Quantitative Inflammation Assessment for Crohn Disease Using Ultrasensitive Ultrasound Microvessel Imaging

A Pilot Study

Ping Gong, PhD, Pengfei Song, PhD, Amy B. Kolbe, MD, Shannon P. Sheedy, MD, Chengwu Huang, PhD, Wenwu Ling, MD, Yue Yu, PhD, Chenyan Zhou, MD, U Wai Lok, PhD, Shanshan Tang, PhD, David H. Bruining, MD, John M. Knudsen, MD, Shigao Chen, PhD

Objectives—Crohn disease (CD) is a chronic inflammation in the digestive tract that affects millions of Americans. Bowel vascularity has important diagnostic information because inflammation is associated with blood flow changes. We recently developed an ultrasensitive ultrasound microvessel imaging (UMI) technique with high vessel sensitivity. This study aimed to evaluate the feasibility of UMI to assist CD detection and staging.

Methods—Ultrasound microvessel imaging was performed on 76 bowel wall segments from 48 symptomatic patients with CD. Clinically indicated computed tomographic/magnetic resonance enterography was used as the reference standard. The vessel-length ratio (VLR, the number of vessel pixels in the bowel wall segment normalized to the segment length) was derived in both conventional color flow imaging (CFI) and UMI to quantitatively stage disease activity. Receiver operating characteristic curves were then analyzed between different disease groups.

Results—The VLR-CFI and VLR-UMI detected similar correlations between vascularization and disease activity: severe inflammation had a higher VLR than normal/mildly inflamed bowels (P < .05). No significant difference was found between quiescent and mild CD due to the small sample size. The VLR-CFI had more difficulties in distinguishing quiescent versus mild CD compared to the VLR-UMI. After combining the VLR-UMI with thickness, in the receiver operating characteristic curve analysis, the areas under the curves (AUCs) improved to AUC1 = 0.996 for active versus quiescent CD, AUC2 = 0.978 for quiescent versus mild CD, and AUC3 = 0.931 for mild versus severe CD, respectively, compared to those using thickness alone (AUC1 = 0.968; P = .04; AUC2 = 0.919; P = .16; AUC3 = 0.857; P = .01).

Conclusions—Ultrasound microvessel imaging offers a safe and cost-effective tool for CD diagnosis and staging, which may potentially assist disease activity classification and therapy efficacy evaluation.

Key Words—Crohn disease staging; microvessel imaging; ultrafast ultrasound imaging

Crohn disease (CD) is a chronic inflammatory condition in the digestive tract that affects more than 1 million Americans, and its incidence is increasing. In more than 90% of cases, CD affects the ileum and colon. A clinically validated index, such as the

Received October 2, 2019, from the Departments of Radiology, Mayo Clinic, Rochester, Minnesota, USA (P.G., P.S., A.B.K., S.P.S., C.H., W.L., C.Z., U.W.L., S.T., J.M.K., S.C.); Department of Ultrasound, West China Hospital of Sichuan University, Chengdu, China (W.L., C.Z.); Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA (Y.Y.); and Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA (D.H.B.). Manuscript accepted for publication March 24, 2020.

This study was partially supported by the National Cancer Institute of the National Institutes of Health under award R00CA214523 and by the National Institute of Diabetes and Digestive and Kidney Diseases under award R01DK120559. Drs Gong and Song contributed equally to this work.

Address correspondence to Shigao Chen, PhD, Department of Radiology, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA.

E-mail: chen.shigao@mayo.edu

Abbreviations
AUC, area under the curve; CD, Crohn disease; CFI, color flow imaging; CTE, computed tomographic enterography; IBD, inflammatory bowel disease; MRI, magnetic resonance enterography; ROC, receiver operating characteristic; UMI, ultrasound microvessel imaging; US, ultrasound; VLR, vessel-length ratio

doi:10.1002/jum.15290

Crohn Disease Activity Index, is based on symptoms and hematocrit measurement. However, the Crohn Disease Activity Index shows a low correlation with biological activity. Imaging strategies that objectively evaluate disease activity with high accuracy and low costs might be beneficial for early disease detection, therapy efficacy evaluation, and the potential need for treatment modification.

