Effectiveness of contrast-enhanced ultrasound for characterisation of intestinal inflammation in Crohn's disease: A comparison with surgical histopathology analysis

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Abstract

Background: Differentiation between predominantly inflammatory versus fibrous-predominant lesions is particularly important in order to decide the optimal therapy in patients with refractory symptoms in Crohn’s disease (CD).

Objective: The purpose of this investigation was to evaluate the accuracy of several US parameters, especially of contrast-enhanced US, for evaluation of mural inflammation in CD, with histopathology as the reference.

Materials and methods: Preoperative ultrasound examination, including contrast-enhanced ultrasound (CEUS) was performed in 25 consecutive patients with Crohn’s disease undergoing elective bowel resection. Ultrasound variables, such as wall thickness, transmural complications, colour Doppler grade, quantitative analysis of the enhancement and the presence and severity of strictures, were prospectively evaluated and compared with the histopathology results. Histopathology grading of acute inflammation using the acute inflammatory score and the degree of fibrostenosis was performed in each segment and the results were compared with all the US variables as well as with a previously defined ultrasound score system for inflammatory and fibrostenotic changes.

Results: 28 segments were analysed. In pathology analysis there were 12 predominantly inflammatory segments, 9 predominantly fibrostenotic and 7 compound lesions. When the pathology score was dichotomised into two groups (inflammatory and fibrostenotic) the number of stenoses correctly classified by US was 23 out of 28, with a substantial agreement (kappa=0.632). There was a good correlation between the sonographic and pathology scores, both inflammation (Spearman’s, r=0.53) and fibrostenosis (Spearman’s, r=0.50). Transmural complications, colour Doppler grade and percentage of increase in contrast enhancement were significantly associated

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1. Introduction

Crohn’s disease (CD) is a chronic inflammatory disease characterised by episodes of inflammation alternating with periods of remission. Therefore, it requires periodic assessment of the inflammatory activity and possible transmural complications in order to plan a proper treatment. However, in CD there is a known discrepancy between characteristics of the lesions and disease course.

In patients with CD, the inflammatory processes active over the course of the disease leads to fibrotic scarring transformation of the intestinal wall. Stenosis occurs in 12–54% of CD patients. Treatment options are based on the differentiation between inflammatory versus fibrous-predominant lesions. Patients with inflammatory lesions can potentially be managed with conservative medical treatment, while patients with fibrous-predominant lesions, especially if associated with obstructive symptoms, may need endoscopic balloon dilatation or surgery.

The anatomic location and length of the affected intestinal segments can be precisely determined with imaging methods such as ultrasonography, CT-enterography or MRI-enterography. A good correlation between all these imaging techniques and the endoscopic severity has been previously demonstrated, but endoscopy can only provide information about the intestinal mucosa, and exclusively in the bowel segments that are accessible by terminal ileoscopy. The transmural extent of the disease has to be evaluated in surgical specimens, because many other factors, such as submucosal oedema, cellular infiltration and fibrosis play a role in the aetiology of the stenosis.

High-resolution bowel ultrasound has emerged as an alternative imaging technique for the diagnosis and follow-up of patients with CD, being as accurate as CT and MR for detecting intramural and extramural extension of the disease.

US has advantages over CT, being free of ionising radiation and non-invasive, and over MRI because it is well tolerated and easily repeatable in follow-ups.

Contrast-enhanced ultrasound (CEUS) is a new technique that involves intravenous administration of an ultrasound contrast agent with real-time examination, providing an accurate depiction of the bowel wall microvasculature and the perienteric tissues. The introduction of imaging quantification techniques enables an objective quantitative measurement of the enhancement.

Several studies have shown that US can play a role in the assessment of CD activity. Previous studies have shown good correlation between several ultrasound findings — for example, mural thickness, colour Doppler grade or contrast enhancement — and clinical or biological scores or with endoscopic findings.

The purpose of this study was to evaluate the effectiveness of several US parameters, especially contrast-enhanced US, for the characterisation of parietal inflammation in intestinal segments with CD, with histopathology as the reference method.

2. Materials and methods

2.1. Patients

Consecutive patients who met the inclusion criteria between October 2007 and March 2010 were included in this prospective study.

