

Standardization of Dynamic Contrast-Enhanced Ultrasound for the Evaluation of Antiangiogenic Therapies

The French Multicenter Support for Innovative and Expensive Techniques Study

Nathalie Lassau, MD, PhD,*† Louis Chapotot, MSc,*† Baya Benatsou, MSc,*† Valérie Vilgrain, MD, PhD,‡ Michèle Kind, MD,§ Joëlle Lacroix, MD,|| Marie Cuinet, MD,¶ Sophie Taieb, MD,# Richard Aziza, MD,** Antony Sarra, MD,†† Catherine Labbe, MD,‡‡ Benoît Gallix, MD,§§ Olivier Lucidarme, MD, PhD,|||| Yvette Ptak, MD,¶¶ Laurence Rocher, MD,## Louis Michel Caquot, MD,*** Sophie Chagnon, MD,††† Denis Marion, MD,‡‡‡ Alain Luciani, MD, PhD,§§§ Joëlle Uzan-Augui, MD,||||| and Serge Koscielny, PhD†¶¶¶

Objectives: The objectives of this study are to describe the standardization and dissemination of dynamic contrast-enhanced ultrasound (DCE-US) for the evaluation of antiangiogenic treatments in solid tumors across 19 oncology centers in France and to define a quality score to account for the variability of the evaluation criteria used to collect DCE-US data.

Materials and Methods: This prospective Soutien aux Techniques Innovantes Coûteuses (Support for Innovative and Expensive Techniques) DCE-US study included patients with metastatic breast cancer, melanoma, colon cancer, gastrointestinal stromal tumors, renal cell carcinoma and patients with primary hepatocellular carcinoma tumors treated with antiangiogenic therapy. The DCE-US method was made available across 19 oncology centers in France.

Overall, 2339 DCE-US examinations were performed by 65 radiologists in 539 patients.

One target site per patient was studied. Standardized DCE-US examinations were performed before treatment (day 0) and at days 7, 15, 30, and 60. Dynamic contrast-enhanced ultrasound data were transferred from the different sites to the main study center at the Institut Gustave-Roussy for analysis. Quantitative analyses were performed with a mathematical model to determine 7 DCE-US functional parameters using raw linear data. Radiologists had to evaluate 6 criteria that were potentially linked to the precision of the evaluation of these parameters: lesion size, target motion, loss of target, clear borders, total acquisition of wash-in, and vascular recognition imaging window adapted to the lesion size.

Eighteen DCE-US examinations were randomly selected from the Soutien aux Techniques Innovantes Coûteuses (Support for Innovative and Expensive Techniques) database. Each examination was quantified twice by 8 engineers/radiologists trained to evaluate the perfusion parameters. The intraobserver variability was estimated on the basis of differences between examinations performed by the same radiologist. The mean coefficient of variability associated with each quality criterion was estimated. The final quality score, ranging from 0 to 5, was defined according to the value of coefficient of variability for each criterion.

Results: A total of 2062 examinations were stored with raw linear data. Five criteria were found to have a major impact on quality: lesion size, motion, loss of target, borders, and total acquisition of wash-in. Only 3% of the examinations were of poor quality (quality of 0); quality was correlated with the radiologists' experience, such that it was significantly higher for radiologists who had performed more than 60 DCE-US examinations ($P < 0.0001$).

Conclusions: The DCE-US methodology has been successfully provided to several centers across France together with strict rules for quality assessment. Only 3% of examinations carried out at these centers were considered not interpretable.

Key Words: dynamic contrast-enhanced ultrasound, antiangiogenic therapy, solid tumors, methodology

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In recent years, targeted antiangiogenic agents have significantly improved outcomes across a wide range of solid tumors.^{1–3} Progression-free survival and overall survival (OS) are the key criteria used to assess response to treatment with these agents. However, with improving survival rates leading to longer treatment duration,¹ assessment of median survival may take longer to achieve. In addition, tumor response criteria such as Response Evaluation Criteria in Solid Tumors have proven to be inadequate in assessing response to targeted agents because tumors often show early necrosis before reduction in tumor size.⁴

Dynamic contrast-enhanced ultrasonography (DCE-US) is a new functional technique that enables a quantitative assessment of