Among CD cross-section imaging techniques, computed tomographic enterography (CTE) and magnetic resonance enterography (MRE) are considered the two techniques with the highest accuracy for CD detection. However, CTE imposes radiation exposure to patients, whereas MRE is expensive and not widely available. Bowel ultrasound (US) is an attractive alternative for a CD follow-up assessment because it is radiation free, cost-effective, and widely available. Features in B-mode US imaging that are related to CD include bowel wall thickening, loss of stratification of the normal bowel wall layers, reduced peristalsis, and reduced compressibility. Bowel wall thickness is a main parameter used for disease activity characterization. However, diagnostic accuracy was insufficient with the use of thickness alone. Other B-mode features listed above are qualitative/subjective and thus not ideal for clinical routine use.

Doppler US has been used for CD evaluations because inflammation is commonly associated with changes in blood flow. However, traditional Doppler US may have limited sensitivity to detect slow flow. Contrast-enhanced US has shown good promise for evaluation of CD. However, contrast-enhanced US parameters for CD assessment (such as the time-intensity curve) often depend on scanner gain settings and bolus doses, which limit the clinical adoption of contrast-enhanced US. The emergence of ultrafast US imaging offers new opportunities for enhanced Doppler sensitivities without using contrast microbubbles. We recently developed an ultrasensitive US microvessel imaging (UMI) technique, which provided advanced vessel sensitivity and superior tissue rejection. Ultrasound microvessel imaging substantially increases the number of echo frames available for Doppler imaging compared to conventional Doppler imaging to improve the detection of small vessels. An advanced clutter filter leverages both spatial and temporal information to robustly separate the blood signal from tissue clutter and background noise. The aim of this study was to investigate whether the microvessels detected in UMI may provide new possibilities for reliable CD activity assessment.

Materials and Methods

An Institutional Review Board–approved prospective study was conducted to investigate the feasibility of using UMI to evaluate inflammatory bowel disease (IBD) activity and severity (ie, normal, mild, and severe). Written informed consent was obtained at the time of enrollment of each participant.

Between October 2015 and January 2018, 48 patients with CD symptoms were enrolled. Seventy-six bowel wall segments were studied from the 48 patients. Patients fasted for at least 6 hours before US imaging. All patients underwent clinically indicated MRE or CTE. Readings from the MRE/CTE images, given by an experienced radiologist, were used as reference standards. The radiologist graded inflammation activities into normal, mild, and severe based on bowel wall thickening and other abnormal signs, such as fibrofatty proliferation or stenosis with prestenotic dilatation.

Bowel US acquisitions were conducted by 1 of 3 experienced sonographers, depending on their availability. The bowel US scan and CTE/MRE were performed within 15 to 30 days of each other. In our study protocol, conventional gray-scale B-mode US and conventional color Doppler examinations were first conducted with a LOGIQ E9 system equipped with a 9 L-D linear array transducer (2–8 MHz; GE Healthcare, Wauwatosa, WI). Conventional US images were acquired from suspicious bowel segments, which were determined by the sonographers during scanning with signs of active inflammation such as wall thickening, increased echogenicity, and increased vascularity. The locations of examined bowel segments were documented by the sonographer during US scanning. Then the radiologist used the US-documented location to look for the best-matched bowel segments on CTE/MRE for IBD activity classification. The bowel wall thickness was also measured with conventional B-mode US. Afterward, UMI was performed on the same view of the studied bowel segment as determined by conventional US, with 3 repeated acquisitions. Ultrasound microvessel imaging was implemented on a Vantage System (Verasonics Inc, Kirkland, WA) equipped with the same linear array 9 L-D transducer.
Instead of the line-by-line scanning typically used in conventional color flow Doppler imaging, UMI is based on ultrafast plane wave imaging (Figure 1A). In a UMI acquisition, 500 ultrafast B-mode frames were accumulated at a frame rate of 500 Hz to generate a single UMI image. This led to greater than 10 times more US frames for blood flow detection than conventional Doppler imaging (which typically uses only 16 B-mode frames). In addition, conventional Doppler imaging typically uses a temporal domain wall filter to reject tissue clutter: echoes with low movement speed are assumed to be tissues and rejected. Therefore, microvessels containing blood flows with a speed lower than the tissue rejection threshold are filtered out and cannot be detected. In contrast, UMI used an advanced clutter-filtering technique that leveraged both spatial and temporal information from the 500 acquired US frames to separate tissue and blood: tissue signals were assumed to be spatially coherent and could be effectively separated from the incoherent microvessel signals with low flow velocities (Figure 1B). The overall enhanced performance allowed imaging bowel vascularity in greater detail. Figure 1C shows an example of a UMI image overlaid on a B-mode image of a neoterminal ileum segment with severe inflammation. A conventional Doppler image is also shown as a reference (Figure 1D). The high Doppler sensitivity offered by UMI reveals bowel wall microvessels that are invisible on conventional Doppler imaging.

