CEUS in the study of bladder, method, administration and evaluation, a technical note

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Abstract

Purpose Contrast-enhanced ultrasound (CEUS) is the application of ultrasound contrast agents (UCAs) to traditional medical sonography. The development of UCAs allowed to overcome some of the limitations of conventional B-mode and Doppler ultrasound techniques and enabled the display of the parenchymal microvasculature. Purpose of this paper is to delineate the elements of a solid and science-based technique in the execution of urinary bladder CEUS.

Methods We describe the technical execution of urinary bladder CEUS and the use of perfusion softwares to perform contrast enhancement quantitative analysis with generation of time–intensity curves from regions of interest.

Results During CEUS, normal bladder wall shows a wash-in time of 13 s, a time to peak (TTP) >40 s, a signal intensity (SI) <45 % and a wash-out time >80 s; Low-grade urothelial cell carcinoma (UCC) shows a wash-in time of 13 s, a time to peak TTP >28 s, a SI <45 % and a wash-out time of 40 s; High-grade UCC shows a wash-in time of 13 s, a TTP >28 s, a SI >50 % and a wash-out time of 58 s.

Conclusions CEUS is a useful tool for an accurate characterization of bladder UCC although it has some drawbacks. To avoid misunderstandings, a widely accepted classification and a standardized terminology about the most significant parameters of this application should be adopted in the immediate future.

Keywords Bladder · CEUS · Time–intensity curves · Urothelial cell carcinoma

Riassunto

Obiettivi l'ecografia con mezzo di contrasto (CEUS) è l'ecografia medica tradizionale con l'utilizzo di mezzi di contrasto ecografici (MDCe). Lo sviluppo di MDCe ha consentito di superare alcune delle limitazioni dell'ecografia B-mode e Doppler convenzionali ed ha consentito la visualizzazione del microcircolo parenchimale. Scopo di questo lavoro è quello di delineare gli elementi di una tecnica solida e basata sull'evidenza scientifica per l'esecuzione della CEUS della vescica urinaria.

Materiali e Metodi Descriviamo l'esecuzione tecnica della CEUS della vescica urinaria e l'utilizzo di software di perfusione per l'esecuzione dell'analisi quantitativa dell'enhancement contrastografico con la generazione di curve intensità tempo (TIC) delle regioni di interesse (ROI).

Risultati Durante la CEUS, la parete della vescica normale mostra un tempo di wash-in di 13 sec., un tempo di picco (TTP) >40sec., una intensità di segnale (SI) <45% e un tempo di wash-out >80 sec.; il carcinoma uroteliale (CU) di basso grado mostra un tempo di wash-in di 13 sec., un tempo di picco (TTP) >28sec., un'intensità di segnale (SI) <45% e un tempo wash-out di 40 sec.; il CU di alto grado mostra un tempo di wash-in di 13 sec., un tempo di picco (TTP) >28sec., un'intensità di segnale (SI) >50% e un tempo di wash-out di 58 sec.

Conclusioni La CEUS è uno strumento utile per una caratterizzazione accurata del CU della vescica anche se possiede alcuni svantaggi. Al fine di evitare incomprensioni, dovrebbero essere adottate in futuro, una classificazione largamente accettata e una terminologia standardizzata sui parametri più significativi di questa applicazione.

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Introduction

Contrast-enhanced ultrasound (CEUS) is the application of ultrasound contrast agents (UCAs) to traditional medical sonography. The development of UCAs allowed to overcome some of the limitations of conventional B-mode and Doppler ultrasound techniques and enabled the display of the parenchymal microvasculature [1].

The use of UCAs is well established in some clinical applications, above all the ones involving liver and focal liver lesions (FLL), while the utility of CEUS in other applications is still under study and it is still object of discussion. The need for recommendations on the use of CEUS brought to an EFSUMB–WFUMB collaboration for the drafting of two documents: “guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver—update 2012” and “The EFSUMB guidelines and recommendations on the clinical practice of contrast enhanced ultrasound (CEUS): Update 2011 on non-hepatic applications” [2, 3].

Among the not well-defined applications of UCAs there is urinary bladder. It has been demonstrated that CEUS improves the differential diagnosis of intraluminal masses, allowing the differentiation between tumors, which are vascularized and show enhancement, and clots, which do not enhance [4–6]. CEUS also resulted to be superior to grayscale ultrasound in assessing infiltration of the muscle wall-layer, but magnetic resonance imaging (MRI) and computed tomography (CT) remain the imaging modalities of choice for local staging of bladder tumors [7–9].

