Contrast-enhanced ultrasonography in clinical practice in Crohn's disease: feasibility of destruction/replenishment method and software analysis

Ecografia con mezzo di contrasto nella pratica clinica nel morbo di Crohn: “feasibility” della procedura distruzione /riempimento e dell'analisi software

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Abstract The aim of this Pictorial Essay is to evaluate the feasibility in the clinical practice of CEUS destruction/replenishment method and time intensity curve (TIC) analysis using the available software in patients with Crohn's disease. The ultrasound (US) procedure using contrast agent is briefly described, elaboration and analysis of the US sequences are explained in detail, and some advantages and disadvantages of the method are highlighted. The Authors suggest that the destruction/replenishment method is useful and time-saving in Crohn's disease if multiple measurements are required on intestinal portions adjacent to each other or in different locations.

Keywords Bowel ultrasound · Contrast-enhanced ultrasound · Time/intensity curve analysis · Crohn's disease

Sommario Lo scopo di questo Pictorial Essay è di valutare la “feasibility” nella pratica clinica e nel morbo di Crohn della CEUS metodo distruzione / riempimento e dell'analisi delle curve intensità tempo elaborate utilizzando il software a disposizione. La procedura ecografica con mezzo di contrasto viene brevemente descritta, le modalità di elaborazione ed analisi delle sequenze ecografiche sono descritte dettagliatamente e sono evidenziati alcuni vantaggi e svantaggi della metodica. E' opinione degli Autori che la metodica distruzione /riempimento nel

morbo di Crohn sia utile e permetta di risparmiare tempo qualora si effettuino più misure su tratti intestinali adiacenti tra loro o situati in differente localizzazione.

Introduction

In Crohn's disease, contrast-enhanced ultrasound (CEUS) accurately documents the macro- and microcirculation of the pathological intestinal tract. Contrast enhancement pattern and intensity depend on the concentration of microbubbles in the ultrasound (US) scanning plane and to some extent to the inflammatory response of the examined bowel [1].

Dynamic CEUS (DCEUS) involves elaboration of recorded CEUS sequences by dedicated software to obtain semiquantitative analysis. The obtained time intensity curve (TIC) thus measures the echo intensity enhancement in a given period of time, in a region of interest (ROI) manually drawn on the bowel wall. A TIC fitted to appropriate mathematical models permits calculation of indices correlating with the vascular volume and flow in the ROI.

The method defined as “bolus” involves injection of a quantity of contrast agent into a peripheral vein and subsequent measurement of changes in contrast concentration in the ROI over a certain period of time (usually > 90 s).

The destruction/replenishment method requires continuous intravenous injection of contrast agent using a dedicated pump and a scanner with specific contrast software that allows targeted microbubble destruction through the use of high mechanical index pulse sequences (Flash) before low mechanical index examination of contrast

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replenishment. The method therefore requires a constant concentration of contrast agent in circulation (steady state), microbubble destruction in the target tissue and subsequent measurement of the enhancement variations in the target tissue (replenishment) until a plateau value is reached (Fig. 1). For further details regarding these methods, please see the recent EFSUMB guidelines and recommendations (2012) [2].

In our Institution, grayscale US, color Doppler US (CDUS), and CEUS are carried out on a high-end US scanner (Mylab Twice, Esaote, Italy) equipped with appropriate probes as well as contrast-specific software and appropriate scanner preset (dual display, dynamic range > 60%, persistence off, post processing off).

The affected bowels are usually evaluated using a LA332 linear probe but a CA541 convex probe is preferred when the bowel is located deep in the abdomen.

CEUS is performed using a second-generation contrast agent currently available in Europe (SonoVue, Bracco, microbubble concentration, 8 $\mu\text{l/ml}$) and a dedicated infusion pump (Vue Ject BR100, Bracco, Switzerland).

SonoVue is used in “off label modality” when digestive tract is assessed and this circumstance has to be specified in the informed consent form.

