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The Role of Contrast-enhanced Ultrasound in Crohn's Disease Activity – Systematic Review

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Abril, 2020

Alberto Carvalho Barbosa

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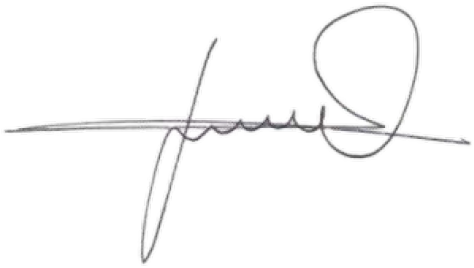

Assinatura do Orientador

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Resumo

Introdução e Objetivos: Os doentes com doença de Crohn têm que ser frequentemente avaliados por métodos de imagem no decorrer da doença, uma vez que os seus sintomas nem sempre correspondem à sua verdadeira severidade. Devido às limitações que outros métodos de imagem apresentam, a ecografia com injeção de contraste de microbolhas (CEUS) tem surgido como uma alternativa válida para a avaliação de doentes com doença de Crohn. Esta revisão sistemática tem como objetivo avaliar que papel pode ter a CEUS na deteção de doença ativa, bem como na sua classificação.

Métodos: Foram pesquisados artigos de 2010 a agosto de 2019 em dois motores de busca que analisassem a eficácia da CEUS na deteção da atividade da doença de Crohn. Foram pesquisadas as seguintes combinações de palavras-chave: "Crohn CEUS", "Crohn contrast enhanced ultrasound" e "Crohn contrast enhanced sonography". Foram excluídos artigos numa língua que não a inglesa, estudos *in vitro* ou em animais, revisões, estudos case-report ou artigos que apenas disponibilizassem Título e *Abstract*. A qualidade dos artigos incluídos nesta revisão foi avaliada usando o Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).

Resultados: Um total de dez artigos foram incluídos na análise final. O maior risco de viés pela análise qualitativa dos estudos estava relacionado com o teste de referência utilizado para definir atividade da doença. Apesar de uma grande heterogeneidade nos parâmetros da CEUS usados, o parâmetro mais avaliado e que mostrou melhores resultados foi a análise quantitativa do realce máximo (PE). A análise específica do realce da camada mais interna da parede intestinal mostrou resultados promissores comparando com o realce da parede intestinal completa. A CEUS parece ser um bom método para distinguir doentes com inflamação ativa de doentes com doença quiescente, no entanto, não apresentou resultados tão favoráveis no que respeita à classificação da severidade da doença.

Conclusões: A CEUS pode ser uma técnica de imagem bastante útil na avaliação da atividade da doença em doentes com doença de Crohn, sobretudo na distinção de inflamação ativa. No entanto, é necessária uma uniformização dos parâmetros utilizados para que se possam utilizar mais amplamente na prática clínica.

Palavras-chave: Doença de Crohn, Ecografia com Contraste, CEUS.

Abstract

Introduction and Aims: Patients with Crohn's disease often have to be evaluated by imaging methods during the course of the disease, as their symptoms do not always correspond to their true severity. Due to the limitations that other imaging methods present, microbubble contrast-enhanced ultrasound (CEUS) has emerged as a valid alternative for the evaluation of Crohn's disease patients. This systematic review aims to assess what role CEUS can play in the detection of active disease as well as in its grading.

Methods: A search was performed on two main databases for articles from 2010 to August 2019 that analyzed the effectiveness of CEUS in detecting Crohn's disease activity. The following keyword combinations were searched: “Crohn CEUS”, “Crohn contrast enhanced ultrasound” and “Crohn contrast enhanced sonography”. Articles in a language other than English, in vitro or animal studies, reviews, case-report studies or articles that only provided Title and *Abstract* were excluded. The quality of the articles included in this review was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).

Results: A total of ten articles were included in the final review. The highest risk of bias by the qualitative analysis of the studies was related to the reference standard used to define disease activity. Despite the great heterogeneity in the CEUS parameters used, the most evaluated parameter and that showed the best results was the quantitative analysis of the peak enhancement (PE). Specific analysis of the enhancement of the innermost layer of the bowel wall showed promising results compared with the enhancement of the complete bowel wall. CEUS seems to be a good modality to distinguish patients with active inflammation from patients with quiescent disease; however, it did not provide such favorable results regarding the grading of disease severity.

Conclusions: CEUS can be a useful imaging technique for assessing disease activity in Crohn's disease patients, especially in distinguishing active inflammation. However, standardization of the parameters used is necessary for the more widely use in clinical practice in the future.

Keywords: Crohn’s disease, Contrast-enhanced ultrasound, CEUS.

List of Abbreviations

AUC – Area Under the Curve
BWT – Bowel Wall Thickness
CD – Crohn's Disease
CDAI – Crohn's Disease Activity Index
CDEIS – Crohn's Disease Endoscopic Index of Severity
CDI – Color Doppler Imaging
CEUS – Contrast-Enhanced Ultrasound
CICDA – Composite Index of Crohn's Disease Activity
CRP – C-reactive protein
CT – Computed Tomography
ECCO – European Crohn's and Colitis Organization
EFSUMB – European Federation of Societies for Ultrasound in Medicine and Biology
ESGAR – European Society of Gastrointestinal Radiology
ESR – Erythrocyte Sedimentation Rate
GS – Gray-Scale
HBI – Harvey-Bradshaw-Index
MPI – Maximum Peak Intensity
MRE – Magnetic Resonance Enterography
MRI – Magnetic Resonance Imaging
mTT – Mean Transit Time
NPV – Negative Predictive Value
PE – Peak Enhancement
PI – Peak Intensity
PPV – Positive Predictive Value
QUADAS-2 – Quality Assessment of Diagnostic Accuracy Studies 2
RBV – Regional Blood Volume
RT – Rise Time
SES-CD – Simple Endoscopy Score for Crohn's Disease
TTP – Time to Peak
US – Ultrasound
WBC – White Blood Cell Count

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Introduction

Crohn's Disease (CD) is a chronic inflammatory bowel disease with alternating episodes of active inflammation and remission, and by several other complications such as strictures, fistulas or abscesses.[1] The disease can affect any part of the digestive tract and may reach its various layers (transmural involvement).[2] Since signs and symptoms do not always reflect disease severity, frequent imaging monitoring is of vital importance as a means of objectively assessing the extent and activity of CD.[3]

Ileocolonoscopy with tissue biopsy remains the gold standard for both diagnosis and follow-up in CD. However, the fact that it is an invasive method, poorly tolerated when repeated often, which does not allow evaluation of the entire bowel or transmural involvement, makes it necessary to use other imaging methods to precisely define the location and extent of the disease as well as the appropriate treatment.[4,5]

Thus, as a complement to endoscopy, other noninvasive imaging exams are currently recommended to allow for cross-sectional evaluation of the bowel, such as computed tomography (CT), magnetic resonance imaging (MRI) and transabdominal ultrasound.[6] Although it is still not widely used, there has been a growing interest in ultrasound. This is mainly due to the fact that MRI is a more expensive exam, but also because of the need to avoid repeated exposure to ionizing radiation as in the case of CT.[7] Comparing with MRI, ultrasound also has important advantages in the pediatric or claustrophobic population, since they often require sedation or even general anesthesia to perform the exam.[8]