The vascularity of each bowel segment in the UMI images was reviewed by an experienced radiologist who was blinded to MRE or CTE readings. The radiologist first adjusted the vessel threshold of UMI to define an optimized threshold, which allowed maximal power Doppler signals immediately above the background noise. Figure 2 shows an example of the same UMI image displayed with 3 different thresholds, adjusted by a control slider. An appropriate vessel threshold

Figure 1. A, Ultrafast plane wave images from a neoterminal ileum segment with severe inflammation. B, Advanced tissue clutter filtering in UMI. C, Ultrasound microvessel image of the bowel segment. D, Conventional Doppler image of the same bowel segment.

Figure 2. The same UMI image displayed with 3 different vessel thresholds: A, −20 dB; B, −30 dB; and C, −40 dB. Regions inside the white dashed lines indicate the studied bowel segment. The blue double arrow in B indicates the length of the bowel segment.
should be selected to best separate the blood signal from tissue or background noise, as shown in Figure 2B (ie, −30 dB). Then image pixels with signal power above the threshold were considered vessels, whereas image pixels with signal power below the threshold were considered noise or tissue. Aggressive thresholding, as in Figure 2A, leads to underestimation of blood signals, whereas background noise arises with a low threshold, as labeled in Figure 2C.

After an optimized UMI threshold was determined, a quantitative parameter, vessel-length ratio (VLR), was derived to characterize disease activity. The VLR is defined as the total number of microvessel pixels throughout the studied bowel segment (such as the region indicated by the white dashed box in Figure 2B) divided by the length of the bowel segment. The bowel segment length in UMI (as indicated by the blue double arrow in Figure 2B) was determined as the length of the suspicious bowel segment based on the acquired conventional B-mode and CFI images. Then an averaged VLR was calculated for each bowel segment from the 3 repeated UMI measurements. Note that vessel density (ie, total number of vessel pixels divided by the area of the bowel segment) is a commonly used metric to assess tumor angiogenesis in the literature. However, that parameter was not applied in this study because the vessels are mainly distributed on the inflamed bowel walls rather than the intestinal lumen. It is not rigorous to include the intestinal lumen area during vessel density quantification. The VLR values of each bowel segment acquired from conventional color flow imaging (CFI) using the GE LOGIQ E9 system were also calculated for comparison to those obtained from UMI.

Statistical analyses were performed with MedCalc version 18.6 software (MedCalc, Mariakerke, Belgium) and R version 3.5.0 software (R Foundation for Statistical Computing, Vienna, Austria). The VLR values acquired from both CFI and UMI were correlated with disease severity readings from MRE/CTE by the Spearman correlation test, with a significance level of 5%. The VLR values (from both CFI and UMI) were also compared between different disease severity groups (ie, normal, mild, and severe) by a 2-tailed t test, with a significance level of 5%. A logistic regression model was applied to the bowel wall thickness measured by conventional B-mode US and VLR measured by CFI or UMI to analyze receiver operating characteristic (ROC) curves in comparison with the ROC curves obtained with the thickness alone. The areas under the curves (AUCs) were then calculated and compared by a 1-tailed t test, with a significance level of 5%.