Time required by SonoVue infusion to reach the steady state was reported in previous papers (< 100 s [3] about 120 s [4, 5]); infusion rate is arbitrary, usually 1 ml/min when a low frequency convex probe is used [4, 5]. We

Fig. 1 Crohn's disease of the left colon: **a** endoscopy shows the presence of a polypoid intraluminal mass at the sigma (histology diagnosed chronic inflammation). **b** Sigma, virtual colonoscopy demonstrates the inflammatory mass which occupies the lumen ***, the sigma walls are thickened. **c** Sigma, CEUS destruction/replenishment method at the end of the infusion phase (steady state) shows homogeneous enhancement of the intestinal walls (arrow) and of endoluminal inflammatory mass(***). **d–g** The same imaging plane as c, shows homogeneous microbubble destruction through acoustic pulses with a high mechanical index (Flash). **h** The same imaging plane as c, at the end of the replenishment phase there is homogeneous enhancement of the intestinal walls and of the endoluminal inflammatory mass

prefer a high-frequency linear probe to scan the bowels and an infusion rate of 1.5 ml/min to obtain a major enhancement of the bowel walls.

At the steady state, measurements on the target portions are carried out in rapid succession using the destruction/replenishment method. Each measurement lasts about 25 s, and the US operator must carefully maintain the scan plane on the target. The clips are saved and exported in DICOM format for analysis. Quantification of the enhancement is calculated using dedicated software Vuebox V 5.1 (Bracco SA, Switzerland).

The US operator (AG) has 15 years' experience in performing US of the bowels, 6 years in performing bowel CEUS, 1 year in using the destruction/replenishment

method, and 3 years' experience in software analysis of CEUS TICs (VueBox V5.1, Bracco Suisse).

The aim of this Pictorial Essay is to assess the feasibility in the clinical practice of CEUS with destruction/replenishment sequences and analysis of TICs using VueBox software in Crohn's disease.

Analysis of TICs using the destruction/replenishment method and VueBox software V5.1

When CEUS has been performed on the target bowel, elaboration of TIC requires dedicated software. VueBox uses linearized data, and after motion compensa-

Fig. 2 TIC and an interpolated mathematical model (green continuous line): **a** the TIC shows a sigmoidal trend with a growth phase (filling phase in the ROI, curved yellow arrow) and a plateau phase (direct yellow line). **b** The value of WIR and mTT indices are influenced by the quality of enhancement data acquisition in the first part of the TIC (rising phase), whereas rBV index value is more influenced by the quality of enhancement data acquisition in the second part of the TIC (plateau phase)

Fig. 3 The same US image as Fig. 1, panel showing the results obtained with CEUS data processing by VueBox. Four quadrants are presented: the first presents the original clip (q1), the second a parametric image of rBV (q2), the third the TIC (q3), and the fourth the rBV value (a.u. and dB, q4)

Fig. 4 Effects of ROI positioning on the anterior or posterior bowel wall: **a** the same US image as Fig. 3, the rBV value of the yellow TIC related to the ROI on the posterior wall is lower than the rBV value of the green TIC related to the ROI on the anterior wall, probably due to attenuation phenomena caused by the inflammatory endoluminal tissue mass. **b** Crohn's disease: virtual colonoscopy, coronal scan of the last ileal loop presenting moderate wall thickening*. **c** Terminal ileum, same patient as **b**, the rBV value of the yellow TIC related to the ROI on the posterior wall is higher than the rBV value of the green TIC related to the ROI on the anterior wall, probably owing to excessive probe compression during the examination

tion and curve fitting it calculates, pixel by pixel in the ROI, TIC values expressed in arbitrary units (a.u.) or in decibels (dB) and related indices. Note that indices are not calculated directly on the TIC linearized data, but on the interpolated mathematical function.

The TIC usually presents a sigmoidal trend and consists of a growth phase and a plateau phase (Fig. 2a). The intensity value of enhancement in the plateau phase (Fig. 2b) is related to “the fractional vascular volume” in the ROI [6]; this parameter is called rBV in VueBox and is expressed in arbitrary units (a.u.) or in decibels (dB).

The TIC slope is correlated to the speed with which the microbubbles fill the ROI after the destruction; in VueBox, the indices wash-in rate (WIR expressed in a.u. or in dB) and mean transit time (mTT expressed in seconds) describe this phase of the curve and are related to the flow in the ROI (Fig. 2b).

