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## Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study with azathioprine and infliximab

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**Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study with azathioprine and infliximab**

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3 **Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study**  
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5 **with azathioprine and infliximab**  
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52 **Keywords:** Crohn's disease; mucosal healing; methotrexate; azathioprine; infliximab.  
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## Abstract

**Background:** Mucosal healing (MH) has become a new therapeutic goal in Crohn's disease (CD) and can be achieved with azathioprine (AZA) or biologics. Methotrexate (MTX) is an effective drug for both the induction and maintenance of remission in CD. However, MH with MTX has been poorly investigated.

**Aim:** to assess the MH rate in patients with CD with clinical response to MTX as compared to AZA or infliximab (IFX).

**Methods:** From October 2007 to May 2009, consecutive patients with CD were prospectively enrolled into a single centre study when they met the following criteria: previous identification of mucosal ulcerations with ileo-colonoscopy, clinical remission within at least three months with MTX, AZA or IFX monotherapy, usual indication for colonoscopy in CD (dysplasia/cancer screening, suspected stenosis) excluding assessment for MH. MH was defined as absence of mucosal ulceration in all segments.

**Results:** Fifty-one patients with CD (38 female; median age: 42 years) were included: 18 receiving MTX, 18 AZA, and 15 IFX. MH was achieved in 2/18 (11%) with MTX, in 9/18 (50%) with AZA ( $p = 0.011$  vs. MTX) and in 9/15 (60%) with IFX ( $p = 0.008$  vs. MTX).

**Conclusion:** In patients with CD in sustained clinical remission, MH is less frequently achieved with MTX as compared to AZA or IFX.

## Introduction:

The natural history of Crohn's disease is characterized by active and remitting periods<sup>1</sup>. For a long time, medical treatment was solely focused on clinical symptoms, aiming to control disease flare and prevent relapse. Healing the gut mucosa was not considered as a therapeutic goal because of the poor correlation between symptom improvement and mucosal healing and the inefficacy of conventional drugs such as steroids to cure endoscopic lesions<sup>2</sup>.

During the past decade, new data **have** highlighted the significance of mucosal healing in Crohn's disease. Firstly, the disappearance of endoscopic inflammatory lesions seems to be related with better long-term disease outcomes - less hospitalization and abdominal surgery<sup>3-5</sup>. Secondly, the absence of mucosal healing in asymptomatic patients is associated with higher relapse rate<sup>5</sup> as positively shown in two recent prospective studies including patients treated with infliximab and immunosuppressants<sup>6,7</sup>. Lastly, retrospective data suggested that Crohn's disease patients with severe endoscopic colonic lesions had increased rates of penetrating complications and colectomy<sup>8</sup>.

Biologics, and especially anti-TNFs – infliximab, adalimumab and certolizumab pegol have brought a major advance in the treatment of both luminal and fistulizing Crohn's disease<sup>9</sup>. They have demonstrated not only clinical **efficacy**, but also that mucosal healing was achievable and could be maintained in patients with active Crohn's disease<sup>3,10-12</sup>.

Therefore, mucosal healing has emerged as a new therapeutic goal and is now considered as an endpoint in clinical trials<sup>11, 12</sup>. It has been less studied with conventional immunosuppressants commonly used in Crohn's disease for many years. Few studies have shown the ability of azathioprine to induce mucosal healing,<sup>13, 14</sup> but recently, complete mucosal healing at week 26 was achieved in 15.6% of the 109 patients assessable in the azathioprine arm of the SONIC trial<sup>12</sup>. Data with methotrexate are more scarce. According to two small retrospective studies – 14 and 8 recruited patients, respectively – mucosal healing

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3 was obtained in 36-37.5% of patients responding to methotrexate <sup>15, 16</sup>. Therefore, the aim of  
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5 the present study was to assess the rate of mucosal healing with methotrexate in Crohn's  
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7 disease, as compared to azathioprine and infliximab.  
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## Patients & Methods

### *Selection of patients and study schedule*

This was a prospective single centre study conducted in the Gastroenterology department of the Haut-Leveque Hospital (south-western France) from October 2007 to May 2009.