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From the *Ultrasonography Unit, Imaging Department, Institut Gustave Roussy, Villejuif; †IR4M, UMR 8081, Université Paris-Sud 11, CNRS, Villejuif; ‡Department of Radiology, Assistance Publique-Hôpitaux de Paris, Hôpital Beaujon, Clichy; Université Paris Diderot, Paris; §Imaging Department, Institut Bergonié, Bordeaux; ||Radiology Department, Centre François Baclesse, Caen; ¶Radiology Department, Centre Léon Bérard, Lyon; #Imaging Department, Centre Oscar Lambret, Lille; **Radiodiagnostics Department, Centre Claudius Regaud, Toulouse; ††Imaging Department, Institut Paoli Calmettes, Marseille; ‡‡Radiodiagnostics Department, Centre René Gauducheau, St-Herblain; §§Department of Abdominal and Digestive Imaging, Hôpital Saint-Éloi, Montpellier; ||||Radiology Department, CHU La Pitié-Salpêtrière, Paris; ¶¶Radiodiagnostics Department, Centre Jean Perrin, Clermont-Ferrand; ##Radiology Department, CHU Bicêtre, Le Kremlin-Bicêtre; ***Radiodiagnostics and Imaging Department, Institut Jean Godinot, Reims; †††Ultrasonography Department, Hôpital Ambroise Paré, Boulogne-Billancourt; ‡‡‡Radiology Department, CHU Hôtel-Dieu, Lyon; §§§Radiology Department, CHU Henri Mondor, Créteil; |||||Radiology Department, Hôpital Cochin, Paris; and ¶¶¶Service of Biostatistics and Epidemiology, Institut Gustave Roussy, Villejuif, France.

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Reprints: Nathalie Lassau, MD, PhD, Institut Gustave-Roussy, 39 rue Camille Desmoulins, 94805 Villejuif, France. E-mail: lassau@igr.fr.

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solid tumor perfusion using raw linear data with a quantitative analysis.⁵ Reduction in tumor vascularization can be detected in responders after 1 or 2 weeks,⁶ and DCE-US has therefore been proposed as an alternative method for measuring early response to treatment that could be predictive of long-term survival.⁷

Several single-center studies previously demonstrated that DCE-US is a useful tool for predicting early efficacy in various tumors with different localizations: sunitinib⁸ (SUTENT; Pfizer Inc, New York, New York) in patients with metastatic renal cell carcinoma (mRCC), bevacizumab (AVASTIN; Roche, Basel, Switzerland) in patients with hepatocellular carcinoma (HCC),⁹ masitinib (AB1010; AB Science, Paris, France) in patients with gastrointestinal stromal tumors (GIST).¹⁰ Correlations were observed between functional parameters measured by DCE-US and disease-free survival and OS.^{8,9} However, multicentric studies with larger sample sizes are warranted to confirm the applicability of these findings across a larger group of patients.

The prospective multicenter French National Program for the Evaluation of DCE-US has studied the technique in metastatic breast cancer, melanoma, colon cancer, GIST, and mRCC as well as in primary HCC to establish the optimal perfusion parameters and timing with which to predict tumor response to different antiangiogenic treatments and to evaluate the cost of DCE-US in a large patient population.

The goals of this study were to describe the standardization and dissemination of DCE-US for the evaluation of antiangiogenic treatments in solid tumors across 19 oncology centers in France and to describe the development of a quality score developed to account for variability in the evaluation criteria used to collect DCE-US results.

MATERIALS AND METHODS

Patients

Patients with metastatic breast cancer, melanoma, colon cancer, GIST, RCC, and primary HCC tumors who are eligible for treatment with approved antiangiogenic molecules or enrolled in a phase 1, 2, or 3 trial of experimental treatments including antiangiogenic molecules, alone or in combination with chemotherapy, were included.

The exclusion criteria were as follows: patients whose age is younger than 18 years and those with heart failure. Patients were excluded if the tumor was inaccessible to ultrasonography or if it was not vascularized at baseline DCE-US examination because such patients cannot be evaluated with this method. One tumor for each patient was studied; the tumor was selected on the basis of size (>2 cm), percentage of necrosis evaluated in B-mode (<50% of total tumor volume), and site (selected for the best acoustic window that enables acquisition more than 3 minutes without losing the tumor).

All patients were informed of the technique and provided written informed consent. The consent forms were included in a national registry, which was declared to the Commission Nationale Informatique et Liberté.