Quality of fit (QOF) index measures the correlation between the TIC data and the interpolated mathematical function (QOF is expressed as a percent and TIC with QOF > 85% is considered suitable for index calculation).

The results related to TIC elaboration and calculation of the selected index are presented in a four-quadrant panel (Fig. 3) including the original clip, the parametric image of the selected index, the TIC, and the numerical value of the selected index (Fig. 3).

Detailed information on how to use the software is reported in the literature [7].

Observations related to the elaboration of TICs using VueBox V5.1 software

ROI placement on the intestinal wall is operator-dependent and may constitute a major cause of divergent test results. ROI size and placement vary to include the entire bowel wall or only certain parts of it and this may affect the results, but there is no consensus on how to perform this procedure.

Fig. 5 The same US image as Fig. 3, the QOF (92.44%) of the yellow ROI related to the yellow ROI (q2, arrowhead) including only processed areas of the parametric image is higher than the QOF of the green TIC (88.52%) related to the green ROI which includes the entire intestinal wall

Recently, Cheng et al. (2016 [8]) stated that ROIs positioned on the anterior and posterior wall may produce slightly to moderately different results, and that ROI placement on the anterior wall is preferable in the presence of intraluminal contents causing attenuation on the posterior wall (Fig. 4a), whereas ROI placement on the posterior wall is preferable in the presence of excessive probe compression during the examination (Fig. 4c).

Wilkens et al. (2017 [9]) stated that TICs with a high QOF are essential for DCEUS reproducibility. The Authors

used the bolus method and selected a large ROI ($> 0.1 \text{ cm}^2$; mean QOF increases with the size of the ROI); they used the parametric image to include areas in which the processing was found to be of the best quality (colored areas) Fig. 5.

On the other hand, analysis of TICs related to ROI drawn on unprocessed areas (gray) of the parametric image (Fig. 6a, arrowhead) shows too low QOF value and a great variation in the distribution of enhancement data (Fig. 6b).

Fig. 6 The same US image as Fig. 3, a the purple ROI is manually drawn on an unprocessed area of parametric image (arrowhead). b The corresponding purple TIC (QOF 20.38%) presents a great variation in the distribution of enhancement data both in the rising phase and in the first part of the plateau phase (arrowheads)

Feasibility of destruction/replenishment method in Crohn's disease

Crohn's disease may affect the gastrointestinal tract in different ways, and it is defined as “extensive” if $> 100 \text{ cm}$ of the bowel is affected (usually with multiple lesions located in different portions) and “localized” if $< 30 \text{ cm}$ of the bowel is affected (usually located in the ileum or ileocecal bowel).

We present images depicting the disease in various portions of the small intestine (Fig. 7a–c). In these cases, evaluation of the inflammatory activity of the bowel wall can be improved by repeated CEUS measurements of the affected portions in the different localizations (Fig. 7d–g).

Also in patients with localized Crohn's disease, US characteristics obtained in the adjacent portions of the affected bowel wall may be different (different wall thickening, vascular signals of different entities at CDUS, and transmural inflammatory exudates of variable intensity) (Fig. 8).

In clinical practice, it may be advisable to repeat the measurement on more targets adjacent to each other for more detailed information on the inflammatory activity of the bowel wall (Fig. 9).

Fig. 7 Crohn's disease affecting the ileum occurring in two sites at diagnosis. **a** Endoscopy, the ileal loop with mildly hyperemic mucosa and two superficial ulcerations (arrowheads). **b** Contrast-enhanced MRI enterography, coronal view shows slight thickening and hyperenhancement of the pelvic ileal loop (arrows). **c** Contrast-enhanced MRI enterography, axial view shows the portion of the ileum upstream of **b**, with mild to moderate thickening of the bowel walls and substenosis of the lumen (arrow). **d–f** TICs related to ROIs drawn on the walls of ileal tracts adjacent to each other, rBV index (53–55 dB) is suggestive of an acute inflammatory response. **g** TIC related to the wall of the ileum substenosis upstream of d,e, f, rBV index (58 dB) is suggestive of an acute inflammatory response

Discussion

US imaging using contrast enhancement (CEUS) was recently introduced in the study of the gastrointestinal tract and it has found a role in the monitoring of inflammatory activity of the intestinal walls in chronic inflammatory bowel disease.