Ultrasound allows the assessment of several relevant aspects of CD, such as bowel wall thickness, echo pattern and stratification of the bowel wall, vascularity, complications and some extra-parietal manifestations such as lymph nodes or mesenteric fat hypertrophy.[9] Within cross-sectional imaging techniques, bowel wall thickness is the most frequently evaluated parameter, as it has a good correlation with disease activity. However, it has the disadvantage that it is not only related to disease activity, but also to its chronicity and associated fibrosis.[10] As part of the inflammatory component characteristic of the disease, it has been described some changes at the microvascular level, such as neoangiogenesis.[11] This increase in blood flow and vascular density is the reason for the use of contrast imaging and color Doppler imaging (CDI), as it is an indicator of inflammation and therefore disease activity.[12] In addition, blood flow measurement may also be important for assessing some complications of CD, with implications for subsequent therapy. It may

allow the distinction between inflammatory or fibrotic strictures, between an abscess and a phlegmon, and may be used in postoperative follow-up as a predictor of recurrence.[9]

Although CDI is a sensitive tool for the assessment of bowel wall vascularity, it only provides a semi-quantitative measure of inflammatory activity and can only measure blood flow from larger vessels, being limited in obese patients or where the bowel lies deeper into the abdominal cavity.[13]

Contrast-enhanced ultrasound (CEUS) consists in the intravascular injection of an ultrasound contrast agent - SonoVue (Bracco, Italy) - made of microscopic gas bubbles that enhance blood vessels on ultrasound imaging.[2] The recommended contrast dose is higher than for other CEUS applications, typically 4,8mL, because of the higher frequencies used when evaluating the bowel wall,[14] and each injection should be followed by a 5mL flush with 9mg / mL (0.9%) sodium chloride solution.[15] This contrast has the ability to reach smaller vessels, and since it is purely intravascular and does not diffuse into the interstitial space, it allows for a much more accurate assessment of tissue perfusion.[16] This type of contrast has another unique advantage over the contrasts used in CT and MRI because it is excreted by the lungs, so there is no risk of nephrotoxicity.[17]

In addition to qualitative assessment using CEUS, which encompasses the pattern of bowel wall enhancement, the use of specific software also allows quantitative assessment using parameters such as peak enhancement (PE) and area under the curve (AUC).[18] This quantitative assessment may be important by decreasing inter-observer variability, which is a frequent problem with ultrasound methods, but may also increase the complexity of the examination. [19]

CEUS can be used to quantify vascularization in a pathological bowel, but also to distinguish vascular structures from avascular structures, which is of great importance in the complications of CD.[16,20] More recently, it has been suggested that CEUS may be a useful tool in determining prognosis in patients starting therapy with biologics and in post-surgical recurrence. [21,22]

Despite its many advantages, CEUS has some important limitations, such as not allowing the evaluation of the pathological bowel in its entirety or not having yet clearly defined cut point values that allow a better classification of CD.[23]

The aim of this study is to review the latest literature regarding the usefulness of CEUS particularly in detecting disease activity in CD, answering the question “Is CEUS an effective technique for correctly assessing and grading the disease activity in CD?”.

Materials and Methods

Literature search and data extraction

The elaboration of this systematic review was based on the 2009 PRISMA Guidelines.[24] The research was conducted through PubMed and Web of Science (Thomson Reuters) up to the 2nd of August 2019. We searched for articles with the following three keyword combinations: “Crohn CEUS”, “Crohn contrast enhanced ultrasound” and “Crohn contrast enhanced sonography”. Filters were added to search only articles from 2010 to 2019, as well as only articles in English. It was also added the search filter to include only studies in humans in PubMed.

Inclusion / exclusion criteria were pre-established, and are presented in Table I. The articles were selected by the author of the present review, with the supervision of his co-advisor. At an early stage the eligibility criteria were applied, and review and case-report articles, articles not referring to CEUS, and an animal study were excluded. Those included in this first selection were analyzed by their title and Abstract, and the inclusion criteria were applied. Finally, the included articles were read in full, from which an article was excluded because it did not correspond to the purpose of this review.

Quality assessment

All included articles were assessed for their risk of bias and applicability using the Revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[25]

Data extraction

Data was extracted by reading and analyzing the complete articles, and then a summary table was constructed for each one, taking into account the study population, the reference method, the characteristics of the analysis with the CEUS and the most important study results.

Results

Search results and selection process

The selection process is detailed in Figure 1. In brief, 169 articles were obtained from the initial search, without duplicates. After applying the inclusion/exclusion criteria, 74 articles were selected. These articles were analyzed for their title and Abstract, and 63 were excluded because they didn't match the review question. The remaining 11 articles were analyzed in full and 1 more article was excluded for not matching the review question. Finally, 10 articles were selected for data extraction.[7,10,12,20,26-31]

Quality of studies

Results of QUADAS-2 evaluation are shown on Figures 2-5. Overall, the studies analyzed showed low risk of bias and high applicability in all the domains. A total of only three studies scored low for both the risk of bias and concerns regarding applicability in all domains. The domain that showed the highest risk of bias is domain 3 – Reference Standard. Two studies used clinical and laboratory parameters as their reference standard, which are known for not always correlating with the actual disease severity in CD.[26,27] Also, in the paper by Medellin-Kowalewski et al.,[12] a global assessment made by ultrasound parameters was used as reference standard and endoscopy results were only available for a couple of patients. The gold standard for defining disease activity in CD is ileocolonoscopy. By poorly defining the disease activity as we are trying to assess in this review, it is possible that they also increase the risk of bias when trying to evaluate the effectiveness of CEUS in this situation.

Data extraction

For each of the 10 included studies, a table with a synthesis of the most important information was made and can be consulted in Tables II-XI.

Study design and demographics

Most studies included in this review followed a prospective design. Only one paper followed a retrospective design[12] and in another one the design was not stated.[29]

Regarding the study population, a total of 654 patients were included, with a mean of 65 patients and a range of 25-180. In the paper by Ripollés et al.,[22] 28 bowel segments were analyzed by histopathology, as 3 of the 25 included patients had more than one bowel segment analyzed,

but we included the number of patients instead of bowel segments. Out of the 654 total patients, 50,3% were male. The mean/median age of the study groups ranged from 33 to 49 years old.

All studies included patients with an already proven diagnostic of CD, except for the paper by Wong et al.,[31] where 5 of the total 30 patients only had suspicion of CD and the diagnosis was only later confirmed by endoscopy and biopsy. In 2 of the studies,[12,26] a diagnostic of CD was the main requirement for the patient selection process, and in other 3 papers,[7,28,31] only patients requiring endoscopy within a time interval of the US scans were included. In 2 other studies,[10,22] patients with scheduled elective surgery for bowel resection were selected. In the remaining 3 studies, even though they included patients with known CD, specific populations were tested: Liu et al. included patients with suspected disease activity,[29] Malagò et al. included patients with only a single small bowel lesion,[27] and De Franco et al. excluded patients with active colonic disease so it didn't interfere with clinical and laboratory activity indexes.[30]

