perfusion using a mathematical model to analyze raw linear ultrasound data.[5] Reduction in tumor vascularization in responders is detectable earlier than what is possible with RECIST,[6] and DCE-US has therefore been proposed as an alternative method of measuring early response to treatment. Moreover, DCE-US has been shown to be predictive of long-term survival.[7] Previously, we conducted several single-center studies that demonstrated the utility of DCE-US for predicting early treatment efficacy with a number of antiangiogenic therapies in patients with solid tumors: sunitinib (SUTENT; Pfizer Inc, New York, NY) in patients with metastatic renal cell carcinoma (RCC),[7] bevacizumab (Avastin; Roche, Basel, Switzerland) in patients with hepatocellular carcinoma (HCC),[8] and masatinib (AB1010; AB Science, Paris, France) in patients with gastrointestinal stromal tumor (GIST).[9] Correlations were observed between several DCE-US criteria and the RECIST response.[7–9] Among these criteria, the change from baseline of the area under the perfusion curve (AUC) was consistently found to be related to the RECIST response. However, multicenter studies with larger sample sizes are warranted to improve the evidence-based medicine level of these findings.

The prospective multicenter French National Program for the Evaluation of DCE-US was established to evaluate DCE-US in patients with a variety of solid tumors. Our objectives were to validate previous findings and to evaluate the cost of the technique.

MATERIALS AND METHODS

Patients and Study Design

Details of the study methods and patients enrolled have been reported previously.[10] In brief, 19 centers across France (11 comprehensive cancer centers and 8 university hospitals) participated in this prospective study, which included patients with metastatic breast cancer, those with metastatic melanoma, those with metastatic colon cancer, those with metastatic GIST, those with metastatic RCC, or those with primary HCC enrolled in a clinical trial of antiangiogenic-based therapy or are otherwise eligible to be started on therapy with an approved antiangiogenic treatment.

All patients provided written informed consent, either specific to this study or in the context of a clinical trial. The study was approved by the ethics committee of each institution and was declared to the French Commission Nationale Informatique et Liberté (CNIL declaration No. 912346).

DCE-US Technique and Quantification

Dynamic contrast-enhanced ultrasound was conducted using an Aplio sonograph (Toshiba, Puteaux, France) in accordance with a standardized procedure published recently.[10] Ultrasonography was performed in 2 steps. First, a morphologic study was conducted, which allowed the target tumor to be identified.[10] The DCE-US step of the examination started with an intravenous bolus injection of 4.8 mL of SonoVue (Bracco S.P.A., Milan, Italy), which was immediately flushed with 5 mL of isotonic sodium chloride solution. For each examination, a perfusion curve was recorded during 3 minutes after the injection of the contrast agent. The DCE-US criteria were quantified using CHI-Q software (Toshiba, Puteaux, France). A quantitative analysis of the time-intensity curve was performed with a mathematical model (patent PCT/IB2006/003742)[11] on the basis of indicator-dilution theory [12] to determine 7 functional criteria: 4 related to blood volume (peak intensity, AUC, AUC during wash-in, and AUC during wash-out), 2 related to blood flow (time to peak intensity and slope of the wash-in), and mean transit time.[4] The quality of DCE-US was determined according to 6 criteria, expressed as a score of 0 to 5.[10] The DCE-US examinations with a score of 0, corresponding to very poor quality, were excluded (3% of all examinations; 48 of 1122 liver metastases and 11 of 936 nonliver metastases).

Assessments

Dynamic contrast-enhanced ultrasound was performed at baseline and at 4 postbaseline time points after treatment (days 7, 15, 30, and 60). For each DCE-US examination, we modeled the tumor perfusion curve with the 7 DCE-US criteria described previously. Changes from baseline in each criterion were calculated for each patient at each postbaseline time point. The change was expressed as the ratio of the postbaseline value over the baseline value.

Patients were to be followed up with a computed tomographic (CT) scan every 2 months for 1 year or until death. Measurements of RECIST were thus performed every 2 months during the first year. Follow-up data were available after the first year. Duration of follow-up was estimated in accordance with the Schemper method.[13]

The main end point was *freedom from progression* (FFP), defined as the time between the baseline DCE-US examination and the date of progression or the date of death of patients who died because of their malignancy without a documented progression. Progression was assessed in accordance with RECIST. Patients who stopped the treatment because of toxicity were censored when the treatment stopped and patients who died without progression were censored at the date of death.