Inclusion criteria were: a) patients with endoscopic and histologically confirmed Crohn’s disease with elective surgery for small bowel or colon CD. The local CD Committee, made up of gastroenterologists, surgeons, and radiologists, indicated elective surgery when medical treatment had failed. b) Ultrasound examination, including colour Doppler and CEUS, within a 60 day period before surgery.

The local Ethics Committee of our hospital approved the study protocol.

2.2. Ultrasonographic examination

US examinations were performed in each patient after the decision to operate. Real-time ultrasound was performed using a Toshiba Aplio 80 (Toshiba, Tokyo, Japan) initially employing a 3–6 MHz convex-array transducer and then a 6–10 MHz probe for a detailed examination.

Each patient underwent abdominal US specifically for the intestine, beginning with an initial grey-scale. Bowel wall vascularity by colour Doppler US with a special preset optimised for slow flow detection was then evaluated. The intensity of the colour Doppler flow was subjectively graded as absent (grade 0), barely-visible vascularity (grade 1), moderate vascularity (grade 2) and marked vascularity (grade 3).

2.3. Contrast-enhanced ultrasound (CEUS)

Patients were examined with a 3–4 MHz convex probe in wideband harmonic contrast mode (pulse inversion-Toshiba Aplio) at low MI (MI<0.10). The second generation echosignal enhancer SonoVue® (Bracco, Milan, Italy) was injected as a bolus in units of 1.2 ml through a three-way 20-gauge catheter into an antecubital vein, followed immediately by injection of 10 ml of normal saline solution (0.9% NaCl).

To assess the vascularity of the involved bowel loop, the contrast uptake was measured over a period of 40 s by

with the pathology inflammatory score ($p=0.018$, $p=0.036$ and $p=0.005$, respectively). There was a significantly negative association between the colour Doppler grade and the pathologic fibrostenotic score.

Conclusions: Ultrasound, including CEUS, can be a useful tool for distinguishing inflammatory from fibrostenotic lesions in CD. This information can be useful in the management of CD.

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means of quantitative analysis of the brightness in regions of interest (ROI) located in the intestinal wall using the software installed in the Aplio 80 system. The software automatically obtains a brightness-time curve (Fig. 1).

Quantitative measurement of contrast enhancement was assessed as the difference between the baseline brightness before contrast injection and the maximal post-contrast or "peak" brightness value. This was considered to be the absolute change of vascularity and was interpreted as the vascularity of the small bowel wall. However, since the absolute change can vary according to the machine type, gain settings and examiner, we calculated the percentage of increase in wall brightness by using the following formula: [(brightness post-contrast – brightness pre-contrast) × 100] / brightness pre-contrast, and this was used for data analysis. We also evaluated the “time-to-peak” in each patient, a parameter which represents the time elapsed between the injection of the contrast agent to its maximum peak.

2.4. Image evaluation

Ultrasound variables, such as wall thickness, parietal stratification, transmural complications (fistula or abscesses), colour Doppler grade, quantitative analysis of the enhancement and the presence, length and severity of strictures, were prospectively evaluated and reported on a standardised form at the end of the US examination. The variables analysed are displayed in Table 1. The threshold brightness values of percentage of contrast enhancement were chosen on the basis of our results in the ROC curve and values previously published.17

An ultrasound score of the maximum severity of inflammation (between 0 and 3) or fibrostenosis (between 0 and 2) of the each stenotic bowel segment based on an adaptation of the CT score developed by Chiorean et al.18 was retrospectively assessed on the basis of the presence and severity of ultrasound abnormalities shown in Table 2. The lesions were classified as; predominantly fibrostenotic if the fibrostenotic score was 2 or if there was ≥ 1 difference from the inflammatory score, predominantly inflammatory if the inflammatory score was 3 or there was ≥ 1 on the fibrostenotic score, and compound lesions when the fibrostenotic and the inflammatory score were similar.