The vessel cutoff threshold for UMI needs to be subjectively determined, which may slightly alter UMI performance in characterizing disease activity between different readers. Therefore, the interobserver variation of the method was undertaken: a second radiologist (blinded to CTE/MRE results) independently reviewed the UMI images. The resultant VLR values were then correlated with those from the first radiologist by the Pearson correlation.

### Table 1. Patient Demographics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19 (40)</td>
<td>40</td>
<td>18–63</td>
</tr>
<tr>
<td>Female</td>
<td>29 (60)</td>
<td>34</td>
<td>14–59</td>
</tr>
<tr>
<td>Reference standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTE</td>
<td>17 (35)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MRE</td>
<td>31 (65)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease Activity</th>
<th>Normal</th>
<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal ileum</td>
<td>30 (39)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Ileum</td>
<td>5 (7)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Colon</td>
<td>36 (47)</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Other</td>
<td>5 (7)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>All segments</td>
<td>76</td>
<td>21 (27)</td>
<td>9 (12)</td>
</tr>
</tbody>
</table>

Data are presented as number (percent) where applicable.
Results

The 48 patients with CD symptoms included 19 male patients (mean age, 40 years; range, 18–63 years) and 29 female patients (mean age, 34 years; range, 14–59 years). Computed tomographic enterographic or MRE readings of bowel segments were used as the reference standards, which revealed 21 bowel segments with no inflammation, 9 bowel segments with mild inflammation, and 46 bowel segments with severe inflammation, as listed in Table 1.

Figure 3 shows 3 representative UMI images, indicating the association between blood flow growth and increased IBD activities (ie, normal, mild, and severe). The bowel segment with no inflammation was relatively avascular or hypovascular. Spots of vascularity were observed in the mildly inflamed bowel segment, whereas the severely inflamed bowel segment contained rich vascularity with irregular branches and chaotic distributions.

The VLR was regarded as a quantification metric to evaluate the performance of vessel detection sensitivity between conventional CFI and UMI (ie, a higher VLR indicates better detection sensitivity). Ultrasound microvessel imaging provided significantly improved vessel detection sensitivity compared to CFI by a 2-tailed Wilcoxon rank sum test ($P < .05$).

Figure 4, A–C, shows the bowel wall thickness and VLR distributions in both CFI and UMI for different disease activity groups: normal, mild, and severe. Table 2 shows the mean and standard deviation values of the thickness, VLR-CFI, and VLR-UMI in

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**Figure 3.** Representative UMI images. **A**, Terminal ileum segment with no inflammation. **B**, Right lower quadrant segment with mild inflammation. **C**, Terminal ileum segment with severe inflammation.

**Figure 4.** Box plots showing the distributions of **A**, bowel wall thickness; **B**, VLR-CFI; and **C**, VLR-UMI for different disease activity groups (ie, normal, mild, and severe). *A statistically significant difference was detected by a 2-tailed $t$-test ($P < .05$).
each group. Strong and significant correlations were detected in both the VLR-CFI versus disease activities and in the VLR-UMI versus disease activities. The Spearman correlation coefficient between the VLR-UMI and disease activities was $r = 0.708$ (95% confidence interval, 0.574–0.805; $P < .05$), which was similar to that between the VLR-CFI and disease activities ($r = 0.697$; 95% confidence interval, 0.574–0.805; $P < .05$). For the VLR-UMI, greater vascularization was detected between bowel segments with mild inflammation versus severe inflammation and between no inflammation versus severe inflammation (2-tailed $t$ test, $P < .05$). Similar performance was also found in the VLR-CFI. However, the VLR-CFI tended to have greater difficulties in differentiating bowel segments with no inflammation from mild inflammation (2-tailed $t$ test, $P = .87$) compared to the VLR-UMI (2-tailed $t$ test, $P = .18$).