Analyses

Statistical analyses were performed using SAS software (SAS, Cary, NC). All statistical tests were 2-sided and the significance level was 0.05.

We performed classic survival analyses to assess the relationship between each criterion and FFP. Each analysis tested the relation between the value of the criterion used as a continuous covariate and FFP with a log-rank test. Separate analyses were performed with the criterion values at baseline and at each of the 4 postbaseline time points; we also used this strategy to test the relationship between changes from baseline in each criterion at each time point. Thus, 9 tests were done for each of the 7 criteria, with a total of 63 tests. To identify criteria/time-point combinations with the strongest correlation with FFP, we focused on those where the association with FFP had a P value less than 0.001 to account for multiple testing.

Criteria/time points with the strongest association with FFP were analyzed further through a systematic search to identify the best cutoff point for each. The best single cutoff point was that with the lowest P value for association with FFP. Correlation between the criteria and the overall survival was studied after the best cutoff point had been estimated. The impact on FFP of the best combination cutoff point/criteria was tested in a multivariate analysis controlling for sex, age, tumor type, and treatment type.

Subgroup Analyses

Given the heterogeneity of the population, results obtained at the overall population level might not be applicable to specific groups of patients. Subgroup analyses according to the treatment and tumor type were therefore performed to identify groups of patients who might contribute most to the heterogeneity. For any such subgroups identified,

TABLE 1. Characteristics of the Patients

Characteristic	Patients (N = 539)
Sex, n (%)	
Male	337 (62.5)
Female	202 (37.5)
Age*, n (%), y	
21–49	121 (22.4)
50–69	306 (56.8)
70–88	110 (20.4)
Tumor, n (%)	
Renal cell carcinoma	157 (29.1)
Hepatocellular carcinoma	107 (19.8)
Colorectal carcinoma	67 (12.4)
Breast cancer	61 (11.3)
Melanoma	52 (9.7)
Gastrointestinal stromal tumor	52 (9.7)
Others	43 (8.0)
Antiangiogenic treatment, n (%)	
Sorafenib	166 (30.8)
Bevacizumab	144 (26.7)
Sunitinib	128 (23.7)
Imatinib	44 (10.6)
Other	57 (8.2)

*Data were missing for 2 patients.

a new search for the best combination criteria/time point and the corresponding best cutoff point was performed.

Economic Evaluation

The total (direct and indirect) cost of DCE-US was assessed from the hospital's perspective. Resource use was evaluated from data collected prospectively for each procedure. It included staff inputs (procedure duration including bolus injection, quantification of criteria, and medical reporting) and the number of vials of contrast medium used. Unit costs applied included wages (radiologist, nurse, engineer, and secretary), machine acquisition and maintenance logistics of an Aplio ultrasound machine, as well as the cost of SonoVue contrast medium (80€, approximately \$110 per vial). Indirect costs were also included (overheads for medical logistics, general logistics, and capital costs). Sensitivity analyses on unit costs were performed using a variation of plus or minus 10%.

RESULTS

A total of 539 patients from 19 centers were enrolled in the study between October 2007 and March 2010. Patient characteristics, including tumor types and antiangiogenic treatments received, are briefly described in Table 1. A total of 2339 DCE-US examinations were performed, of which 371 were excluded from the analysis because of the following: raw data were not quantified because of technical problems (total or partial loss of data, n = 277) and the quality of examination was poor (n = 59). Finally, examinations performed at day 1 (n = 35) were too rare to be included in the present analyses. Thus, 1968 DCE-US examinations were evaluated: 463, 401, 412, 392, and 300 at baseline as well as on days 7, 15, 30, and 60, respectively (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/RLI/A163). Examinations with a corresponding baseline were available for 360 patients at day 7, for 370 patients at day 15, for 353 patients at day 30, and for 266 patients at day 60. The median follow-up was 1.65 years (95% confidence interval [CI], 0.02–3.58). A total of 190 patients (35%) were still alive after 12 months of follow-up.