Reference method

Regarding the reference method, only 4 out of the 10 studies included used the gold standard, ileocolonoscopy, as their main reference standard,[7,28,29,31] even though De Franco et al. used it as a secondary reference standard.[30] Both Ripollés et al. and Wong et al. used the Crohn's Disease Endoscopic Index of Severity (CDEIS)[32] which is based on endoscopic assessment of the extent of mucosal ulceration and luminal stenosis.[28,31] For the patients that did have a temporal related colonoscopy, Medellin-Kowalewski et al. also used the CDEIS.[12] Horje et al. used the Simple Endoscopy Score for CD (SES-CD)[33] adjusted to score ileal disease activity, the same score used by De Franco et al. for the endoscopy index.[7,30] This score is based on the scoring of four variables: size of ulcers, proportion of ulcerated surface, surface of any other lesion and presence of stenosis. Liu et al. used the Rutgeerts's modified grading system[34] which scores the patients from 0 to 4 according to the lesions found and then divided them into the mild disease group (score 1 and 2) and severe disease group (score 3 and 4).[29]

Two papers used histopathology as their reference standard.[10,20] They both scored the specimens for their active inflammation and fibrostenosis/chronic inflammation according to the same indexes. Both assessed active inflammation according to the Borley et al. method[35] which is based on mucosal inflammation, edema and quantity and depth of neutrophilic infiltration. Chronic inflammation was assessed according to the Chiorean et al. method[36] which is based on the presence and grade of strictures, submucosal fibrosis, muscular hyperplasia, transmural fibrosis and structural layers of the bowel.

There were two studies who used clinical and laboratory parameters as their reference standards.[26,27] Malagò et al. used the Crohn's Disease Activity Index (CDAI) and Girlich et al. used the Harvey-Bradshaw-Index (HBI) as their clinical scoring methods.

Medellin-Kowalewski et al. used an "US global assessment" as their reference standard.[12] Patients were classified as having absent, mild, moderate or severe disease. The scoring was made mostly according to the BWT and mural blood flow on CDI. Patients were then divided in two groups: concordant when the CDI score was equal to or greater than the wall thickness score and indeterminate when the CDI score was less than the BWT score.

Finally, De Franco et al. used the Composite Index of CD Activity (CICDA) as their main reference standard. With this scoring method, the disease was classified as active when at least three out of four criteria were met: (1) CDAI of at least 150; (2) C-reactive protein (CRP) level > 5mg/dL, white blood cell count > 10000 cells/µL and fibrinogen level > 400mg/dL; (3) presence of ileal ulceration at retrograde ileoscopy; and (4) small bowel enema or small bowel follow-through examination showing aphtous or linear ulcers, cobblestone mucosa, sinus tracts, fistulas with extra luminal fluid collections, and perienteric fat with increased attenuation at CT and/or high signal intensity at T2-weighted MRI.

Most studies performed the CEUS examinations in a month or less from the reference standard, exceptions made for the paper from Ripollés et al., where the bowel resection surgeries were performed within 60 days after the CEUS examinations,[20] and the paper from Malagò et al., that doesn´t state the time interval between CEUS and the clinical and laboratory assessment.[27] In the paper from Medellin-Kowalewski et al., colonoscopies were considered temporal related to the CEUS examinations when they were performed within 3 months.[12]

In most papers, the investigators performing either the CEUS examinations or the reference standard were blinded to other test results. There are three papers where the investigators' blindness is not stated.[20,26,29]

In 4 out of 10 studies included in this review, only the small bowel disease activity was assessed.[7,10,27,30]

Characteristics of the CEUS examinations

Regarding more specific characteristics of the sonographic study with CEUS, the authors used convex or linear probes with a frequency range of 1-9 MHz and a mechanical index range of 0,05-0,1, even though 3 papers did not report the used mechanical index.[10,26,29] There was a big heterogeneity in the US devices used and also with the software used in the analysis.

Most studies reported the scan time using the CEUS, with a range of 40-180s. Even though most papers used SonoVue as their sonographic contrast agent, with doses ranging from 1,2mL to 4,8mL, both papers from Medellin-Kowalewski et al. and Wong et al. used Definity, which is composed of perflutren lipid microspheres and is the sonographic contrast agent approved for CEUS in some countries.[12,31]

Only four studies reported having experienced operators doing the sonographic studies with CEUS.[10,28-30] Also, three studies reported having two operators analyzing the CEUS examinations.[28,30,31]

Any of the studies used pre-defined thresholds for detecting disease activity with CEUS. All studies used quantitative parameters to assess the efficacy of CEUS in detecting or grading disease activity in CD, which are less user dependent than the qualitative parameters normally used in sonographic evaluation. The only paper to also include a qualitative analysis with CEUS was the paper from Liu et al.,[29] where they divided the enhancement of the intestinal wall in 4 patterns: 1, transmural hyper-enhancement; 2, hyper-enhancing inner bowel layers and isoenhancing outer bowel layers; 3, isoenhancing of both inner and outer layers; 4, isoenhancing inner layers and hypo-enhancing outer layers. Even though the qualitative analysis showed good sensitivity (100%), it showed low specificity (57,9%). In the same study, several quantitative parameters were analyzed, but the only ones that showed ability to differentiate mild from severe disease were the maximum of intensity (Imax) of the entire bowel wall and the Imax of the inner bowel wall, with the Imax of the inner bowel wall showing better results with a cutoff value of 3356 (100% of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and a Youden's index of 1.0).

Both papers from Ripollés et al. used the percentage increase in wall brightness, calculated using the following formula: ([brightness post-contrast – brightness pre-contrast] x 100)/brightness pre-contrast.[20,28] In the study that compared the sonographic study with the histopathologic scores, using a threshold of percentage > 65%, CEUS had a sensitivity of 93%, specificity of 69%, accuracy of 82%, PPV of 78% and NPV of 90% for differentiating between inflammatory and fibrostenotic bowel lesions.[20] In the other study that compared CEUS with endoscopic disease activity an optimal cutoff value of 47% for the percentage increase in wall brightness showed sensitivity of 99,3%, specificity of 60,5%, PPV of 90,4%, NPV of 95,8% and accuracy of 91,1% for detecting disease activity (moderate or severe grade).[28]

Wilkens et al. compared several relative CEUS parameters such as PE, AUC, wash-in and wash-out AUC with histopathology inflammation and fibrosis scores, f-calprotectin, CRP, CDAI and HBI but didn't find any statistical significant correlation between any of them, except with f-calprotectin, but the correlation was weak.[10]

Medellin-Kowalewski et al. found a correlation between the PE and the AUC for differentiating mild, moderate and severe diseased based on the US global assessment, even though the PE showed better results.[12] Comparing the results in the concordant group, the PE with a cutoff value of 18,2 dB for differentiating mild from moderate disease showed 89,2% sensitivity and 90,9% specificity, and a cutoff of 23,0 dB for differentiating moderate from severe disease showed 89,5% sensitivity and 83,1% specificity; for the AUC, a cutoff of 33,8 dB/s for mild versus moderate disease showed sensitivity of 83,8% and specificity of 81,0% and a cutoff of 36,9 dB/s for moderate versus severe disease showed sensitivity of 94,7% and specificity of 72,4%. Also, for the patients that had a temporally related colonoscopy, CEUS correctly classified 31 in 34 patients as having active disease (moderate or severe) using a PE of 17 dB or greater. Horje et al. also found a correlation for the PE but this time with endoscopic disease activity.[7] For a cutoff value of 10% PE for detecting active inflammation on the terminal ileum, CEUS showed a sensitivity of 100%, specificity of 92% and accuracy of 99%. They also found a relationship between regional blood volume (RBV) and disease activity with a cutoff value of 200cm3 showing a sensitivity of 93%, specificity of 83% and accuracy of 88%.