Dynamic Contrast-Enhanced Ultrasonography

Standardized DCE-US examinations (Fig. 1) were performed with an Aplio sonograph (Toshiba, Puteaux, France). The same type of machine was used at all centers included in the study. All the Aplio sonographs had access to the raw linear data, and all were equipped with the same software:

1. Vascular recognition imaging (VRI) perfusion software that enables enhanced detection of the signal generated by microbubbles; it combines grayscale-coded fundamental B-mode imaging (providing anatomical information), Doppler imaging (providing

vascular information), and harmonic imaging on the basis of pulse subtraction mode.

2. iASSIST software, which allows the automatic recording of 3 minutes of raw linear data.
3. CHI-Q quantification software (Toshiba, Puteaux, France) was used to determine and follow the region of interest (ROI) using VRI and B-mode

Two different probes were used depending on the localization of the target: either a 3.5-MHz convex-array abdominal probe or an 8-MHz superficial linear-array probe. The gain and acoustic power were fixed to 32 and 0.8% for the abdominal probe and to 37 and 0.8% for the superficial probe, respectively. The mechanical index (<0.1) was low with both settings.

Ultrasonographic examinations were performed in 2 stages. First, a morphologic study was conducted in B-mode, which allowed the target tumor to be identified. The tumor was measured with electronic calipers. The DCE-US stage of the examination started with a single intravenous bolus injection of 4.8 mL of SonoVue (Bracco S.P.A., Milan, Italy), a contrast medium consisting of sulfur hexafluoride-filled microbubbles, and flushed immediately thereafter with 5 mL of normal saline. The investigation recordings and timing were triggered when the contrast agent was injected.⁸ A total of 720 images (ie, 4 images per second) in raw linear data were acquired during 3 minutes using the VRI mode.

Nine of the 19 centers transferred the DCE-US data to the Institut Gustave Roussy (IGR) using a national secured network (SMN router; Etiam, Rennes, France). The other 10 centers used a Toshiba server to store their data locally; the data were subsequently transferred to the IGR. All DCE-US examinations were archived for 10 years at the IGR on a Centera archive system (EMC, Becons, France). This archive automatically replicates data, which are stored securely without the possibility of deletion.

Quantification of DCE-US Parameters

After each examination, the DCE-US parameters were quantified using CHI-Q software. The ROI, including the total lesion, was defined manually. Depending on the motion of the lesion, several frames were selected to adjust the position of the ROI. The ROI was defined by frame, and if the ROI did not fit the position of the lesion after initiating the recording, it was moved (to fit the position of the lesion) on the next selected frame. The CHI-Q software was then used to interpolate between the 2 ROI positions. The process was repeated for a period of 3 minutes. The time-intensity curve (TIC) of the total ROI was calculated as the mean of the TIC of all the pixels using linear raw data. A quantitative analysis of the TIC (Fig. 1) was performed using a mathematical model (patent PCT/IB2006/003742) to determine 7 DCE-US functional parameters: peak intensity; area under the curve (AUC), area under the wash-in, area under the wash-out (AUWO) (all of the previously mentioned corresponded to blood volume), time to peak intensity, slope of the wash-in (both of the previously mentioned corresponded to blood flow), and mean transit time.⁵

Statistical Analysis

Evaluation of Quality

To define which criteria affected the quality of the DCE-US data, 18 examinations were selected by an engineer from the Soutien aux Techniques Innovantes Coûteuses (Support for Innovative and Expensive Techniques) (STIC) database. All examinations were first classified (subjectively) into 1 of 6 categories (ranging from “very bad” to “excellent”) by the engineer responsible for quantification. Three examinations from each class were selected at random from all examinations in the STIC database. These examinations were then