Receiver operating characteristic curves were then analyzed under 5 conditions: (1) using the VLR-CFI alone; (2) using the VLR-UMI alone; (3) using bowel wall thickness alone; (4) combining thickness with the VLR-CFI; and (5) combining thickness with the VLR-UMI via a logistic regression model. In general, the VLR-CFI and VLR-UMI provided comparable AUC performances: no significant differences were found between the VLR-CFI and VLR-UMI among all disease

**Table 2. Mean and Standard Deviation Values of Thickness, VLR-CRI, and VLR-UMI**

<table>
<thead>
<tr>
<th>Disease Activity</th>
<th>Thickness, mm</th>
<th>VLR-CFI</th>
<th>VLR-UMI</th>
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</thead>
<tbody>
<tr>
<td>Normal (n = 21)</td>
<td>2.93 ± 0.06</td>
<td>0.17 ± 0.21</td>
<td>2.12 ± 1.52</td>
</tr>
<tr>
<td>Mild (n = 9)</td>
<td>4.61 ± 1.45</td>
<td>0.19 ± 0.28</td>
<td>3.00 ± 1.87</td>
</tr>
<tr>
<td>Severe (n = 46)</td>
<td>7.08 ± 2.07</td>
<td>1.45 ± 1.10</td>
<td>6.59 ± 3.18</td>
</tr>
</tbody>
</table>

**Figure 5.** Receiver operating characteristic curve analysis between different disease activity groups: **A**, normal versus disease; **B**, normal versus mild; and **C**, mild versus severe.

**Figure 6.** Pearson correlation ($r = 0.90$) of the VLR between 2 readers who independently reviewed the UMI images.
activity groups. In addition, using the VLR-CFI or VLR-UMI alone provided worse AUC values compared to those obtained after combining with thickness, especially for the normal-versus-mild CD case (Figure 5B). Therefore, vessel information may not be suitable as a standalone biomarker for characterizing disease activities. For characterizing normal versus diseased bowel segments (Figure 5A), the AUC was 0.968 when using thickness alone. The AUC improved to 0.977 after combining thickness with the VLR-CFI but with no significance detected ($P = .20$). In contrast, significant AUC improvement was achieved after combining thickness with the VLR-UMI ($AUC = 0.996; P = .04$). Figure 5B shows the ROC curves for differentiating normal and mildly inflamed bowel segments. The AUC improved from 0.919 (thickness alone) to 0.950 (thickness + VLR-CFI) and 0.978 (thickness + VLR-UMI). However, due to the small sample sizes of quiescent and mild CD (29 cases in total), no significance was found in either AUC. Among inflamed cases (Figure 5C), the addition of vessel information from both CFI ($AUC = 0.940$) and UMI ($AUC = 0.931$) provided significantly improved performance in differentiating mild and severe CD as compared to using thickness alone ($AUC = 0.857$).

Figure 6 shows the Pearson correlation of VLR values given by 2 readers who independently reviewed the UMI images and were both blinded to CTE/MRE results. The Pearson correlation coefficient ($r = 0.90$) showed that reader-induced variability should be small, and comparable diagnostic performance of UMI can be expected from different examiners.