Some authors (including our group) also demonstrated that in most cases it is possible to predict bladder tumor grading by the pattern of CEUS time–intensity curves (TICs); however this application needs to be confirmed by studies on larger patient populations [4, 8].

EFSUMB guidelines state that CEUS may play a role in urinary bladder diagnostic field, even though it cannot replace cystoscopy and pathologic staging. The principal diagnostic role of CEUS is the differential diagnosis of bladder cancer from clots in patients with hematuria when the diagnosis is equivocal on conventional B-mode and Doppler ultrasound; this application has been assigned a Recommendation Level C; 2b, which means that the recommendation is based on experts opinions and that the studies were performed in a set of non-consecutive patients or confined to a narrow spectrum/number of patients all of whom have undergone both the diagnostic test and the reference standard. Moreover, on the basis of experts opinion, CEUS resulted to be contraindicated (Recommendation Level X; 5) in patients with a poor urinary bladder visualization due to anatomical or pathological situations [3].

Nowadays in this field of research, there are still many points which need to be accurately studied to reach a high

methodological level accepted worldwide. Therefore, the purpose of this paper is to delineate the elements of a solid and science-based technique in the execution of urinary bladder CEUS.

UCAs

In the last decades several types of UCAs have been developed, including gas–liquid emulsions (microbubbles), liquid–liquid emulsions (nanodrops), liposomes, and other particles. Nowadays, microbubbles are almost the only particles to be used in common clinical practice because they resulted to be the ideal UCA by virtue of they are extremely echogenic, as well as being biocompatible, multifunctional, and economical. They represent the second generation of UCAs. We determined an increase of specificity, sensibility and diagnostic accuracy of ultrasonographic examination [10].

Microbubbles are filled with air and/or insoluble inert gas (perfluorocarbon, sulfur hexafluoride, etc.) and have a biodegradable lipidic or protein shell. The utilization of UCAs containing inert gas instead of air is preferable because of the major resistance of these microbubbles to pressure. Indeed, defining as critical pressure (CP) the pressure at which 50 % of microbubbles is destroyed, CP for air microbubbles is 47 mmHg, while CP of sulfur hexafluoride microbubbles is 127 mmHg. This difference is related to the high molecular weight of sulfur hexafluoride and to its reduced solubility in water.

The lipidic composition of the shell has also an important role in the resistance to pressure. Indeed it confers to the microbubbles a major elasticity. This characteristic allows the emission of a harmonic signal by the microbubbles even when a low insonation power is used. Therefore, the utilization of lower power ultrasound and the higher microbubbles resistance to pressure determine a longer contrastographic effect.

UCAs’ pharmaceutical form is usually powder and solvent for dispersion and injection. After the injection UCAs dilute in blood until they reach the highest concentration (principle of dilution) and the enhancement generated by microbubbles is visible along all the vascular system.

UCAs go through three phases: arterial phase (12–25 s after injection), portal/venous phase (30–80 s after injection) and late phase (>90 s after injection). UCAs do not show a parenchymal phase because they are blood pool agents and so they do not seep through vessel wall into the extracellular space. This attribute ensures that the enhancement is strictly connected to the district’s vascularization, without interferences by parenchymal contrastographic signal.

Microbubbles should have a diameter lower than the ultrasound wavelength. The most part of UCAs shows a global microbubble diameters range between 1 and 10 μm , with a mean value of 3 μm . This 3 μm diameter is considered crucial for two reasons: (1) it allows microbubbles resonance and the emission of harmonic frequencies at the normal B-mode operative frequencies (3.5–7 MHz) (2) it allows microbubbles to cross the pulmonary capillary filter.

UCAs contrastographic effect lasts for about 8 min; microbubbles gas is progressively exhaled through pulmonary breathing, while the external shell is degraded by different metabolic pathways depending on its composition [11].

UCAs show both linear and non-linear ultrasound reflection. Linear reflection of ultrasound is related to the utilization of low mechanical index (MI) (<0.1); in these conditions microbubbles are exposed to low pressures and go through equally partitioned compression/expansion cycles. Therefore, they oscillate symmetrically and principally reflect the fundamental insonation frequency; this behavior generates a linear reflection phenomenon, which strengthens blood scattering.