Eligible Crohn's disease patients were consecutively recruited when they met the following criteria: age 18 years or more, previous diagnosis of luminal Crohn's disease established on usual criteria including identification of mucosal ulcerations with ileo-colonoscopy, clinical remission off steroids within the last three months with a single immunomodulator (methotrexate or azathioprine or infliximab) as maintenance treatment at stable doses, requiring a colonoscopy based on usual indication in Crohn's disease (dysplasia/cancer screening according to the recommendations from the French endoscopic society, suspected stenosis) excluding assessment for mucosal healing. Subjects with ulcerative colitis, indeterminate colitis, active luminal Crohn's disease (CDAI>150), isolated ano-perineal location or ileal disease not assessable with colonoscopy were excluded.

Inclusion date was defined as that of colonoscopy which was routinely performed by three experienced gastroenterologists (D.L., E.C., C.S.) not blinded to the medications taken by the patients. Data were collected at inclusion and the following characteristics were recorded: date of birth, gender, disease duration, age at diagnosis, disease location and behavior according to the Montreal classification<sup>17</sup>, perianal disease, extraintestinal manifestation, smoking history, previous history of intestinal surgery, previous response to steroids, clinical (CDAI) and biological (CRP level in mg/L) disease activity at baseline, past treatment with immunosuppressants and biologics, type of immunomodulator (methotrexate, azathioprine, infliximab) given as maintenance and duration of this treatment, indication for colonoscopic examination. Endoscopic and clinical data were available in all patients.

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3 Biopsy samples were taken in the most **inflamed** areas and for the screening of dysplasia.  
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5 Samples were fixed in formalin and embedded in paraffin, and 3µm thick sections were  
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7 stained with HES. All biopsy specimens were analyzed independently by one experienced  
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9 pathologist (G.B.) blinded to the treatment. The goal of this examination was to assess  
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11 microscopic activity of Crohn's disease according to the Scoring System for Crohn's disease  
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13 **constructed by Geboes and** adapted from D'Haens *et al*<sup>18</sup>. The most inflammatory lesions  
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15 observed within the most **inflamed** area at colonoscopy were retained **for scoring**.  
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20 During the follow-up period, from inclusion to December 2009, Crohn's disease clinical  
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22 relapse, defined as worsening of symptoms coupled with endoscopic, radiographic, and/or  
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24 biological evidence of inflammation, was recorded.  
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27 All patients received treatment according to clinical need. Drugs used were those normally  
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29 employed in Crohn's disease, according to licensed or published doses and frequency  
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31 (parenteral methotrexate: 15-25 mg/week; azathioprine: 2-3 mg/kg/d; infliximab: 5  
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33 mg/kg/8weeks). All patients received treatment and had colonoscopy only after full and  
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35 informed consent.  
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### 38 ***Outcomes and objectives***

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40 Mucosal healing was defined as the total disappearance of all mucosal ulcerations in all  
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42 segments<sup>11,12</sup>. Two indices of endoscopic activity, the Crohn's Disease Endoscopic Index of  
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44 Severity (CDEIS)<sup>19</sup> and the Simple Endoscopic Score for Crohn's Disease (SES-CD)<sup>20</sup>, were  
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46 calculated at inclusion. Considering the CDEIS, two cut-off values were retained: <6 as  
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48 endoscopic remission and <4 as complete mucosal healing<sup>10</sup>. Microscopic Crohn's disease  
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50 activity was assessed with the Scoring System for Crohn's disease adapted from D'Haens *et*  
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52 *al*, ranging from 1 (no activity) to 13 (table 1)<sup>18</sup>.  
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3 The primary objective of the study was to establish the proportion of mucosal healing in  
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5 Crohn's disease patients with sustained remission by monotherapy with methotrexate, as  
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7 compared to azathioprine or infliximab.  
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10 Secondary objectives were: i) to compare CDEIS and SES-CD in Crohn's patients in  
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12 remission receiving methotrexate to those treated with azathioprine or infliximab; ii) to  
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14 identify factors associated with mucosal healing; iii) to compare Crohn's disease microscopic  
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16 activity in patients in remission with methotrexate to those receiving azathioprine or  
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18 infliximab; iv) to look for an association between mucosal healing at baseline and relapse rate  
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20 during follow-up.  
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### 24 *Statistics*