In 2011, the EFSUMB guidelines [10] suggested the use of CEUS in inflammatory bowel disease, and in 2015 two meta-analyses [11, 12] reported the diagnostic accuracy of the method (sensitivity 93–94% and specificity 78–87%).

The ECCO guidelines on “diagnosis and management of Crohn disease” (2017 [13]) state that CEUS may increase the accuracy of US in the documentation of inflammatory activity and may also have a role in the characterization of bowel stenosis.

To the best of our knowledge, studies in the literature reporting on the effectiveness of CEUS in the investigation of the gastrointestinal tract are focused on the bolus method. CEUS destruction/replenishment method was initially used in the field of cardiology [14] and more recently in studies of renal perfusion [4, 5]; it has proved to be easily performed and it can therefore be suggested for use in clinical practice [4, 5].

The aim of this Pictorial Essay is to assess the feasibility in clinical practice of CEUS destruction/replenishment method in Crohn's disease and the feasibility of analysis of the relative TICs using the proposed software. The method seems appropriate for the study of the intestinal loops whose arterial blood flow is similar to renal blood flow and different from simultaneous hepatic arterial and portal inflow.

The destruction/replenishment method requires a scanner with adequate contrastographic properties and a dedicated infusion pump. The method is highly feasible and compatible with the activity of an outpatient clinic dedicated to US examination of the gastrointestinal tract in a provincial hospital. It is easy to perform and relatively fast, as repeated measurements can be made on the target bowels in about 10–15 min.

The destruction/replenishment method makes it possible to avoid bolus of contrast agent which is an operator-dependent procedure. Moreover, the short duration (approximately 20–25 s) of the “destruction/replenishment US scanning” makes it possible to avoid breath-related motion artifacts.

Fig. 8 Crohn's disease affecting the terminal ileum, extension about 25 cm (current corticosteroid therapy). **a** Contrast-enhanced CT, coronal view, depicts the entire affected loop with varying degree of wall thickening. Wall enhancement occurs mainly in the mucosa and submucosa of the proximal and middle portions (***) compared to the distal portion (arrow) adjacent to the ileocecal valve. **b**, **c** CDUS of the middle portion (***) of the affected bowel presenting wall thickening (7–10 mm) with vascular signals arising from the bowel wall classified Limberg 2°–3°. **d** CDUS of the distal portion adjacent to the ileocecal valve with moderately thickened walls (5 mm), and vascular signals arising from the bowel wall classified Limberg 2°

Fig. 9 Same case as Fig. 8, CEUS carried out on adjacent ileal portions characterized by wall thickening. **a, b** At diagnosis (untreated). **c, d** After about 30 days of corticosteroid therapy. **e, f** After biologic induction therapy. **a–f** rBV index values are similar; **a** artifact caused by endoluminal contents (arrowhead); **b** transmural inflammatory exudates (arrowheads)

A second advantage is that the measurements can be repeated in quick succession, whereas the bolus method requires waiting for microbubble wash-out between one measurement and the next (about 10 min) with a substantial loss of time.

TIC processing and calculation of parameters using the selected software (VueBox V5.1) is furthermore facilitated by the simplicity of the replenishment curve and the fact

that only three parameters are derived from the interpolated function (rBV, WIR, mTT).

However, a disadvantage compared to the bolus method was the reduced QOF of the TICs (Fig. 9), although large ROIs were drawn on the target carefully avoiding the visceral lumen and the larger vessels of the serosa and despite the solutions offered by the software (automatic motion compensation and manual removal of inadequate images).