Girlich et al. compared several quantitative parameters such as PE, time to peak (TTP), RBV and the ratio TTP/PE with clinical and laboratory parameters.[26] They found a negative correlation between the CRP and TTP (the higher the CRP, the shorter the TTP) and also a significant negative correlation between the HBI and both the TTP and TTP/PE. Wong et al. also compared several quantitative parameters such as AUC, PE and TTP but with endoscopic disease activity, but found no correlation between them.[31] A subgroup of patients was reassessed after treatment, and they found a significant reduction in TTP similar to the one observed in the CDAI and CRP. Another interesting finding in this paper was that the PE was significantly higher in the submucosa/muscularis propria compared to the mucosa (13,9 vs. 12,5 dB, respectively).

De Franco et al. used two different parameters: maximum peak intensity (MPI) and the β-coefficient, which describes the slope of the initial curve and is related to the TTP.[30] Using the clinical index CICDA as the reference standard, a cutoff value for the MPI of 24 VI showed 97% sensitivity and 83% specificity for differentiating active versus inactive CD; a cutoff value for the β-coefficient of 4,5 VI/s showed 86% sensitivity and 83% specificity. A relationship between these two parameters and the secondary reference standards (CRP, fibrinogen level, CDAI and SES-CD) was also demonstrated, even though it was weaker than the correlation with CICDA.

Finally, Malagò et al. compared CEUS activity curves with MRE activity curves, using clinical and laboratory parameters as reference standard.[27] There was no statistical significant correlation between both activity curves, and also no correlation with the clinical and laboratory parameters.

Discussion

It is now recognized that the disease severity does not always match the signs and symptoms in CD, making clinical indexes less reliable when trying to assess the activity of the disease which is something that can have an impact for example when trying to establish the right therapeutic in a specific patient. Also, some laboratory parameters such as inflammatory markers that are usually used on the clinical assessment of CD activity are being increasingly disputed.[37] Due to the natural course of the disease, patients need to be frequently assessed for acute inflammation, complications, therapeutic adjustments and even post-surgical recurrence. Because of this, the choice of the right diagnostic tool is very important, as it needs to be easily repeatable without the inconvenience for the patient, or even harming him with ionizing radiation. US overcomes several limitations of other diagnostic tools and has already gained its space in the assessment of patients with CD, even though it has its own disadvantages.[38] The fact that the inflammatory component of CD provokes neoangiogenesis of the bowel wall makes other imaging modalities that are able to assess regional perfusion useful in this regard.[39] CDI and CEUS have more recently emerged as valid techniques for the assessment of bowel wall perfusion, with an increasing interest in the quantitative assessment that CEUS can offer, overcoming some of the limitations of other ultrasound techniques, such as the interobserver variability.

According to the latest consensus guidelines by the European Crohn's and Colitis Organization (ECCO) and European Society of Gastrointestinal Radiology (ESGAR) for diagnostic assessment in IBD, cross sectional imaging is recommended for diagnosis, detection of disease activity or other complications related to the disease.[38] US techniques have some unique advantages over other cross sectional imaging modalities such as being less expensive, being well tolerated and radiation free. The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) in their latest paper on gastrointestinal US for IBD states that CEUS can be used to estimate endoscopic disease activity, with Level of Evidence 1b and with a Grade of Recommendation level of A.[9]

To date, only one other systematic review was done specifically assessing the role of CEUS for the detection of CD activity.[40] In their meta-analysis, a pooled sensitivity of 0,94 [95% CI 0,87-0,97] and pooled specificity of 0,79 [95% CI 0,67-0,88] was found. Their biggest limitations were the size of the study population and the use of different enhancement thresholds for defining disease activity.

Among the US parameters for assessing disease activity, BWT is the most commonly used, since it has shown the most consistent results in systematic reviews and meta-analysis in this regard. Even though the cutoff value of BWT still varies among studies, the EFSUMB Guidelines

recommend a value of > 3 mm when a high sensitivity is preferred and a value of > 4 mm when a high specificity is preferred for detecting CD activity. A recent meta-analysis showed a sensitivity of 89% and specificity of 96% for the cutoff value of > 3mm and a sensitivity of 87% and specificity of 98% for the cutoff value of > 4mm.[41] However, the fact that some patients without active inflammation continue to show an increased BWT, probably due to fibrosis, make the quantification of the bowel wall vascularity important in the assessment of these patients.

Color Doppler US is one of the methods used to assess bowel wall vascularity in the affected bowel segment. One of the problems related to this technique is the subjectivity of the analysis, which may have contributed to the introduction of combined scoring systems based on thickening of the bowel wall and CDI analysis.[42] In one of the studies included in this systematic review,[28] CDI grades 2 and 3 showed a very high specificity in identifying patients with active disease on endoscopy, but lower grades on CDI showed less positive results. This can be due to the fact that CDI does not detect the blood flow in smaller vessels and vascularity in deep-lying bowel wall segments, and in these cases CEUS can be a useful tool.

In this review, we included a total of ten papers, most of them with a prospective design, which tried to determinate the validity of CEUS as a reliable method for assessing the disease activity in CD. Some papers tried to find a direct correlation between CEUS parameters and the reference standard, while others tried to distinguish active from inactive disease or even distinguish mild from severe disease finding the optimal cutoff values for different parameters. The results varied significantly among studies, except for the fact that CEUS quantitative parameters do not correlate well with clinical and laboratory parameters for disease activity. This comes as an expected result because, as mentioned earlier, active disease can occur without very expressive symptomatology and laboratory parameters for inflammation can be affected by many other causes besides CD. One paper included in our review also used CEUS qualitative parameters to assess active inflammation, but its specificity was very low (57,9%) in differentiating mild from severe disease.[29] In fact, the qualitative analysis in CEUS does have some advantages over the quantitative assessment, like its simplicity or not being influenced by peristalsis, but the fact that it is user dependent, makes it a less accurate method. There were only two studies that used endoscopy or histology as their reference standard and did not find any relationship with CEUS quantitative parameters,[10,31] even though Wong et al. did find a similar reduction in the TTP as in the clinical and laboratory parameters following treatment. Most studies used different parameters in the quantitative analysis, but the most consistently used and the one who showed the best results was PE, and other parameters calculated from it, like the percentage increase in wall brightness used by Ripollés et al.[20,28] The test results differed among studies, but even though most studies found a high sensitivity,[7,12,20,28-30] some found lower values for specificity,[12,20,28,29] with

results ranging from 89,2-100% for sensitivity and from 57,9-100% for specificity in the PE parameters. One thing that is important to mention is that CEUS appears to be an effective method for distinguishing active from inactive inflammation in the bowel, but doesn't seem to have the same results when trying to grade disease activity, as the only two studies who tried to find a direct correlation between CEUS quantitative parameters and disease activity on endoscopy[31] or histology[10] couldn't find any significant statistical correlation. Liu et al. analyzed the different quantitative parameters of CEUS in two different layers of the bowel wall: the inner layer, which included the mucosa, muscularis mucosa and submucosa, and exterior layer, which included the muscularis propria and serosa.[29] The PE (Imax) of the internal layer showed very interesting results, as it was superior to the Imax of the entire wall in differentiating mild from severe disease, and it was the parameter with the highest scores across all studies included in this review. The fact that this quantitative analysis was only performed in 15 patients, and also being the first and only study to use this parameter, makes it important to further investigate it in more and larger studies, but the quantification of enhancement of specific bowel layers is a promising CEUS parameter for future investigations.