2.5. Histopathology analysis

Within 2 h after surgical resection we perform a sonographic exam of the fresh specimen (Figs. 1c and 2). This exam was used to locate anatomic landmarks such as the ileocecal valve and identify any abnormal finding, as stenosis, fistulae or abscesses, that were visible on the specimen ultrasound exam. Using the annotated ultrasound images and the macroscopic

Figure 1  US images in 43-year old woman with severe ileal stenosis. a) Longitudinal US scan of the intestinal thickened segment before contrast agent. b) Measurement of bowel wall vascularity, after second generation contrast agent injection, in a manually defined ROI, obtaining the brightness-time curves over a period of 40 s. In this case, absolute and percentage of increase of enhancement were 64 and 93%, respectively (baseline value 69, maximum value 133). c) Longitudinal US compound scan of the surgical specimen. d) Photograph of the divided gross specimen confirms the presence of mural thickening of a long ileal segment with congested mucosa, eroded appearance and abundant linear ulcers that tend to converge over the entire surface. This lesion was scored as sonographic inflammation (3/3) and fibrostenosis (0/2). Pathology score was inflammation (8/13) and fibrostenosis (1/3).
analysis of the specimen by the pathologist, the radiologist and pathologist reached a consensus regarding the location of the sample. Histological slices were obtained at the identified locations for subsequent analysis.

Acute inflammation was assessed by the pathologist in each slice according to the Borley et al. method. This acute inflammatory score assigns up to a maximum of 13 on the basis of grades of mucosal inflammation, oedema and quantity and depth of neutrophilic infiltration (between 0 and 13). Fibrostenosis was graded using a modification of the Chiorean et al. method. This scoring system is based on the evaluation of inflammatory and fibrostenotic features in the intestinal segments examined.

<table>
<thead>
<tr>
<th>Inflammation (score)</th>
<th>Absent (0)</th>
<th>Mild (1)</th>
<th>Moderate (2)</th>
<th>Severe (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No findings</td>
<td>1 or 2 positive findings, any severe features</td>
<td>≥2 positive findings, any severe</td>
<td>≥3 positive findings, at least one severe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrostenosis (score)</th>
<th>Absent (0)</th>
<th>Mild (1)</th>
<th>Severe (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No findings</td>
<td>1 or 2 positive findings, any severe features</td>
<td>≥2 positive findings, at least one severe or proximal dilation</td>
</tr>
</tbody>
</table>

Findings are described in Table 1.

2.6. Statistical analysis

The correlation between the ultrasound findings and pathologic variables (inflammation and fibrostenosis according to the score system previously defined) was analysed using the Mantel–Haenszel $\chi^2$ test and Spearman rank correlation coefficient. The correlation between CD pathological inflammation score and fibrosis score were estimated using Spearman rank correlation coefficient.

The agreement between ultrasound and pathology scores was calculated by using the Cohen's $k$ coefficient. A $k$ value of 0–0.20 indicates slight agreement; 0.21–0.40 fair agreement; 0.41–0.60 moderate agreement; 0.61–0.80 substantial agreement; and 0.81–1 almost perfect agreement.

The accuracy of the how the percentage of increase in wall brightness discriminated between inflammation and non-inflammation was evaluated by using the area under the receiver operating characteristic (ROC) curve. A ROC curve was constructed to determine the best US enhancement cutoff value for predicting inflammatory activity. The best cutoff value was determined while balancing the best sensitivity with the lowest false-positive rate. Sensitivity, specificity, positive predictive value and negative predictive value were evaluated, with 95% confidence intervals by means of the best cutoff value of the increase in wall brightness.

The Statistical Package for the Social Sciences (SPSS) version 15.0.1. for Windows (SPSS, Chicago, Ill) was used to describe and analyse the data, considering values of $p<0.05$ as significant.

3. Results

Over a 30-month period, 25 consecutive patients coming from a single centre satisfied the inclusion criteria; median age: 37.3 years (range: 21–64). The main demographic characteristics of the 25 patients included in the study are shown in Table 3. Five patients (18%) had previous Crohn’s-related surgeries. In the pathologic analysis there were 28 affected bowel segments, one segment in 22 patients and two segments in 3 patients (ileum and colon). Indications for surgery were: 15 bowel obstruction, 7 refractory nonobstructive disease and 3 perforating disease. The median time interval between ultrasound and surgery was 34.5 ±17.3 days (range: 10–59), 40% less of a month. In many cases, surgery was delayed mainly due to the hospital waiting lists. Patient treatments were not modified during the period elapsed between the US assessment and surgery.