When a higher MI is used (0.1–1), the oscillation becomes non-symmetrical since the compression phase gets faster than the expansion phase. This distortion of the oscillatory phenomenon determines changes in the release of acoustic energy by microbubbles. Indeed during the expansion phase, microbubbles release not only acoustic energy equal to the fundamental insonation frequency (f_1) but also harmonic and sub-harmonic frequencies, or rather multiple (f_2, f_3) and sub-multiple ($f_{0.5}$) of the fundamental one. The quote of second harmonic frequency generated by microbubbles is higher than the quote generated by stationary tissues, so this is a very favorable way to exploit UCAs properties.

TICs

Contrastographic enhancement can be quantified using perfusion softwares. To perform quantitative analysis, clips must be taken during the CEUS examination. Quantitative softwares analyze the temporal sequence of the images. The signal intensity is calculated for every pixel for every second, thus generating perfusion maps.

TICs are extracted from regions of interest (ROIs) in the lesion and may be compared with ROIs from normal tissue areas. Therefore, TICs qualitative and quantitative analysis can be performed. The quantitative parameters most commonly taken into consideration are: wash-in time, time to peak (TTP), maximum signal intensity (SI), and wash-out time. Other parameters which are less commonly used are: regional blood volume, mean transit time, regional blood

flow, area under the curve, replenishment time and replenishment velocity.

In many applications different TICs shape can be identified. Several authors tried to find correlations between TICs shape and type/grading of lesions [12, 13]. Nevertheless, these correlations still need to be confirmed by studies on larger patient populations.

Gray scale US, CDUS and CEUS of the bladder: exam technique

Gray scale ultrasound (US) and color-Doppler (CDUS) must always precede CEUS. Urinary bladder has to be adequately full (neither empty nor overfilled, but sufficiently full not to overestimate the lesions). The examination should be performed using a 3.5–5 MHz multifrequency broadband convex transducer.

Grayscale conventional US is used to characterize the lesions of the bladder wall as sessile or polypoid masses. Lesions with a broader base compared to their height are classified as sessile masses, while lesions which are taller than they are wide at their base are classified as polypoid masses. The location and size of the lesions detected during grayscale US must be recorded.

CDUS must be performed using scanning parameters set for maximum sensitivity for slow flow while the power output has to be increased to maximum. Color gain must be increased to the point just before the appearance of random noise. The pulse repetition frequency has to be set at the lowest possible levels. It could be useful to assign a simple score to images, as follows: absent (score 0), mild (one vascular pole; score 1) or strong (more than one vascular pole; score 2).

CEUS should be performed in the same session and written informed consent must be obtained from all patients before the examination.

UCAs usually have a preparation kit including a vial with lyophilised powder, a pre-filled syringe containing sodium chloride and a transfer system. The operator has to:

1. Connect the plunger rod by screwing it clockwise into the syringe.
2. Open the transfer system blister and remove syringe tip cap.
3. Open the transfer system cap and connect the syringe to the transfer system by screwing it in clockwise.
4. Remove the protective disk from the vial. Slide the vial into the transparent sleeve of the transfer system and press firmly to lock the vial in place.
5. Empty the contents of the syringe into the vial by pushing on the plunger rod.
6. Shake vigorously for 20 s to mix all the contents in the vial to obtain a white milky homogeneous liquid.

7. Invert the system and carefully withdraw UCA into the syringe.
8. Unscrew the syringe from the transfer system [11].

The total amount of UCA should be split in two equal doses. Each dose should be administered as an intravenous hand-injected fast bolus, followed by 5 ml of a saline flush. The second dose should be injected 15 min after the first one. The injection should be preferably performed through an 18 GA needle; the use of lower needle calibers could result in a high quote of microbubbles mechanical rupture.

Table 1 TICs parameters of normal bladder wall, low-grade UCC and high-grade UCC

	Wash-in time (s)	TTP (s)	SI (%)	Wash-out time (s)
Normal bladder wall	13	>40	<45	>80
Low-grade UCC	13	<28	<45	~40
High-grade UCC	13	>28	>50	~58

TTP time to peak, SI signal intensity, UCC urothelial cell carcinoma

The utilization of a low MI (range 0.04–0.1) is crucial to obtain true subtraction effect. This technique is based on signal amplitude subtraction (not only frequency subtraction) and is possible thanks to the combination of two different components: the harmonic signal coming from the UCA and the dynamic threshold of low amplitude signals which suppress the low amplitude signals returning to the transducer.

Images of the ideal scanning plane must be displayed in real-time by slightly changing the scanning plane to portray the whole area of the lesion. The entire examination is digitally recorded with still images recorded on the optic disk; start of recording is initiated simultaneously with the start of contrast injection. All time measurements started from this moment, which is “time-0” in all recorded video-clips.