There are some limitations to the studies included in this systematic review that should be pointed out. First of all, there were some limitations regarding the study populations used. Even though larger studies were included comparing to the previous systematic review by Serafin et al., only three papers included more than 100 patients,[7,12,28] with the others including substantially smaller study groups. Besides that, some studies used rigid inclusion criteria and assessed specific subsets of patients with CD that could have an impact in the results, for example, using patients requiring surgery for bowel resection. These patients probably have much more severe disease activity than the others, since bowel resection surgery is indicated in cases where medical therapy has failed or when there are severe complications associated with CD. Another important limitation already mentioned, is the choice of the reference standard. Ileocolonoscopy with tissue biopsy remains the first line method for diagnosing and assessing CD. Some studies still included as their reference standard clinical and laboratory parameters, introducing a potential risk of bias since it is possible that the clinical presentation on a specific patient does not match the actual bowel inflammation measured by CEUS. It is also important to refer that even studies that included endoscopy or histopathological analysis of biopsy specimens as their reference standard used different reference definitions for assessing disease activity, which can have implications for the results of the index test. There are also limitations regarding the use of CEUS. First, even though the recommended dose of the contrast agent SonoVue is 4,8mL, due to the high frequencies used when evaluating the bowel wall, only two papers referred using this dose,[28,30] with the others using smaller doses, which can have an impact on the results, since fewer microbubbles of the

appropriate size resonate at higher frequencies.[14] Besides that, two studies used the contrast agent Definity,[12,31] which is different from SonoVue and there is no study on the comparison of both in the assessment of IBD. Lastly, and probably the most important limitation regarding the use of CEUS, is the use of different parameters and thresholds for defining disease activity in CD. This is probably an important cause for the non-consensual results and conclusions about the role of CEUS as a valid method for diagnosing and assessing CD activity, and can be an obstacle to the definition of clear cutoff values for this method to be validated.

This review also has some limitations that are important to refer. A limitation that can affect the validity of any systematic review is the publication bias that occurs because a paper with favorable results has a better chance of being publish than one with negative results. Even though there isn't enough evidence of publication bias in reviews about the role of a specific diagnostic method, this should always be mentioned.[43] Specifically about this systematic review, only articles from two main databases and written in English were used. Also, the fact that only papers from 2010 until 2019 were considered, may have excluded some important articles. Finally, the review included articles with a wide range of reference standards, and also articles with different thresholds for the diagnosis of disease activity, which was also an important limitation on the first systematic review assessing the role of CEUS in the detection of CD's activity.

Conclusion

CEUS appears to be an effective technique for differentiating patients with active inflammation from patients with quiescent disease, even though it doesn't seem to be so promising in grading patients with active disease. Larger prospective studies are starting to emerge, but the heterogeneity of methodologies used is still an obstacle to the definition of a validated diagnostic threshold. Without that, it is difficult to implement CEUS in everyday practice. For the future, it would be important a standardization of CEUS characteristics to clearly define its role in the assessment of CD's activity and establish clear guidelines for clinical practice. In parallel with CEUS, other techniques for assessing CD's activity are showing promising results, such as the fecal calprotectin level, so it would be useful to also include them in future studies comparing both techniques.