The pathological inflammatory score was inversely correlated with the fibrosis score ($r=-0.537, p=0.03$). Only seven out of 28 segments had both a high grade of inflammation (≥5) and fibrosis (>1).
Acute inflammatory scores of the evaluated bowel segments by the pathologist ranged from 1 to 11, with a score of 5.68±2.9 (mean±SD). Nineteen segments had an inflammatory score ≥5. The associations between individual ultrasound variables and the pathological inflammation and fibrostenosis scores are shown in Table 4. There was a significant association between transmural complications, colour Doppler grade or percentage of contrast enhancement and pathologic inflammation score (Mantel–Haenszel $\chi^2$ test $p$ values 0.018, 0.036 and 0.005, respectively). The fibrostenosis score for the evaluated bowel segments ranged from 0 to 3, with a score of 1.5±0.69 (mean±SD). Fifteen segments had a fibrostenosis score ≥1. The colour Doppler grade had a significant negative association with the fibrostenotic score (Mantel–Haenszel $\chi^2$ test $p$ value 0.002). There was an association trend between the presence of stenosis, prestenotic dilatation or "time-to-peak" and fibrostenotic score ($p$ values of 0.071, 0.056 and 0.068, respectively).

The Spearman rank correlation coefficients with significant associations between ultrasound variables and the pathology scores were: correlation between transmural complications and inflammatory score ($r=0.464$, $p=0.01$), and between...
segments into only two groups: inflammatory or fibrostenotic, with 15 (54%) inflammatory lesions and 13 (46%) fibrostenosis.

The bowel segments were classified by ultrasound as predominantly inflammatory lesions in 15 cases (54%), predominantly fibrotic in 6 (21%) and compound in 7 (25%). The associations and correlations between ultrasound and pathology scores are shown in Table 5. When the stenoses were classified into three groups (inflammatory, compound and fibrostenotic) the number of stenoses correctly classified by US were 19 out of 28 and the agreement between US and pathology scores was moderate (kappa = 0.497). When the pathology score was dichotomised into two groups (inflammatory or fibrostenotic) the number of stenoses correctly classified were 23 out of 28 bowel segments, with a substantial agreement between US and pathology scores (kappa = 0.632). All the 15 bowel segments diagnosed as inflammatory in the pathology score had an inflammatory ultrasound score. However, 5 out of 13 bowel segments diagnosed as fibrostenotic by the pathologic analysis were considered inflammatory by the ultrasound score (Fig. 3) (Table 6). 4 out of these 5 sonographically misinterpreted segments categorised as fibrostenotic in the pathological analysis also had a high inflammatory score (>5). Using the ultrasound scores, we obtained a sensitivity, specificity and accuracy for detecting inflammation of 100%, 61.5% and 82.1%, respectively, with a PPV and NPV of 75% and 100%, respectively. When the inflammatory histological score was dichotomised as none/mild versus moderate/severe, the accuracy of ultrasound reached 82.1% (95% CI 64.4–92.1), with 81.8% of sensitivity, 83.3% of specificity, 94.7% of PPV and 55.6% of NPV.

The percentage of increase in contrast enhancement of the bowel wall in patients with inflammatory lesions (82.07 ± 17.36) was significantly greater in comparison with that of patients with fibrotic lesions (63.08 ± 28.01) (p = 0.03). The ROC curve for performance of percentage of increase in wall brightness as a predictor of inflammation showed a mean area under the ROC curve of 0.844 ± 0.086 (range, 0.675–1.012) (p = 0.002). We extracted from the ROC curves of the contrast enhancement measurements the, optimal cutoff value for predicting inflammatory activity at the pathology score. With the use of a threshold value of 65% for the percentage of enhancement increase, contrast-enhanced US had a sensitivity of 93% (95% CI 66–99), specificity of 69% (95% CI 39–90) and accuracy of 82% (95% CI 62–93)
for differentiating between inflammatory and fibrostenotic bowel lesions, with PPV of 78% and NPV of 90%. Six out of 15 patients with grade 2 or 3 pathologic fibrosis had a percentage of enhancement increase ≥65. In 5 out of these 6 patients the inflammation score was ≥5. Mean "time-to-peak" was 21.8±4.6 s (range: 15–31), without significant differences between inflammatory and fibrostenotic bowel lesions.