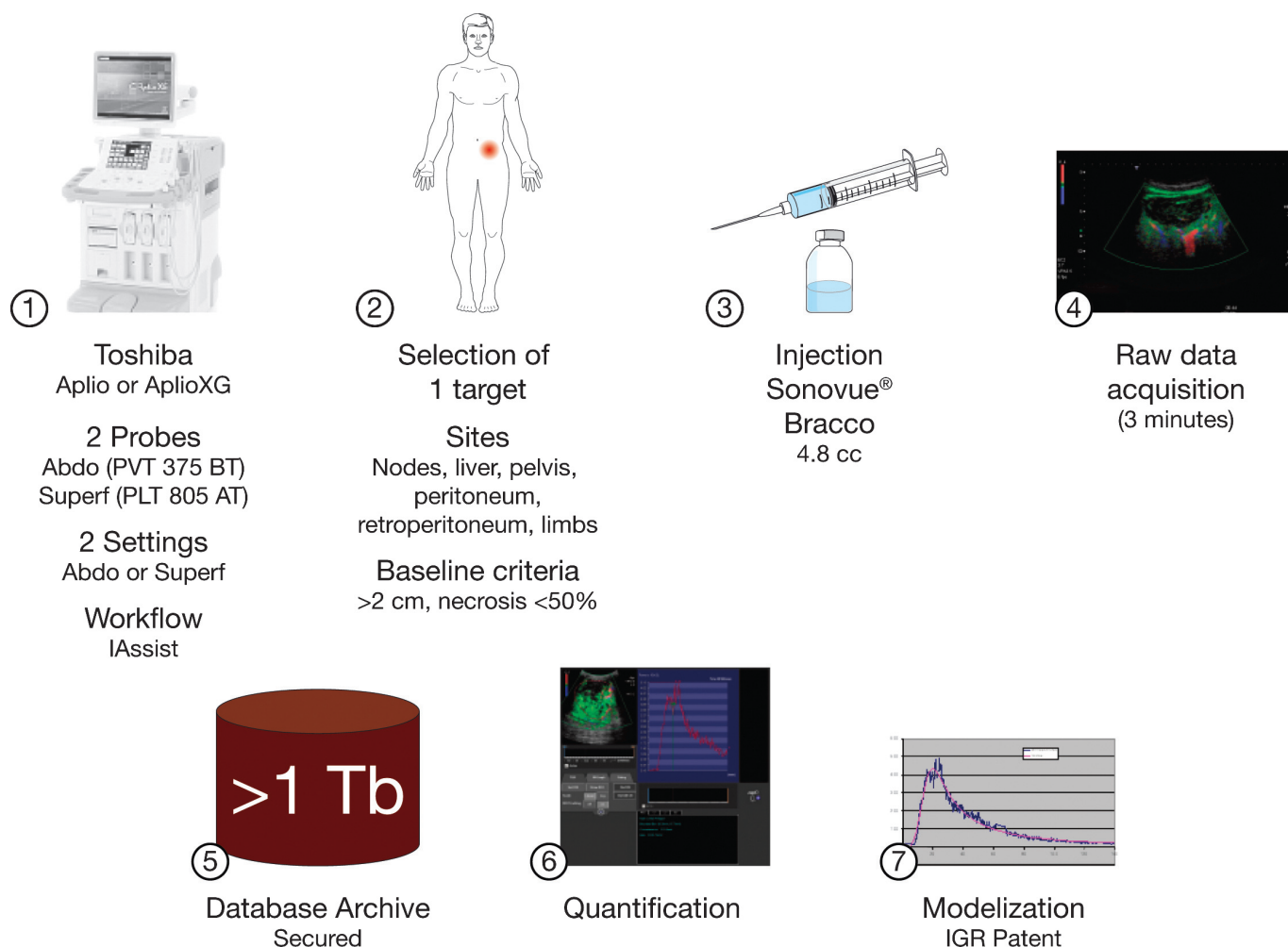


FIGURE 1. Standardized DCE-US methodology used at each study center with centralized DCE-US data quantification.

assessed twice by 8 quantifiers (3 engineers and 5 radiologists) trained to quantify the perfusion parameters. During this assessment, the order of the quantifications was defined at random. Six quality criteria were examined during each assessment, and each criterion was assigned a value of 1 if the condition was fulfilled; otherwise, a value of 0 was assigned. The criteria, which were examined for their quality, were: size (>2 cm = assigned a value 1), motion (intensive tracking was not required = 1), loss of target (the target was not lost for <20 seconds = 1), contour (clear borders = 1), wash-in (total data acquisition during the wash-in period = 1), and VRI window adapted (VRI window adapted to the lesion size = 1).

The intraobserver coefficient of variation (CV) was then estimated on the basis of differences between examinations performed by the same quantifier. In practice, we estimated the difference between the 2 measures of the same parameter for each examination. The distribution of this difference has a mean of 0 and a variance equal to twice the intrapatient variance of the parameter. The intrapatient SD of the parameter was estimated as the square root of half the variance of the difference. The CV was estimated as the intrapatient SD divided by the parameter mean. The final quality score was defined according to the difference between the CV when the condition was fulfilled and when it was not. The components of the quality score were then assessed for all the examinations present in the STIC database.