At baseline, there was no significant association between any of the DCE-US criteria values and FFP.

Analysis of changes from baseline revealed significant associations with FFP at day 30 for several criteria (Table 2). Change in AUC at day 30 showed the strongest association ($P = 0.00002$), and a systematic search for the best cutoff point for this criterion identified an $AUC_{day30}/AUC_{baseline}$ value of 0.6 (Supplementary Table 2, Supplemental Digital Content 2, http://links.lww.com/RLI/A164). This corresponds to a decrease of 40% from baseline in AUC at day 30. The difference between the groups defined by this cutoff point was significant for both FFP ($P = 0.005$) and overall survival ($P = 0.05$; Fig. 1). In the multivariate analysis controlling for age, sex, tumor type, and treatment type, the cutoff point remained significant (Table 3). Subgroup analyses according to treatment suggested heterogeneity, which could be attributed either to patients treated with sunitinib or

TABLE 2. Significance Level (*P* Value) of the Association Between Criteria Values and Freedom From Progression at Different Time Points

	Baseline	Day 7	Day 15	Day 30	Day 60
Area under the curve	—	—	—	—	—
AUC_{WI}	—	—	—	—	—
AUC_{WO}	—	—	—	—	—
MTT	—	0.002	—	—	—
PI	—	—	—	0.006	—
Slope	—	—	—	0.004	—
T_{PI}	—	—	—	—	—
Ratio between AUC and baseline AUC	—	—	—	0.00002	—
Ratio between AUC_{WI} and baseline AUC_{WI}	—	—	—	0.00003	—
Ratio between AUC_{WO} and baseline AUC_{WO}	—	—	—	0.00002	—
Ratio between MTT and baseline MTT	—	—	—	—	—
Ratio between PI and baseline PI	—	—	—	0.00003	—
Ratio between slope and baseline slope	—	—	—	0.0002	—
Ratio between T_{PI} and baseline T_{PI}	—	—	—	—	—

Empty cells indicate nonsignificant associations ($P > 0.01$).