Figures

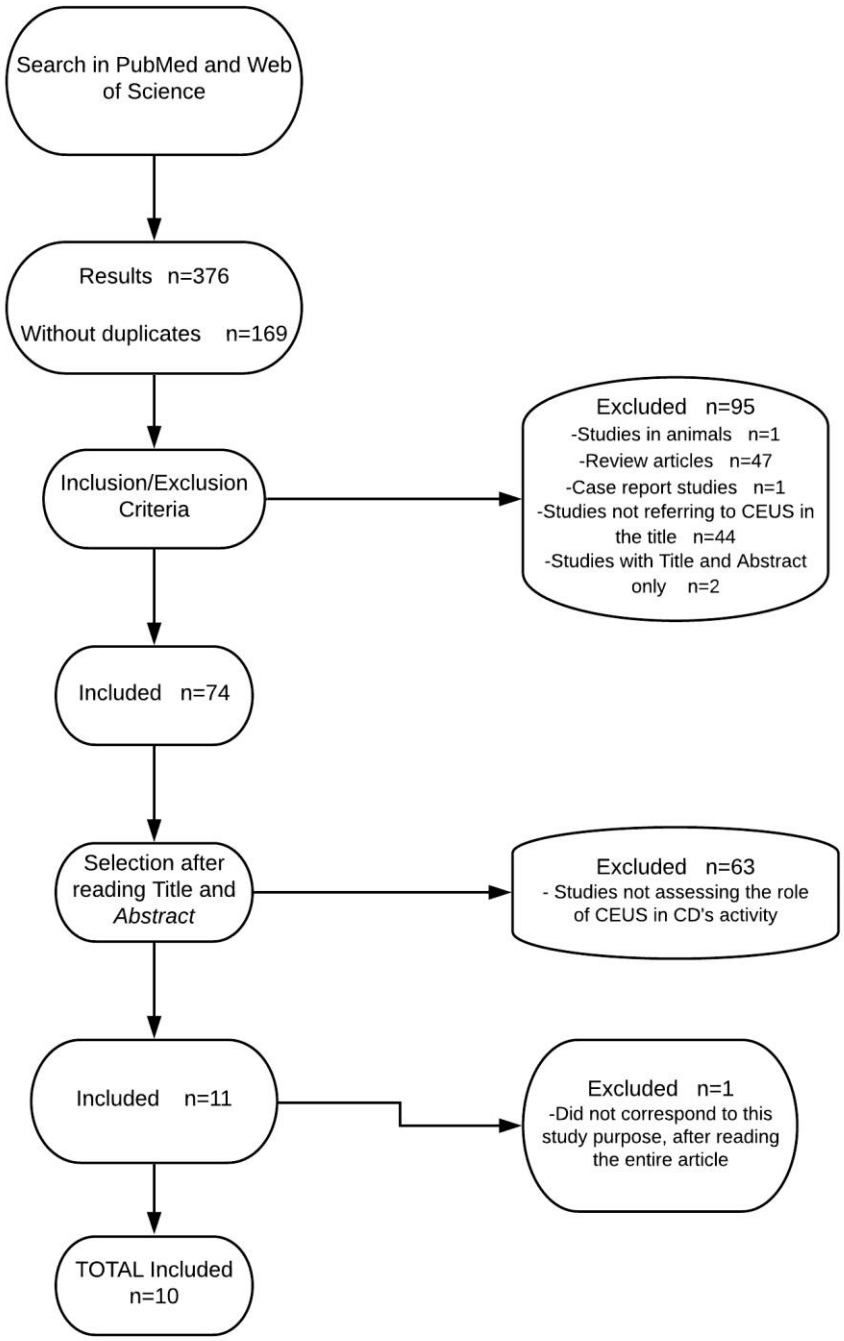

Figure 1 - Flow chart of the literature search

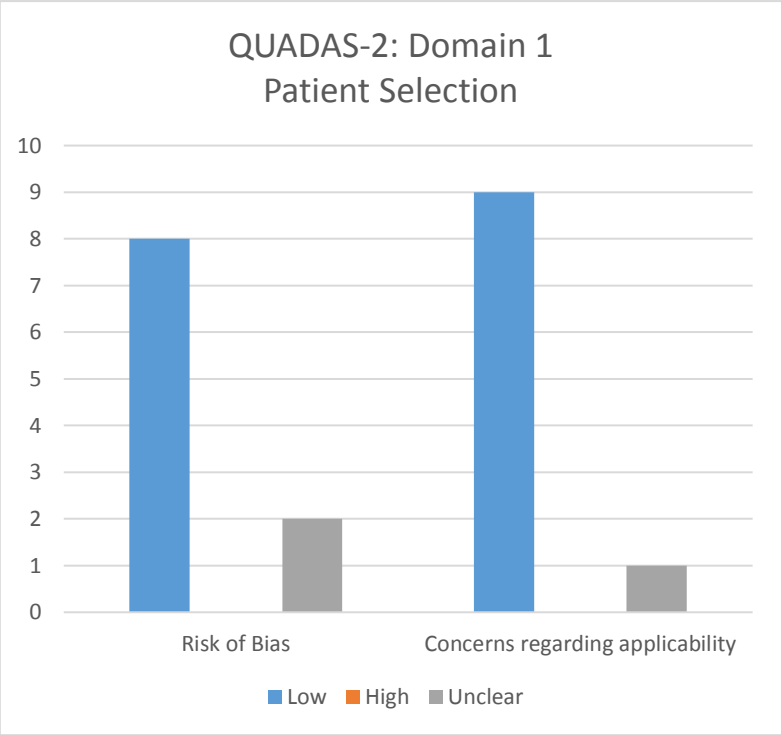

Figure 2 - QUADAS-2: Domain 1

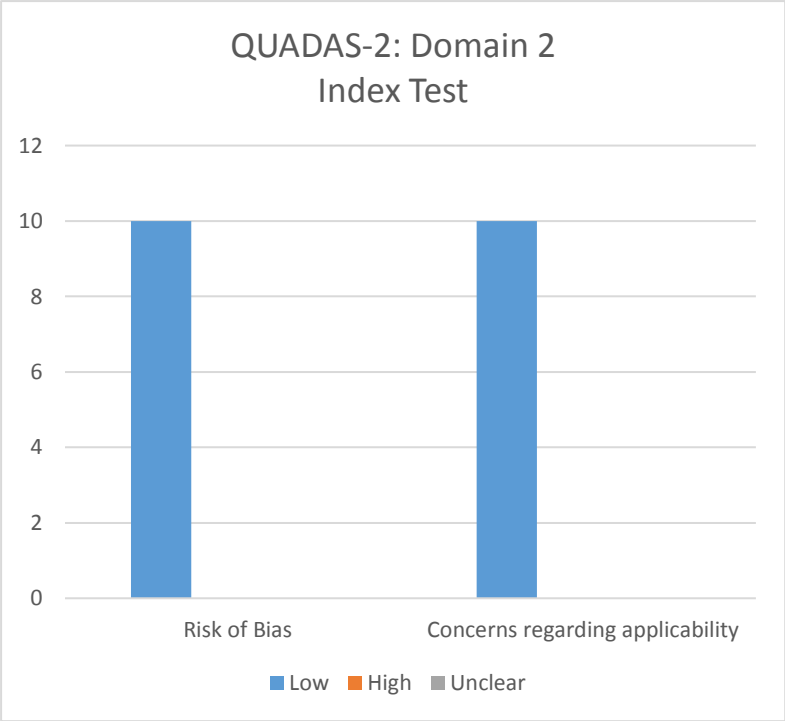

Figure 3 - QUADAS-2: Domain 2

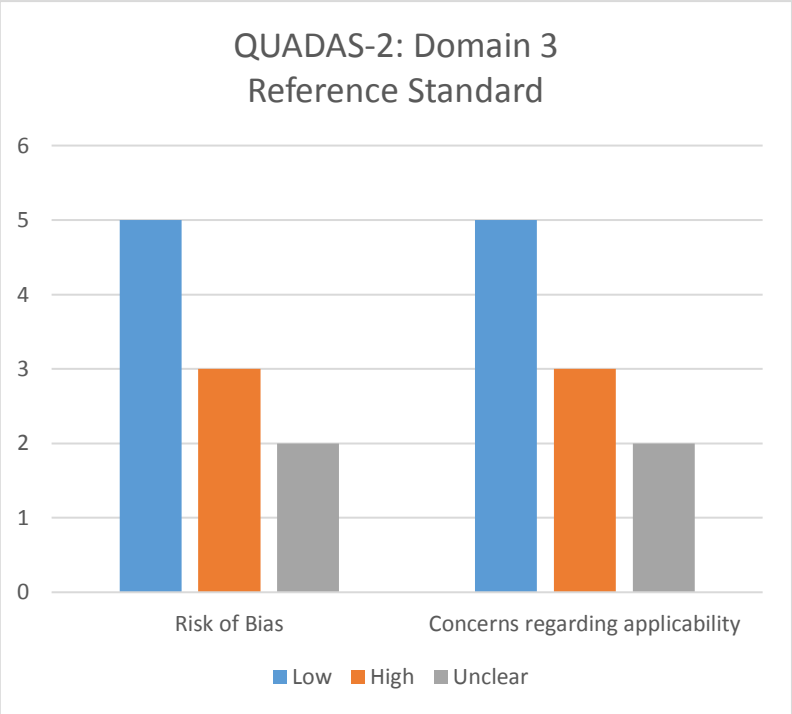

Figure 4 - QUADAS-2: Domain 3

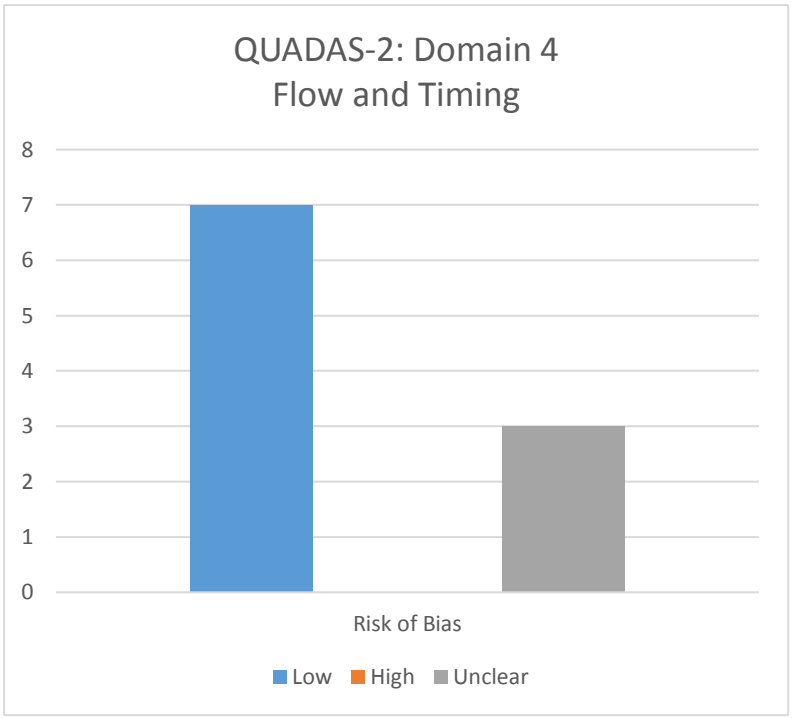

Figure 5 - QUADAS-2: Domain 4

Tables

Table I - Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Published between 2010-2019	Articles not written in English
Study assessing the role of CEUS in Crohn's Disease activity	*In vitro* or animal studies
	Review articles
	Case report studies
	Articles with only Title and *Abstract*

Table II - Ripollés et al., The Role of Intravenous Contrast Agent in the Sonographic Assessment of Crohn's Disease Activity: Is Contrast Agent Injection Necessary?