Quality According to Radiologists' Experience and Nature of Target

The examinations performed by each radiologist were recorded and numbered. The level of experience possessed by a radiologist when he/she performed an examination was defined as the number of previous examinations he/she had performed. The relationship between the quality of an examination and the experience of the radiologist was analyzed using a logistic regression model. Quality was compared according to the type of target: hepatic and nonhepatic targets.

RESULTS

Population

The study enrolled a total of 539 patients from 19 centers across France (11 comprehensive cancer centers and 8 teaching hospitals) between October 2007 and March 2010. Patient baseline characteristics are listed in Table 1. The most common tumor types included in the study were mRCC (29% of the patients), HCC (20% of the patients), and metastatic colorectal cancer (12% of the patients). Thirty-one percent of the patients received treatment with sorafenib, 27% with bevacizumab, 24% with sunitinib, and 8% with imatinib. The DCE-US evaluations of the liver were conducted in 55% of the patients. A total of 2339 DCE-US examinations were

TABLE 1. Patient Characteristics at Baseline

Characteristics	No.	%
Total patients	539	100
Tumor type		
mRCC	157	29
HCC	107	20
mCRC	67	12
mMelanoma	52	10
mGIST	52	10
mBC	61	11
Others	43	8
Main treatment received		
Bevacizumab	144	27
Sorafenib	166	31
Sunitinib	128	24
Imatinib	44	8
Others/combinations	57	11

mBC indicates metastatic breast cancer; mCRC, metastatic colorectal cancer; mGIST, metastatic gastrointestinal stromal tumors; and mMelanoma, metastatic melanoma.

performed: 277 were not quantified because of technical problems (total or partial loss of data), leaving 2062 DCE-US examinations as quantifiable (Fig. 2).

Study Treatments

The main antiangiogenic treatments administered to patients included sunitinib, sorafenib, bevacizumab, and imatinib. Sunitinib was self-administered by patients at 50 mg/d orally in 6-week cycles of 4 weeks on treatment, followed by 2 weeks off treatment (Schedule 4/2). Imatinib was administered orally at 400 mg/d; oral sorafenib, at 800 mg/d; and bevacizumab, by intravenous infusion at a dosage of 15 mg every 3 weeks or 10 mg every 2 weeks in combination with chemotherapy, according to the approved indications (colon cancer, breast cancer, and RCC).

Evaluation of Quality According to Radiologists' Experience

The variability, depending on the criterion of quality under consideration, was plotted for the 18 examinations randomly selected for assessment from the STIC database. Figure 3 illustrates the impact of the different criteria on the intrapatient CV.

Among the 6 criteria examined, 5 were found to have the greatest impact on quality. These included the size of the target, the motion, the loss of the target, the contour, and the total acquisition during the wash-in. The remaining criterion, the VRI window adapted to lesion size, had a limited impact on CV.

After completion of this initial variability study, all 2062 DCE-US examinations were analyzed for quality and yielded a mean quality score of 2.84 (range, 0–5). Three percent of the examinations ($n = 59$) were of poor quality, that is, a quality score of 0, on the basis of value attributed to each criterion examined (Fig. 4).

The mean quality was significantly improved when the radiologists' experience increased ($P < 0.0001$; Fig. 5). In addition, compared with hepatic targets, quality was significantly better when nonliver targets were assessed (Table 2; mean quality score 3.4 versus 2.4, respectively; $P < 0.0001$).

DISCUSSION

There is a need for more sensitive evaluation measures of response to newer targeted agents. To date, DCE-US has been used in