AUC indicates area under the curve; AUC_{WI}, AUC during wash-in; AUC_{WO}, AUC during wash-out; MTT, mean transit time; PI, peak intensity; T_{PI}, time to PI.
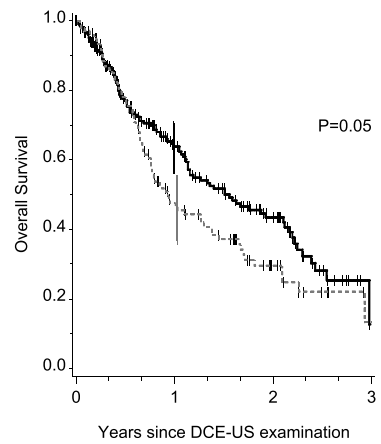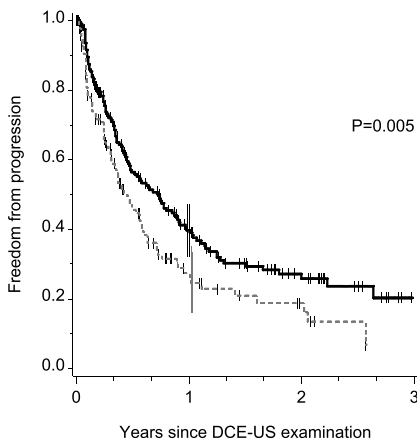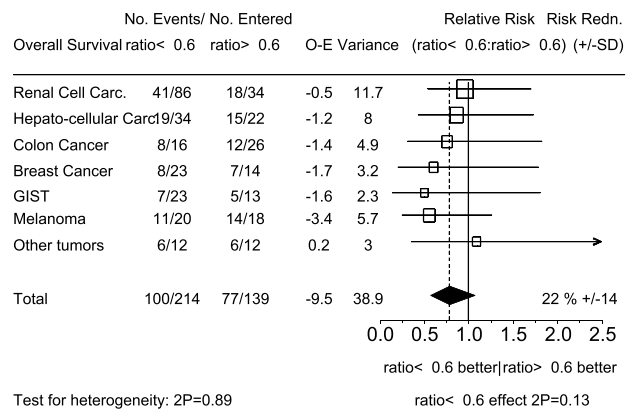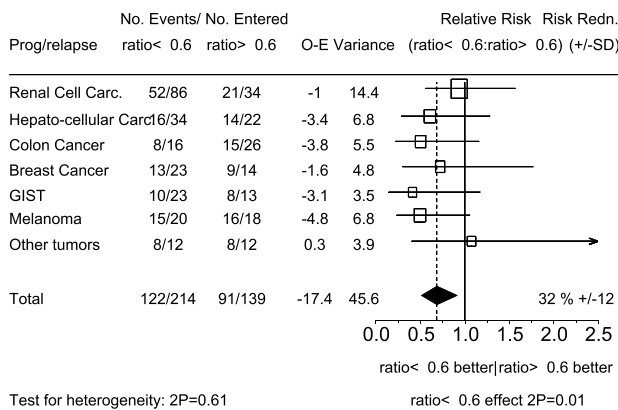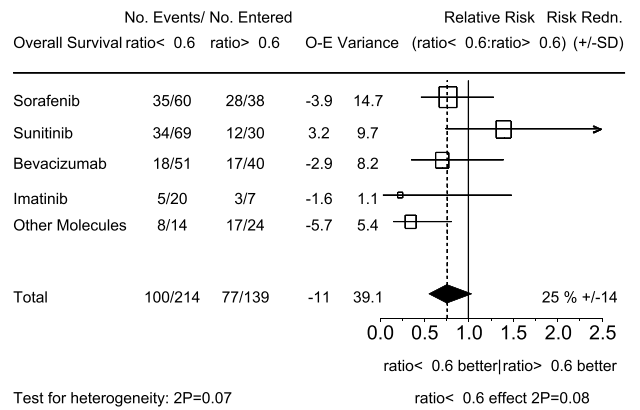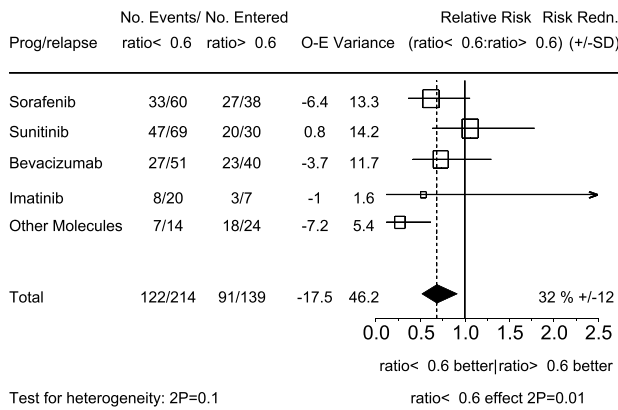
FIGURE 1. Impact of change in AUC from baseline to day 30 on FFP (left-hand panels) and on overall survival (right-hand panels). A ratio $AUC_{day30}/AUC_{baseline}$ smaller than 0.60 indicates a decrease in AUC greater than 40%. The upper panels display the results for the overall population. The middle panels show forest plots of relative risks according to specific antiangiogenic agents. The lower panels show forest plots of relative risks according to tumor type.

TABLE 3. Multivariate Analyses of Freedom From Progression

Criteria	Value	Hazard ratio (95% Confidence Interval)	P
Ratio AUC day 30/baseline	<0.6	1*	0.008
	>0.6	1.45 (1.10–1.92)	
Pathology	Renal cell carcinoma	1*	<0.001
	Breast	2.36 (0.97–5.73)	
	HCC	1.58 (0.89–2.81)	
	Colorectal Cancer	2.07 (0.87–4.92)	
	GIST	0.87 (0.42–1.79)	
	Melanoma	4.07 (2.23–7.43)	
	Other	2.02 (1.16–3.52)	
Treatment	Sunitinib	1*	0.03
	Bevacizumab	0.35 (0.16–0.79)	
	Imatinib	0.44 (0.19–1.02)	
	Sorafenib	0.84 (0.49–1.42)	
	Other	1.11 (0.68–1.81)	

Sex, age, pathology, treatment, and the AUC ratio between AUC at day 30 and the AUC at baseline were included in the multivariate analysis of freedom from progression; only the pathology, the treatment, and the AUC ratio were found to be significantly associated with FFP.