First Author /Date	Study Population	Study Design	Relevant Results
T. Ripollés, 2019	- 180 patients were included. - Inclusion criteria: - complete ultrasound (US) examination (gray-scale (GS), color Doppler imaging (CDI), contrast-enhanced ultrasound (CEUS)); - colonoscopy within 30 days of the US scans. - Excluded: - patients < 18 years old; - pregnant women; - patients with contraindications for the CEUS contrast.	- Prospective study. - Two experienced radiologists performed all the exams; they measured the bowel wall thickness (BWT) of the affected segment, wall vascularization with CDI and measured the percentage increase of enhancement in wall brightness with CEUS. - The intensity of the color Doppler flow was subjectively graded as Grade 0, 1, 2 or 3. - Ileocolonoscopy was used as the reference standard; it was performed by two experienced gastroenterologists who were blinded for the US results; they used the Crohn's Disease Endoscopic Index of Severity (CDEIS) for grading.	- The optimal cutoff values for identifying active disease by the ROC curve were CDI grade > 1 and percentage of enhancement > 47%. - CEUS alone is the most reliable US technique to detect disease activity. - CDI grade 2 and 3 has a high specificity (92,1%) for detecting disease activity with a positive predictive value (PPV) of 97%. - By adding CEUS to CDI grade 0 and 1, the combined results were sensitivity 100%, specificity 60,5%, negative predictive value (NPV) 100%, PPV 90,4% and accuracy 91,7%.

Table III - Wilkens et al., Validity of Contrast-enhanced Ultrasonography and Dynamic Contrast-enhanced MR Enterography in the Assessment of Transmural Activity and Fibrosis in Crohn's Disease

First Author /Date	Study Population	Study Design	Relevant Results
R. Wilkens, 2018	- Included 25 patients with known Crohn's Disease (CD) scheduled for elective ileo-caecal or small intestine resection within 30 days of the US scans. - Inclusion criteria: - > 18 years; - detectable inflammation on US, defined as BWT > 3mm and/or visualization of small bowel stricture. - Excluded: - pregnant or breastfeeding women; - contraindications for MRE or CEUS.	- Prospective study. - In a maximum period of 4 days between exams, patients were assessed by both CEUS and MRE. - During CEUS analysis, several perfusion parameters were assessed on the more affected areas; on the day of the US examination, patients were also assessed for their Crohn's Disease Activity Index (CDAI), Harvey-Bradshaw Index (HBI), collected blood samples for inflammatory markers and stool samples for f-calprotectin. - Histopathology was used as the reference standard; the pathologists graded the specimens for their inflammation and fibrosis; they were blinded to other test results.	- There was no statistically significant correlation between any CEUS or MRE perfusion parameter and the inflammation or fibrosis scores on histopathology. - There was a moderate correlation between CEUS parameters and f-calprotectin although no correlation was found between CEUS and CDAI, HBI or C-reactive protein (CRP).

Table IV - Medellin-Kowalewski et al., Quantitative Contrast-Enhanced Ultrasound Parameters in Crohn Disease: Their Role in Disease Activity Determination With Ultrasound

First Author /Date	Study Population	Study Design	Relevant Results
A. Medellin-Kowalewski, 2016	- 127 patients with known CD were included. - Inclusion criteria: - > 18 years; - complete US examination of the bowel (GS, CDI, CEUS). - Excluded: - patients who started a new pharmacologic therapy that could alter CEUS results; - technically poor US scans in which visualization was limited or failed; - unexpected bowel findings, including tumors.	- Retrospective study. - On the complete US scan, patients were classified as having inactive, mild, moderate or severe disease activity according to their BWT or mural blood flow; they were then divided in two groups: the concordant group when the CDI score was equal or greater than the BWT score, and the indeterminate group when the CDI score was lower than the BWT score. - On the day of the US scans, patients were assessed for their HBI. - CEUS was performed on the bowel areas that showed the most disease activity, and some quantitative parameters such as peak enhancement (PE) and area under the curve (AUC) were measured. - 43 had a temporally related colonoscopy, even though that in 9 of those patients the CEUS scan assessed an area proximal to the endoscopy.	- There was a good correlation between PE and BWT with an incremented trend of PE to increase in proportion to BWT. - There was also a relationship between the AUC and continuous BWT, but not as strong as the one with the PE. - Of the 34 patients with colonoscopy, 31 were correctly identified as having active disease using a cutoff of PE of 17dB or greater (cutoff for the entire group), and 28 were correctly identified using a cutoff value of 18dB or greater (cutoff value for the concordant group).

Table V - Horje et al., Contrast Enhanced Abdominal Ultrasound in the Assessment of Ileal Inflammation in Crohn's Disease: A Comparison with MR Enterography

First Author /Date	Study Population	Study Design	Relevant Results
C. S. Horje, 2015	- 105 patients with known CD were included. - Inclusion criteria: - > 18 years; - clinical indication for ileocolonoscopy to assess location, extent and severity of the disease. - Excluded: - pregnant women; - history of heart disease; - contraindications for CEUS or MRE. - 11 patients had a stenosis disallowing endoscopic intubation of the terminal ileum.	- Prospective study. - At inclusion, all the patients had their HBI assessed and collected blood samples to measure the CRP. - All patients underwent ileocolonoscopy and the disease activity was graded by the Simple Endoscopic Score for Crohn's Disease (SES-CD). - Within 2 weeks of endoscopy, patients were assessed by CEUS and MRE on the same day; the radiologist who performed the exams was blinded to the endoscopy results. - After a GS US, CEUS was performed in the area with the greatest BWT and the quantitative parameters peak intensity (PI), time to peak (TTP) and regional blood volume (RBV) were measured.	- The CEUS parameter that best correlated with disease activity was PI, with a cutoff value of 10% (indicated by area under the ROC curve). - Using this value of PI, CEUS appears to be superior to MRE for detecting active endoscopic inflammation, with 100% sensitivity, 92% specificity, 99% PPV, 100% NPV and 99% accuracy. - Neither CEUS nor MRE correlated with the clinical and laboratory parameters.