Discussion

This study investigated the feasibility of combining UMI and conventional US to facilitate a more accurate CD diagnosis. Doppler US has been used for CD evaluation because inflammation usually is associated with increase of blood flow. Semiquantitative methods, such as the Limberg score, have also been used to analyze the correlation between vascularity and bowel inflammation. In this study, a quantitative parameter, VLR, was derived to analyze bowel wall vascularity. Compared to conventional CFI, UMI provided enhanced vessel detection sensitivity (ie, higher VLR) in all disease activity groups. Note that slight acquisition view mismatches existed between CFI and UMI. However, such differences may lead to a minor impact on the VLR comparison between CFI and UMI. The improved sensitivity in UMI was mainly attributed to more image frames and advanced clutter-filtering methods used for Doppler image formation. Both CFI and UMI detected significantly increased blood flows in severe CD compared to those in quiescent or mild CD (Figure 3). However, considerable overlaps existed between the VLR calculated in quiescent and mild CD using both CFI and UMI. In addition, CFI tended to have more difficulties in differentiating quiescent and mild CD compared to UMI, as shown by the 2 tailed $t$ test. One possible reason is that mildly inflamed bowels mainly contain small and slow flows (ie, microvessels), which could not be detected by CFI because of its low sensitivity. In contrast, the improved sensitivity of UMI allowed better detection of microvessels from tissue and background noise. However, because of the limited sample size (only 9 cases of mild CD), no significant difference was found between bowel segments with no inflammation and those with mild inflammation on UMI. A larger sample size would be beneficial in future studies to explore the additional clinical values of UMI. Another reason for the considerable VLR-UMI overlap between quiescent and mild CD may have been caused by an inappropriate vessel cutoff threshold selection. The threshold determination was a subjective process, which was largely affected by the background noise (Figure 2). Thresholding with only the power signal amplitude may not be sufficient to accurately distinguish vessels from noise. More advanced signal-processing methods (such as using both the signal power and phase) will be considered in the future. In addition, better transmission design with improved penetration or noise suppression technologies can be applied in the future to further improve the performance of UMI. With regard to ROC curves, the combination of the VLR-UMI and bowel wall thickness offered significantly improved AUC values compared to using thickness alone in the differentiation of active versus quiescent CD and mild versus severe CD. Considering that the symptoms of CD are often nonspecific and show a poor correlation with disease activity, the AUC improvement demonstrates the potential of combining the vascularity and bowel wall thickness to evaluate bowel disease activity. In future studies, more vessel features in UMI (such as vessel diameter) can be explored for CD diagnosis and treatment management.

Crohn disease characterization with UMI had small interobserver variation ($r = 0.9; $Figure 6) so
that comparable diagnostic performance can be expected from different readers. Small discrepancies between readers occurred for bowel segments with higher vascularity. This may have been because the readers had no previous experience with UMI and did not properly determine the UMI threshold for bowel segments with higher vascularity. Ultrasound microvessel imaging is a newly developed technology, and to our knowledge, this is the first study applying UMI in bowel inflammation characterization. Due to the limited sample size, we did not provide additional UMI images to train the readers before they reviewed the images. This was a limitation of our study. Better inter-reader agreement may be reached in the future with more training. In addition, we acknowledge that the process of region of interest and intestine length selection in UMI was subjective, which may have affected the VLR calculation. It may also have limited the reproducibility and reliability of IBD activity staging using UMI. More rigorous quantification metrics needs to be investigated in future studies. An inter-reader comparison should also be conducted in the future to explore the effect of intestine length selection on VLR quantification.

Another limitation was that the results lacked a comparison with pathologic results. Readings from CTE and MRE were used as reference standards because they are considered the cross-sectional imaging techniques with the highest accuracy for CD detection. However, the readings may be reader dependent, and variations may exist between the readings from CTE and MRE. Future studies with pathologic results serving as reference standards may provide a more rigorous evaluation of UMI.

Ultrasound microvessel imaging offers high detection sensitivity of power Doppler signals from microvessels. It would be more relevant to compare UMI to conventional power Doppler imaging rather than color Doppler imaging. However, in this study, conventional US scans were performed as part of clinical care before the UMI acquisitions. Therefore, the sonographers followed clinical protocols, in which color Doppler images rather than power Doppler images were acquired. As a result, power Doppler images were not available for this study. We acknowledge that this was a limitation of the study, and a straight head-to-head comparison between UMI and conventional power Doppler imaging should be performed in the future.

In summary, UMI provided improved vessel detection sensitivity compared to conventional Doppler imaging. The combined use of vascularity in UMI and wall thickness in conventional US offered improved diagnostic accuracy compared to using thickness alone, demonstrating the potential of UMI in CD detection and staging. The microvessel information provided by UMI may be valuable to clinicians for assessing disease activity and evaluating therapy responses.

References