1* indicates reference category; AUC, area under the curve; HCC, hepatocellular carcinoma; GIST, gastrointestinal stromal tumor.

to the group of patients treated with "other molecules." We performed a separate analysis of patients treated with sunitinib. Most of these patients (81/128) had RCC. In this subgroup, the best cutoff for change in AUC at 30 days was 0.1, corresponding to a decrease of 90% in the AUC. Twenty-seven patients of 81 had an AUC less than 0.1. Curves of FFP for this cutoff point are shown for patients with RCC in Figure 2.

Economic Evaluation

The mean duration of the DCE-US procedure was estimated to be 28 minutes (95% CI, 13–50). The radiologist's intervention, including the medical report, lasted 23 minutes (95% CI, 8–45). Where reinjection was required, the procedure took an additional 7 minutes. The overall reinjection rate was 10%, but this was highest in baseline procedures (17%). The mean cost of a DCE-US procedure was estimated at 180€ corresponding to 250$ because of the current exchange rate (95% CI, 145€ [approximately $200] to 278€ [approximately $381]). The main cost drivers were the cost of Sonovue (49% of total cost) and the reinjection rate. Staff costs accounted for 29% of the total cost of the procedure. In the sensitivity analyses, the cost of DCE-US ranged from 177€ (approximately $243) to 223€ (approximately $306), being most sensitive to the manufacturer's price of the contrast agent and to the reinjection rate.

DISCUSSION

Currently, there is a need to improve the evaluation of patients treated with antiangiogenic treatments. Imaging biomarkers for the evaluation of tumor vascularization have been developed using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) or dynamic contrast-enhanced CT (DCE-CT). Concerning the DCE-US, the monitoring of antiangiogenic treatments was added to the European Federation of Societies for Ultrasound in Medicine and Biology guidelines on the clinical practice of contrast-enhanced ultrasound in 2011[14] and to the World Federation for Ultrasound in Medicine and Biology guidelines in 2012.[15]

Compared with other imaging techniques, the advantages of DCE-US are its suitability for use in patients with renal failure and its availability because of the pulmonary elimination of the contrast agent. However, DCE-US cannot be used for metastasis in the lung or brain because ultrasound waves are stopped by gas and bone structures.

For CT scan, a perfusion acquisition adds approximately 10% to 20% of radiation dose to a standard abdomen and pelvis CT (ie, approximately 20 mSv), and this must be taken into account when considering longitudinal follow-up.[16]

With DCE-US and DCE-CT, the relationship between the concentration and the intensity of the signal is linear. In DCE-US, the contrast medium remains solely intravascular compared with DCE-CT and DCE-MRI.

Dynamic contrast-enhanced magnetic resonance imaging is the most complex technique with respect to data interpretation because results depend on the relationship between concentration and intensity as well as on the method of data analysis.[17] To date, more than 100 clinical trials have been performed using DCE-MRI to evaluate antiangiogenic or antivascular drugs.[18] The latest consensus statement for DCE-MRI[19] recommends that lesions smaller than 3 cm be avoided, that acquisition be performed for at least 10 minutes, and that the Tofts model be used to evaluate K_{trans}, the recommended volume transfer criteria.

We used the standardized DCE-US methodology in 19 oncology centers in France.[10] Only 3% of the tests had to be excluded because they were not interpretable. We validated the change in AUC from baseline to day 30 as a criterion correlated with FFP.