25  
26 Continuous variables are presented as medians and range; categorical variables are presented  
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28 as percentages. Continuous data were analysed using Mann-Whitney's test. Categorical data  
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30 were analysed using the Pearson's chi-squared test, or Fisher's exact test if any cell number  
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32 was <5, for frequencies. Pearson's coefficient of correlation ( $\kappa$ ) and their probabilities (p)  
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34 evaluated the relationship between CDEIS, SES-CD and microscopic Crohn's disease  
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36 activity.  
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41 Analysis of predictive factors of mucosal healing was performed. Univariate and multivariate  
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43 analyses were performed to assess impact of clinical, disease, and treatment variables on  
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45 mucosal healing. Continuous variable were dichotomised according to the median. Variables  
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47 analysed were age at inclusion, gender, disease duration, active tobacco smoking, Crohn's  
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49 disease location and behaviour, associated perineal disease, extra-intestinal manifestations,  
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51 previous intestinal resection, current treatment type and duration, indication for colonoscopy,  
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53 CRP level (mg/L). Difference between mucosal healing and non-healing groups was assessed  
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55 by  $\chi^2$  or Fisher's exact tests when appropriate. A logistic regression model was created using  
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57 significantly associated variables (p<0.10), and the odds ratios (OR) for the variables that  
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3 remained significant ( $p < 0.05$ ) in the model determined. Considering complete mucosal  
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5 healing at baseline (CDEIS $<4$ ), relapse-free survival rates in relation to observation time were  
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8 estimated using Kaplan-Meier life-table method.  
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## Results

### *Study population*

During the study period, 1 796 consecutive colonoscopies were performed in the Gastroenterology department of the Haut-Leveque Hospital, including 266 in patients with Crohn's disease. Two-hundred-and-fifteen patients were excluded: 161 with active disease, 49 in remission not treated with a single immunomodulator at stable dose within the last three months, and 5 in whom colonoscopy aimed to assess mucosal healing. Therefore, 51 patients met the inclusion criteria (38 women; median age: 42 years): 18 receiving methotrexate, 18 azathioprine, and 15 infliximab. Their main characteristics are presented in Table 2. There was no difference between the methotrexate group and the two others except regarding median treatment duration that was shorter with methotrexate as compared to azathioprine (24 and 57 months, respectively,  $p=0.014$ ).

### *Mucosal healing*

The primary objective of the study – complete disappearance of mucosal ulcerations – was achieved in 2/18 (11%) patients treated with methotrexate, in 9/18 (50%) with azathioprine ( $p=0.011$  vs. methotrexate) and in 9/15 (60%) with infliximab ( $p=0.008$  vs. methotrexate) (Figure 1). Considering the CDEIS, patients receiving methotrexate had a higher median value than those from the two other groups (5.5, range [0.5-11.2] with methotrexate, 1.5, range [0-9.4] with azathioprine –  $p=0.002$  vs. methotrexate – and 1.2, range [0-23.6] with infliximab –  $p=0.03$  vs. methotrexate). Individual CDEIS values are presented in Figure 2A. According to the pre-specified CDEIS cut-off for endoscopic remission, rates of patients with  $CDEIS < 6$  were 50% with methotrexate, 89% with azathioprine ( $p=0.011$  vs. methotrexate) and 80% with infliximab ( $p=0.074$  vs. methotrexate). With the cut-off for complete mucosal healing ( $CDEIS < 4$ ), rates were 39% with methotrexate, 83% with azathioprine ( $p=0,006$  vs. methotrexate) and 67% with infliximab ( $p=0,112$  vs. methotrexate).

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3 The median SES-CD value was higher in patients receiving methotrexate as compared to the  
4 two other groups (6, range [2-18] with methotrexate, 3, range [0-16] with azathioprine –  
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The median SES-CD value was higher in patients receiving methotrexate as compared to the two other groups (6, range [2-18] with methotrexate, 3, range [0-16] with azathioprine – p=0.008 vs. methotrexate – and 2, range [0-27] with infliximab – p=0.032 vs. methotrexate). Individual values are shown in Figure 2B. The two indices of endoscopic activity - CDEIS and SES-CD – were closely correlated ( $\kappa=0.927$ ;  $p<0,001$ ).

In univariate analysis, factors associated with mucosal healing were treatment at baseline and Crohn's disease location. In multivariate analysis, three factors were positively associated with mucosal healing: current treatment with azathioprine (OR=7.13, 95% confidence interval (CI): [1.11-45.68] vs. methotrexate), current treatment with infliximab (OR=19.02, 95%CI [2.49-145.52] vs. methotrexate), and pure ileal disease (OR=5.77, 95%CI [1.27-26.28] vs. ileo-colonic location; Table 3).