Table VI - Liu et al., Conventional ultrasound and contrast-enhanced ultrasound in evaluating the severity of Crohn's disease

First Author /Date	Study Population	Study Design	Relevant Results
C. Liu, 2015	- 37 patients with known CD and suspected active disease by the CDAI and endoscopy were included.	- All patients underwent ileocolonoscopy and the severity of the disease was graded by the modified Rutgeerts score; they were then divided in the mild disease group and severe disease group. - Within a week they underwent the US examination, were they were assessed for BWT; the radiologists identified an internal bowel layer (mucosa, muscularis mucosa and submucosa) and external layer (muscularis propria and serosa). - CEUS assessed the most suspicious area for disease activity; all patients underwent the qualitative analysis where they were graded by their pattern of enhancement; on the quantitative analysis, patients were assessed for maximum intensity (Imax), rise time (RT), TTP and mean transit time (mTT); 22 patients weren't assessed by the quantitative analysis due to peristalsis.	- On qualitative analysis, CEUS showed good sensitivity and specificity for identifying patients with active disease (93,5% and 93,7% respectively), but showed lower specificity to distinguish mild from severe disease (sensitivity 100%, specificity 57,9%). - On quantitative analysis, the only relevant parameters were Imax of entire bowel wall and Imax of inner bowel wall; Imax of entire bowel wall showed sensitivity 100%, specificity 67,7%, PPV 100%, NPV 88,9% and Youden's index 0,677 to distinguish mild from severe disease; Imax of inner bowel wall scored 100% in all parameters with a Youden's index of 1.

Table VII - Ripollés et al., Effectiveness of contrast-enhanced ultrasound for characterization of intestinal inflammation in Crohn's disease: A comparison with surgical histopathology analysis

First Author /Date	Study Population	Study Design	Relevant Results
T. Ripollés, 2013	- 25 patients were included. - Inclusion criteria: - endoscopic and histologically confirmed CD with elective surgery for small bowel or colon CD; - US examination, including CDI and CEUS within a 60 day period before surgery.	- Prospective study. - Patients were examined by the GS US, CDI and CEUS; they were classified as having predominantly inflammatory, fibrostenotic or compound disease; CEUS assessed the percentage of increase in wall brightness and the TTP. - After surgical resection, histological slices were taken from 28 specimens and graded for their inflammation and fibrostenosis; they were then classified as being predominantly inflammatory, fibrostenotic or compound.	- The percentage of contrast enhancement was significantly associated with the inflammatory score at histology. - Using a value of 65% percentage of enhancement as the cutoff value from the ROC curve, CEUS had a sensitivity 93%, specificity 69% and accuracy 82% for differentiating inflammatory from fibrostenotic bowel lesions. - There was a high correlation between US scores and pathology scores as the US scores correctly detected inflammation in all segments classified as predominantly inflammatory in the pathology analysis; even though it classified 5 out of 13 with fibrosis as inflammatory, 4 out of these 5 were initially classified as compound.

Table VIII - Malagò et al., Contrast-enhanced ultrasonography (CEUS) vs. MRI of the small bowel in the evaluation of Crohn's disease activity

First Author /Date	Study Population	Study Design	Relevant Results
R. Malagò, 2012	- 30 patients with known CD and with a single small bowel lesion were included. - Excluded: - contraindications for MRE; - suspected acute abdomen; - multiple bowel lesions; - Body Mass Index (BMI) > 30.	- Prospective study. - At inclusion, each patient was assessed for the CDAI and laboratory parameters such as CRP, white blood cell count (WBC), hematocrit and erythrocyte sedimentation rate (ESR) were obtained. - Both MRE and CEUS were performed in a maximum of 2 days between them; the pathological intestinal loop was located by a B-mode US scan before CEUS examination. - On the contrast enhanced exams, time-intensity curves were classified as reflecting CD in an active state (quick wash-in and wash-out) or in a chronic phase (slow wash-in, plateau and slow wash-out); several other parameters were analyzed for both techniques.	- Both MRE and CEUS had a low correlation with CDAI and laboratory parameters. - There was a poor correlation between MRE and CEUS activity curves; the differences were mainly observed in chronic lesions, which showed moderate enhancement at MRE but did not reveal significant enhancement with CEUS.

Table IX - De Franco et al., Ileal Crohn Disease: Mural Microvascularity Quantified with Contrast-enhanced US Correlates with Disease Activity

First Author /Date	Study Population	Study Design	Relevant Results
A. De Franco, 2012	- 54 adult patients with endoscopically confirmed ileal CD were included. - Excluded: - pregnant women; - heart disease history; - ileal disease that was not appreciable at US; - recent endoscopic findings of active colonic CD. - 17 of these patients did the ileocolonoscopy in a different hospital so the SES-CD was not available.	- Prospective study. - The main reference standard was the Composite Index of CD Activity (CICDA), and the CDAI and SES-CD were used as secondary reference standards. - Both the CICDA and the ileocolonoscopy were assessed < 2 weeks before the CEUS examination; on the day of the CEUS examination, the CDAI was calculated and blood was drawn for complete blood counts and measurement of CRP and fibrinogen levels. - CEUS exams were performed by two experienced radiologists blinded to other test results; the areas that showed aperistalsis, BWT > 3mm and intramural Doppler signal were examined for maximum peak intensity (MPI) and the coefficient of the enhancement wash-in slope (β coefficient).	- The MPI and the β coefficient were both significantly higher in patients with active disease as determined by the CICDA; they also showed significant (albeit weaker) positive correlation with the CRP level, CDAI and SES-CD. - The MPI was superior to the β coefficient with a 97% sensitivity and 87% specificity for differentiating active from inactive CD defined by the CICDA.

Table X - Girlich et al., Comparison between a clinical activity index (Harvey-Bradshaw-Index), laboratory inflammation markers and quantitative assessment of bowel wall vascularization by contrast-enhanced ultrasound in Crohn's disease

First Author /Date	Study Population	Study Design	Relevant Results
C. Girlich, 2012	- 45 patients with proven CD were included. - 4 were later excluded due to incomplete sonographic findings.	- Prospective study. - All patients were evaluated by a B-mode US, CDI and CEUS, and the HBI was calculated in the same day. - Laboratory assessment including CRP, leukocytes and hematocrit was performed within 24h. - The parameters assessed by CEUS were Peak (%), TTP, RBV and the TTP/Peak ratio. - The data of 34 patients with a mean Peak > 25% was further analyzed.	- The CRP had a negative correlation with TTP, as none of the other CEUS parameters had a significant correlation with the inflammatory markers. - There was a correlation between the HBI and the TTP and TTP/Peak; this correlation was stronger in the Peak > 25% group.

Table XI - Wong et al., Crohn's disease activity: quantitative contrast-enhanced ultrasound assessment

First Author /Date	Study Population	Study Design	Relevant Results
D. Wong, 2012	- 30 symptomatic patients with known or suspected CD requiring either a colonoscopy or flexible sigmoidoscopy were included. - Excluded: - known heart or pulmonary disease; - abdominal surgery within the last 6 months; - pregnant or lactating women; - hypersensitivity to US contrast agents. - Only 15 patients were available for re-scan after treatment as 2 suffered adverse reactions from CEUS, 2 underwent surgery and 11 were lost to follow-up.	- Prospective study. - At recruitment, the CDAI was calculated and blood samples were taken to measure the CRP. - Colonoscopy and flexible sigmoidoscopies were performed and the CDEIS was calculated as it was used as reference standard. - US scans were undertaken within 7 days of endoscopy and repeated following completion of treatment for the exacerbation; the location analyzed by CEUS was independently selected by the radiologists as they assessed the AUC, PI and TTP. - All exams were repeated at follow-up after treatment except the endoscopies.	- None of the three CEUS parameters showed a significant correlation with endoscopically active disease. - There was a similar significant reduction in TTP compared with CDAI and CRP following treatment.

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