The elapsed time since diagnosis of CD to surgery was 77.4±65.4 months (range 1–222 months). The correlation between disease chronicity (in months) and contrast enhancement was not significant (correlation coefficient, 0.13, p=0.51).

A total of 11 abdominal fistulae and 9 abscesses in 14 intestinal segments were found in the surgical or in the pathological specimen reports. All the transmural complications except one fistula were visualised on US. Eleven out of the 14 segments (78.5%) with transmural complications (fistulas or abscesses) had a high grade (≥5) of inflammation in the pathology score. Six out of the 14 segments (42.8%) also had a high grade (>1) of fibrostenosis in the pathology score. Among the 11 patients with abdominal fistulae, only 3 (27%) had sono-graphic signs of obstruction.

There was no significant correlation between treatment of patients (with anti-TNF versus other treatments) and the ultrasound or pathology score (χ² Fisher exact test, p values 0.41 and 0.705, respectively). There was no statistically significant difference in grade of inflammation or pathologic score between patients with and patients without treatment with steroids (p values 0.947 and 0.319, respectively). There was no statistically significant difference in the degree of inflammation or pathological score between the different location of the involved segments (ileum, colon or anastomosis) (p values 0.996 and 0.885, respectively). There was no significant relationship between the time interval from the ultrasound exams to surgery and the pathology score correlation for either inflammatory or fibrostenosis (Mann–Whitney test, p values 0.192 and 0.982, respectively).

### Table 6  Relationship between dichotomized pathology and sonographic score of the surgical specimens: 2×2 contingency table.

<table>
<thead>
<tr>
<th>Pathology score</th>
<th>Fibrostenosis</th>
<th>Inflammatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonographic score</td>
<td>Fibrostenosis</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Inflammatory</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>13 (46%)</td>
<td>15 (54%)</td>
</tr>
</tbody>
</table>

4. Discussion

Previous studies have shown that B-mode ultrasonography, colour Doppler and CEUS correlate with inflammatory activity based on endoscopic activity in CD. Vascularity within the...
diseased bowel wall assessed by colour Doppler detects only macrovascularisation and the hallmark of active CD is the new proliferation of microvessels. It has been shown that CEUS can detect small vessels: quantitative measurements of enhancement at CEUS or semi-quantitative evaluation have shown a good correlation with inflammatory activity as shown by endoscopy. Other techniques, like CT or MRI, have demonstrated that a greater degree of mural enhancement is seen when inflammatory activity increases. However, a histological analysis of intestinal specimens is necessary to evaluate CD activity because the inflammation and fibrosis in CD are often transmural, including fistulas or abscesses and deep neutrophilic infiltration, and therefore cannot be properly evaluated by endoscopy.

Identification of inflammation is important because it can potentially be managed using conservative medical therapy, while the presence of fibrosis requires surgical therapy. Our results show that several sonographic variables were significantly associated with the inflammatory activity shown at histology, including transmural complications, colour Doppler grade and quantitative measurements of enhancement at CEUS. In our study, patients with inflammatory lesions had a greater increase in contrast enhancement of the bowel wall in comparison with patients with fibrotic lesions. This finding is in keeping with previous CT or MRI-based reports in surgical specimens, as in our study. Zappa et al. showed a greater degree of wall enhancement on delayed T1-weighted sequences in patients with a moderate or severely-active CD inflammatory score than in patients with non-active score. Moreover, in our series using a value of 65% of percentage of enhancement increase, CEUS had a sensitivity of 93% and accuracy of 82% for differentiating between inflammatory and fibrostenotic bowel lesions.

US showed excellent accuracy for the detection of transmural complications (sensitivity and specificity of 95% and 100%, respectively), similar to the results of previous studies, confirming the usefulness of the US to detect fistulas and abscesses. Moreover, our results agree with those of previous studies confirming that the presence of fistulas or abscesses is significantly associated with histologic inflammation. There was probably a bias in our results because people included in the study represent a subgroup of patients that need surgery and therefore have more severe inflammatory bowel disease. On the other hand, high grade of fibrostenosis in our series was found in 42.8% of the segments with transmural complications, confirming that fistula or abscesses can develop over time transmural fibrosis.