The reduced QOF of the TICs originates from the excessive dispersion of the enhancement data distribution (Fig. 6b) and may be linked to the CEUS procedure: excessive probe compression on the target bowels, heterogeneous microbubble destruction within the intestinal wall due to Flash, excessive acoustic signal

Fig. 10 The same US image as Fig. 6, rBV index (38.89 dB) related to the green TIC (QOF > 85%, green ROI covering the entire wall) is congruent with rBV index (39.39 dB) related to the purple TIC (QOF:20.38%, purple ROI drawn on an unprocessed area of the parametric image). The two TICs (q3) present different trends in the rising phase, but similar trends in the plateau phase

Fig. 11 Crohn's disease of the distal ileum at diagnosis. **a** Contrast-enhanced MRI enterography coronal view, the ileal wall is 7–8 mm thick, wall enhancement occurs mainly in the mucosa and submucosa (arrow). **b** The green ROI is manually drawn on the anterior ileal wall to avoid endoluminal content artifacts (arrowhead). The corresponding green TIC (QOF 48.37%) presents a great variation in the

distribution of enhancement data in the plateau phase. (*) These artifacts may be due to “out of plane” US scanning caused by visceral movements with passage of endoluminal contents. In this case, rBV value is not reliable. It is worth noting that in this case the TIC in the short filling phase is free from movement artifacts and the QOF(89.77%) is high

Fig. 12 The same US scan as Fig. 4c, parametric images related to mTT (**a**) and rBV (**b**) indices. **a** The values (arrow) of the mTT index range from 0.0 s (dark red) to 32 s (dark blue). The distribution of mTT values within the ROI is heterogeneous. **b** The values (arrow) of the rBV index range from 23 dB (dark blue) to 43 dB (dark red). The

fluctuations in some areas of the bowel wall in the initial replenishment phase, and low signal to noise ratio in the initial replenishment phase. The values of the two parameters, WIR and mTT, are directly correlated to replenishment of the ROI after microbubble destruction (Fig. 2b) and seem to be influenced by some of the described artifacts.

The rBV index value depends on the plateau phase of the TIC (Fig. 2b) in which dispersion of enhancement values is usually reduced. In our opinion, WIR and mTT indices are reliable only if the TIC presents a high QOF ($> 85\%$), while rBV value seems to be less influenced by QOF index, if the plateau phase of TIC is not altered by movement artifacts (Fig. 10). However, if imperceptible movements of the US probe, or visceral wall movements prevent proper CEUS execution and subsequent TIC processing, also rBV index value appears incongruous (Fig. 11b).

Comparison of the parametric images related to the different indices may provide additional information on the vascular network and flow within the bowel wall portion encircled by the ROI. Parametric images (Fig. 12) show lack of homogeneity of the tested index within the ROI (a, mTT index; b, rBV index). Moreover, the dark blue and turquoise areas (Fig. 12a—mTT, arrowheads), and the corresponding bright or dark red areas (Fig. 12b—rBV, arrowheads) suggest a slower blood flow in areas with high vascular density (high rBV).

distribution of rBV values within the ROI is heterogeneous, particularly on the endoluminal surface of the bowel wall. Two areas showing particularly slow blood flow (Fig. 11a, arrowheads) correspond to areas with high rBV index values (Fig. 11b, arrowheads)

Conclusion

Our initial experience shows that CEUS destruction/replenishment method may be useful in clinical practice in Crohn's disease in selected patients. Some individual parameters, such as blood pressure, frequency, and heart output, cannot be standardized, but the necessary condition for a lesser variability of the procedure are appropriate scanner presets, a diligent execution of CEUS, and an accurately drawn ROI on the bowel wall paying particular attention to QOF of the elaborated TICs.

It should be pointed out that CEUS 2D mode allows the procedure to be repeated on target intestinal tracts when these are clearly identifiable, whereas accurate retrieval of previously used scanning planes on the bowel wall is too difficult.

Based on the above observations, we believe that a relative heterogeneity of the results should be expected in practice. Targeted studies are required, however, in the meantime, in agreement with the recommendations related to studies of renal perfusion [5], we believe that only value index variations exceeding 25% should be considered clinically significant.

Finally, CEUS destruction/replenishment method which allows repeated measurements on multiple scanning planes of the same target or on adjacent visceral sections will likely provide more detailed information, and it may be advisable especially if the aim is to monitor response to a medical therapy.

Compliance with ethical standards

Conflict of interest Authors have no conflict of interest to declare related to this study.

Ethical Statement 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.

Informed consent The patient provided written informed consent to enrolment in the study and to the publication of information that could potentially lead to his identification.

Human and animal studies The study described in this article does not involve the use of animal subjects.

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