To record all the phases (arterial, venous and late phase) of contrastographic enhancement, it is very important that the first clip is at least 2 min long. Furthermore it is crucial that the transducer is kept as still as possible, during the recording of this first clip; this expedient is fundamental to the correct construction of ROIs during the further quantitative analysis.

Fig. 1 Image from Qontrast™ software; normal bladder wall. TIC shows: wash-in time 13 s, TTP 47 s, SI 43 %, wash-out time >80 s

Fig. 2 Image from QontrastTM software; low-grade carcinoma. TIC shows: wash-in time 13 s, TTP 21 s, SI 36 %, wash-out time 35 s

Fig. 3 Image from QontrastTM software; high-grade carcinoma. TIC shows: wash-in time 13 s, TTP 30 s, SI 58 %, wash-out time 55 s

Images are then analyzed off-site at consensus conferences to determine the size of the lesion, baseline and CDUS appearance as well as enhancement characteristics after UCA administration. In this phase it could be also useful to assign the enhancement pattern a score, as follows: 1 (mild enhancement) or 2 (strong enhancement) as compared to the surrounding urinary bladder wall. Quantitative analysis is subsequently performed using perfusion software.

Quantitative analysis

There are many different commercial perfusion softwares and so it is not possible to describe the operating principles for each one of them, thus just the common elements will be described. In most cases manual demarcation of the ROI is needed. Once the ROI is demarcated, the software will calculate the contrast enhancement for every pixel for every second, thus generating a TIC and in some cases a perfusion map. A minimum of two ROIs should be taken; one in the lesion and one in the closest bladder wall, so that the respective TICs may be compared.

Since each one has its own features, some misunderstanding could emerge comparing results from different study; e.g. the maximum signal intensity reached is called peak intensity (PI) and is expressed in dB by QLAB (Philips medical system, Bothell, WA, USA), while the same parameter is called SI max and it is expressed in % by QontrastTM (Bracco, Milan, Italy) [12].

To avoid subsequent misunderstandings, a widely accepted terminology about CEUS parameters should be adopted. These are the parameters more commonly taken into consideration when performing a quantitative analysis on CEUS of the urinary bladder:

- 1) Wash-in time: the length of time preceding the moment in which the TIC starts rising from the bottom of the chart recording a signal intensity different from 0;
- 2) SI: the maximum signal intensity reached;
- 3) TTP: the time elapsed before reaching the peak of the TIC;
- 4) Wash-out time: the length of time that precedes the moment in which the TIC signal returns to 0 or a value close to 0.

We would also like to emphasize that all time measurements should start from “time 0” corresponding to the time of injection of UCA.

In Table 1 wash-in time, TTP, SI and wash-out time of high-grade UCC, low-grade UCC and normal bladder wall are reported. These data come from our group’s clinical records, which include 224 patients (age range 51–76;

mean age 68), 84 (37.5 %) women and 140 (62.5 %) men. Among them 80 (35.7 %) were found negative for bladder neoplasms and 144 (64.3 %) were affected by UCC (8 high-grade Ta, 56 low-grade Ta, 72 high-grade T1 and 8 high-grade T2). The perfusion software program used for the quantitative analysis of all these patients was QontrastTM (Figs. 1, 2, 3).

Conclusions

CEUS is a useful tool for an accurate characterization of bladder UCC. This technique is characterized by high cost-effectiveness, and seems to be able to provide specific contrast enhancement patterns. This technique can also be used in patients who cannot undergo CT and/or MRI, or in patients in whom there is a poor indication for conventional cystoscopy.

Nevertheless CEUS has some drawbacks. One is the limited ability to detect flat lesions. Another major limitation is that CEUS provides no tissue for histological evaluation. However, the combination of enhancement pattern, contrastographic parameters and the TIC shapes could indicate the histopathology of bladder lesions, thus increasing the specificity of Color-Doppler US without a statistically significant reduction of sensitivity.

To avoid misunderstandings, a standardized technical procedure should be introduced. Furthermore, a widely accepted classification and terminology about the most significant parameters of this application should be adopted in the immediate future.

Conflict of interest F. M. Drudi, N. Di Leo, F. Maghella, F. Malpassini, J. Iera, A. Rubini, N. Orsogna and F. D’Ambrosio declare that they have no conflict of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). All subjects provided written informed consent to enrolment in the study and to the inclusion in this article of information that could potentially lead to their identification.

Human and animal studies The study was conducted in accordance with all institutional and national guidelines for the care and use of laboratory animals.

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