Several authors have found that mural thickness increases with acute inflammation assessed in surgical specimens. However, no relationship was found in our study between wall thickness and acute inflammation. This is not surprising given that a thickened bowel wall can represent inflammation or fibrosis; furthermore, chronic fibrosis and acute inflammation may coexist. In fact, 25% of the bowel segments assessed in this study had high levels of inflammation and fibrosis.

An important aspect of our study was the ability to evaluate the accuracy of ultrasound imaging for fibrostenotic lesions. The severity of fibrosis cannot be evaluated with the endoscopic biopsies because biopsies are not deep enough to assess the pathologic changes that occur especially in the submucosa and muscularis propria. In our study, there was a strong negative correlation between colour Doppler grade and the pathological fibrostenosis score; the higher the fibrostenosis score, the lower the degree of colour Doppler. Other parameters, including wall thickness, prestenotic dilatation, length of stenosis, “time-to-peak” or contrast enhancement, showed no correlation with the scoring. Other authors have reported similar results regarding the wall thickness or contrast enhancement using CT or MRI.

To our knowledge, only one previous study has compared CEUS and surgical pathological findings. As in the study of Girlich et al. we found a negative correlation between the “time-to-peak” and the pathological fibrostenotic score, although without significant differences. This result suggests that the time-taken to reach peak enhancement is lengthened as the fibrosis increases. However, in contrast to our results, these authors could not find any relationship between percentage of contrast enhancement and pathological scoring. This discrepancy could be explained because their pathological score mixed chronic and acute inflammation.

In this study, we also used an ultrasound scoring system to evaluate the bowel segments with CD and we found that there were high correlations between ultrasound scores and pathologic inflammatory or fibrostenotic scores (Spearman’s r=0.69 and 0.68, respectively, p<0.0001). The ultrasound score detected inflammation in all the segments categorised as inflammatory in the pathological analysis (100% of sensitivity), while 5 of 13 with fibrosis were classified as inflammatory in the ultrasound score (specificity of 61.5%). Interestingly, 4 out of these 5 segments misclassified by ultrasound also had high-grade inflammation in the pathological analysis. In our opinion, this fact suggests that in cases where both components are present in the same intestinal segment, ultrasound detects the inflammation accurately (transmural complications, colour Doppler, contrast enhancement) but has difficulty identifying the fibrotic component, which is a possible limitation of the ultrasound examination.

Patients receiving tumour necrosis factor antibody (anti-TNF) treatment between imaging studies (MRI) and surgery were excluded in a previous study because it has been published that rapid anti-TNF-induced tissue healing may result in excess scar tissue leading to an increased risk of intestinal stenosis or obstructive events. However, data from the observational TREAT Registry and ACCENT I study have not confirmed this relationship. Moreover, no significant correlation was found in our study between treatment of patients (with anti-TNF versus other treatments) and the ultrasound or pathology score.

Limitations of our study include a small number of cases (n=25), though this number is reasonable coming from a single-centre. Second, probably there was a bias due to the inclusion criteria since people included in the study represent a group with more severe inflammatory bowel disease. Third, ultrasound scores were retrospectively evaluated. Although the heterogeneity of the tested CD population and the involved segments included in our study could have led to another bias, this fact did not seem to affect our results. Finally, we performed all the examinations with the same machine and so far no studies have analysed if quantitative measurements of the enhancement obtained with different commercial US equipments are comparable.

In conclusion, ultrasonography including CEUS is a promising tool for distinguishing inflammatory from fibrostenotic lesions in CD. Using a sonographic score system, it is possible
to differentiate both kinds of lesions with high sensitivity and accuracy. Transmural complications, colour Doppler grade and percentage of increase in contrast enhancement correlate with histologic inflammation markers, while colour Doppler grade is associated with pathological features of fibrostenosis. This information can be useful in the management of CD as well as to measure the effectiveness of newer therapies.

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References