The AUC is calculated by integration of the signal longer than 3 minutes and is indicative of blood volume.[20] In vitro and in vivo studies have shown that the variability in the AUC is smaller than the variability in other criteria associated with blood flow, such as the slope and time to peak.[21]

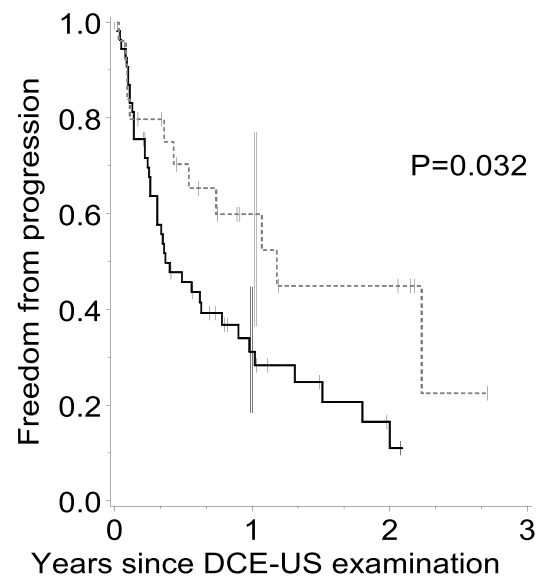| AUC day30/AUC day0	Patients at risk			
>0.1	54	11	3	0
<0.1	27	8	5	0

FIGURE 2. Time to progression (in years) according to $AUC_{day30}/AUC_{baseline}$ less than 0.1 in RCC.

Since 2006, published studies using qualitative analysis of tumor perfusion[22,23] have suggested that DCE-US may be useful in evaluating early tumor response. More recently, 5 studies, including a total of 145 patients, used quantitative DCE-US to monitor response to antiangiogenic therapies in solid tumors.[7–9] In these studies, AUC was associated with response according to RECIST in patients with HCC treated with bevacizumab[9] or sorafenib,[24] in patients with RCC treated with sunitinib,[8] in patients with GIST treated with masatinib,[10] and in patients with phase 1 with sorafenib.[25] The current large multicenter study extends this association to FFP and overall survival.

Until now, the majority of studies that used functional imaging as biomarkers[26,27] were conducted on patients with RCC, HCC, and glioma.

In RCC, significant correlations with overall survival were found in 3 studies: 2 with CT (size and density in Hounsfield units)[28,29] and one with DCE-US.[7] In all of these studies, the population was small (<70 patients) compared with the 141 patients with RCC included in the present study. Another small study (<50 patients) showed a significant association with progression-free survival using DCE-MRI.[30]

In HCC, only 2 studies identified imaging criteria correlated with overall survival. One study using DCE-MRI[31] included 38 patients and found a relationship between K_{trans} at day 15 and survival. The other study on 42 patients used DCE-US[8] and found that AUC at day 3 was a significantly associated criterion. Several other studies, all including fewer than 32 patients, showed correlations between time to progression and K_{trans} (for DCE-MRI)[31,32] or between time to progression and modified Choi criteria (for CT scan).[33] The present study is consistent with the DCE-US study, with a population more than twice as large (107 patients).

We have confirmed that the AUC may be used to predict progression and survival after treatment with tyrosine kinase inhibitors. These inhibitors block neoangiogenesis, inducing the destruction of neovessels and resulting in a decrease in blood volume within tumors. In our parameterization of the perfusion curve, 4 criteria were linked to tumor blood volume. We have shown in mice that AUC is the criterion with the smallest intraoperator variability (<15%). The good precision in the estimation of this criterion could explain why AUC was always significant in previous studies and in this multicenter study.

To increase the robustness of the technique, in the future, several further directions should be followed: the first is the use of an arterial input,[11] as is done with DCE-CT, to improve the precision of the measurements. This process takes into account the hemodynamics of the patient and has been implemented with DCE-US in research work in mice.[11] The second direction involves 3-dimensional quantification of the tumor[34,35] because, in 2-dimensional, it is difficult to ensure the same area of the tumor measured during follow-up. Furthermore, a new utilization of DCE-US demonstrated on mice[36] could be proposed to evaluate patients with osteolytic bone metastasis. To finish, a very promising new approach described by Burns should be adopted to study vascular heterogeneity as a prognostic factor.[37]

CONCLUSIONS

Dynamic contrast-enhanced ultrasound is a new functional imaging technique that provides a validated criterion, namely, the area under the time-intensity curve, which predicts tumor progression in a large multicenter cohort. Because of its low cost, it should be considered for the routine evaluation of solid tumors treated with antiangiogenic therapy.

ACKNOWLEDGMENTS

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