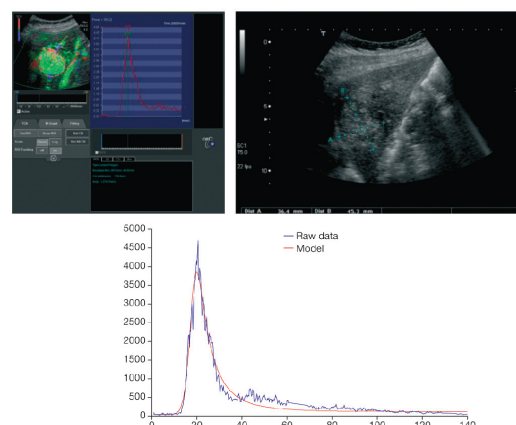
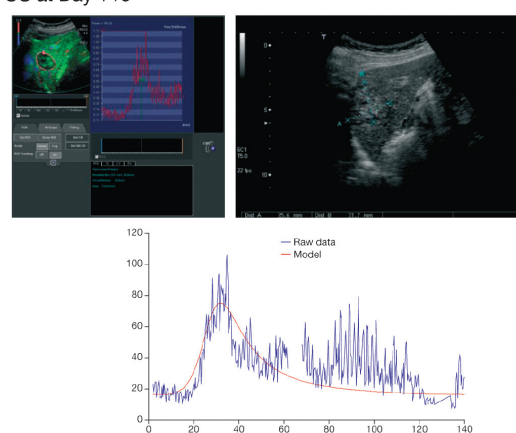
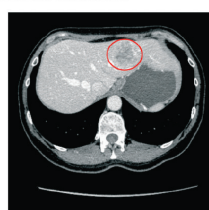
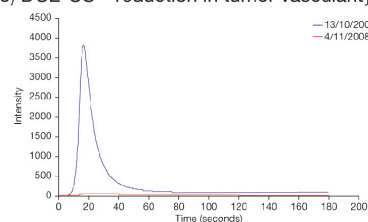
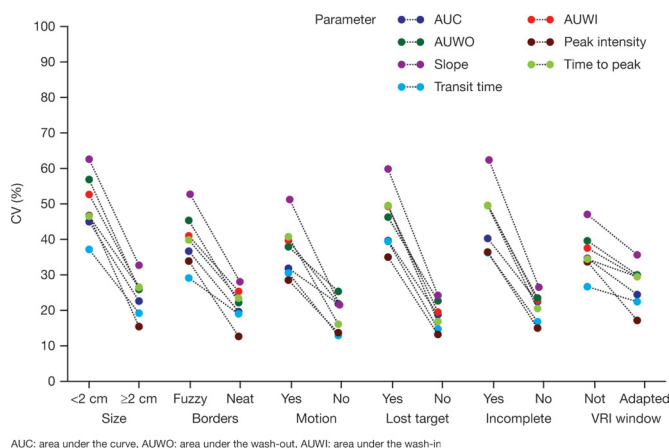
1A) DCE-US at baseline**1B) DCE-US at Day +15****2A) CT scan at baseline****2B) CT scan at 2 months****3) DCE-US – reduction in tumor vascularity**

FIGURE 2. Example of quantification: female patient (50 years old) who received 10 treatment cycles of sunitinib, 50 mg/d, Schedule 4/2. The patient presented with liver metastases that were measured by DCE-US at baseline (1A) and 15 days after the start of treatment (1B). In addition, the patient had a computed tomographic scan at baseline (2A) and after 2 months of treatment (2B). A DCE-US examination revealed a significant decrease in tumor vascularity (3), and the tumor was also smaller when examined using a computed tomographic scan at 2 months. The results obtained in this patient support a role for DCE-US in monitoring response to treatment with targeted therapy.



AUC: area under the curve, AUWO: area under the wash-out, AUWI: area under the wash-in

FIGURE 3. Coefficient of variation of 7 functional parameters according to the components of quality scores. AUWI indicates area under the wash-in.

a number of preclinical¹¹ and clinical trials with targeted agents. Initially, qualitative analyses in several clinical studies have indicated that DCE-US correlated with tumor responses, for example, in RCC treated with sorafenib^{12,13} or in GIST treated with imatinib.¹⁴

After an improvement in DCE-US methodology using quantitative analysis, we have conducted several different studies using DCE-US. One study demonstrated that DCE-US is a useful tool for predicting the early efficacy of sunitinib in mRCC.⁸ Here, robust correlations were observed between functional parameters measured by DCE-US and disease-free survival plus OS.⁸ In a study of patients with HCC treated with bevacizumab, a correlation with progression-free survival and OS was also observed.⁹ The preliminary results of this study, which included 400 patients, demonstrated that AUC and AUWO were correlated with response per Response Evaluation Criteria in Solid Tumors.¹⁵ Subsequently, DCE-US methodology has been included in the new European guidelines on the use of ultrasound in clinical practice.¹⁶

In this article, we have described the dissemination of the DCE-US methodology for the evaluation of antiangiogenic treatments in solid tumors across multiple oncology centers in France to improve evidence-based medicine across these centers. Our study demonstrates that an Internet-based network can be successfully used to record data in real time and that DCE-US can be successfully used across different metastatic sites, including the liver.

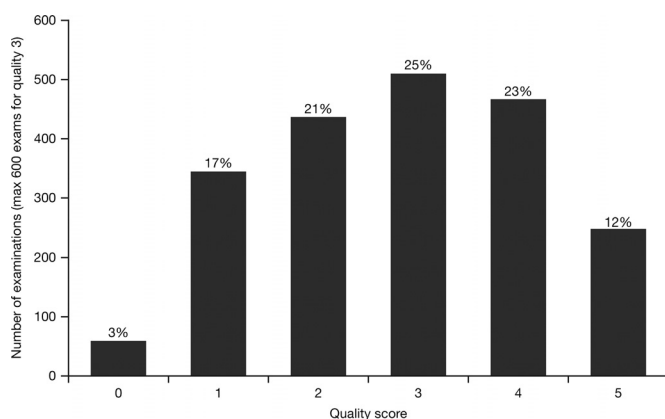


FIGURE 4. Distribution of quality scores based on the number of DCE-US examinations conducted by radiologists.

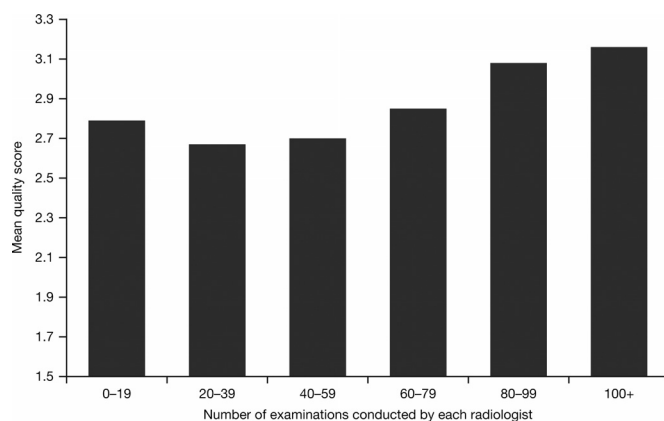


FIGURE 5. Changes to the quality scores distribution in accordance with radiologists' experience.

Fixed settings were used in all centers. Standardization was achieved without difficulty. Overall, 65 radiologists were trained in the use of the DCE-US methodology. The training was carried out on-site by engineers specializing in DCE-US, and the first (1–5) examinations were performed by the radiologist, assisted by the engineer. Strict rules were established to evaluate the quality of DCE-US, using a quality score ranging between 0 and 5. Evaluation of all the DCE-US tests conducted demonstrated that, of these, only 3% needed to be excluded (ie, those assigned a quality score of 0, where quality could not be verified). In addition, 5 of 6 criteria used to assess quality were found to have a major impact on quality. In fact, a lesion less than 2 cm in size is very difficult to quantify because of the difficulties associated with tracking it. This is also the case for dynamic contrast enhanced-magnetic resonance imaging when used to assess target lesions of a similar size.¹⁷

The quality of assessments increased with an increase in the radiologists' experience; in clinical practice, it is relatively easy to achieve experience by assessing at least 60 examinations. As such, the learning curve required to successfully implement this technique in new centers is relatively short. Finally, the results relating to the assessment of fixed versus mobile lesions may assist radiologists in selecting the best target lesion where the patient presents with several different metastases. A system is strongly recommended to track mobile lesions. In fact, a study by Goetti et al¹⁸ showed that without tracking, only 70% of the examination could be analyzed. Tracking of some new devices using a real-time motion compensation algorithm¹⁹ could improve their quality, whereas 3-dimensional acquisition²⁰ and ultrasound molecular imaging targeting $\alpha V\beta 3$ ²¹ may also improve the quality of this technique in the near future.

In summary, this study, conducted across different centers in France, confirms that DCE-US is a feasible tool that can be relatively easy to implement. The study also helps establish the rules for evaluating the quality of results obtained.

TABLE 2. Quality Score According to the Nature of the Targets

Quality Score/No. DCE-US Examinations*	0	1	2	3	4	5
Liver (total = 1122; 55%)	48	257	304	291	178	44
Nonliver (total = 936; 45%)	11	87	132	216	286	204

Total for liver and nonliver = 2058 (the nature of the target was not specified for 4 lesions).

*Number of DCE-US examinations per quality score, by target (ie, liver/nonliver)

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