### ***Histological response***

Biopsy samples were available for 40 patients (12 methotrexate, 14 azathioprine, and 14 infliximab). Considering microscopic activity, no significant difference was observed between patients receiving methotrexate (median 2.5, range [1-12]) and those treated with azathioprine (3, range [0-11];  $p=0.622$ ) infliximab (3.5, range [0-12];  $p=0.583$ ). No correlation was found between microscopic inflammation and CDEIS (Pearson's coefficient: 0.154;  $p=0.344$ ) or SES-CD.

### ***Follow-up***

During the follow-up period (median duration: 13.2 months, range [0-25.2]), 8 patients experienced Crohn's disease relapse with a median time of 4 months, range [0-23] (one patient without mucosal healing was lost to follow-up just after the inclusion and was considered to have relapsed at baseline). All but one had endoscopic ulcerations at entry. The last patient with mucosal healing at baseline experienced Crohn's disease worsening 23 months after inclusion. Relapse rates at 6, 12 and 24 months were 9.8%, 12.1% and 23.9%,

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3 respectively. Considering complete mucosal healing at baseline according to CDEIS cut-off,  
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5 patients with CDEIS<4 experienced fewer relapses during the follow up period than patients  
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7 with CDEIS $\geq$ 4 at entry (Figure 3).  
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## Discussion

Until recently, symptom improvement has been considered as the main therapeutic issue in Crohn's disease. With the advent of biologics, new objectives have been determined: mucosal healing, bowel damage, disability or surgical and hospitalisation rates<sup>21</sup>. Mucosal healing has become an endpoint in clinical trials and emerges as a desirable goal in current practice<sup>22</sup>. However, endoscopic response to conventional Crohn's disease treatments has been less studied than with anti-TNFs. When considering methotrexate, the efficacy of which has been proven in controlled trials<sup>23, 24</sup>, little is known about its ability to heal inflammatory lesions<sup>15, 16</sup>. In this prospective study conducted in Crohn's disease patients with sustained clinical remission, complete healing of endoscopic ulcerations was observed in 11% of methotrexate-treated patients. This rate was significantly lower compared to patients receiving monotherapy with azathioprine or infliximab: 50% and 60%, respectively.

Although mucosal healing is an important goal of treatment in Crohn's disease, no recognized definition exists. The disappearance of all mucosal ulcerations defined mucosal healing in controlled studies<sup>11, 12</sup>, and should be very simple in practice. However, this binary outcome – healed-not healed – does not reflect the great variety of endoscopic signs of Crohn's disease activity, and two endoscopic quantitative scoring systems are available at this time. Firstly, the Crohn's disease endoscopic index of severity (CDEIS) has been prospectively established in order to detect changes in disease activity<sup>19</sup>. It has been validated as a reproducible index. Despite its complexity and the learning curve required, the CDEIS is considered as the gold standard for classifying severity of disease. Furthermore, two cut-offs defining endoscopic remission and complete mucosal healing have been proposed<sup>10</sup>. Secondly, the simple endoscopic score for Crohn's disease (SES-CD) correlates well with the CDEIS<sup>20</sup> and has been validated. No threshold defining remission has been established at this time.

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3 Although the series reported here involved a modest number of patients, mucosal healing  
4 rates observed with azathioprine and infliximab are comparable to that previously published  
5 in patients in clinical remission with these drugs <sup>3, 25</sup>. Therefore, the small number of patients  
6 endoscopically healed with methotrexate in the same setting illustrates probably a lower  
7 mucosal **efficacy** of this drug. However, a more refractory Crohn's disease in patients  
8 receiving methotrexate cannot be excluded. Indeed, 78% previously received azathioprine  
9 **which was discontinued because of failure or intolerance**, and 83% infliximab. One can argue  
10 that this discrepancy can also be related to the treatment duration, which was shorter with  
11 methotrexate as compared to azathioprine. Nevertheless, duration of treatment was not  
12 associated with mucosal healing in the multivariate analysis. Furthermore, Crohn's disease  
13 inflammatory lesions seem to heal rapidly as suggested in a recent study with adalimumab  
14 showing similar endoscopic rates of response after 12 and 52 weeks of treatment. In the same  
15 study, shorter Crohn's disease duration was related to better endoscopic response <sup>11</sup>. This  
16 correlation was not confirmed in the current study, even if there was a trend for longer disease  
17 duration in patients treated with azathioprine and infliximab as compared to those receiving  
18 methotrexate.

19  
20 Methotrexate is a folate anti-metabolite used at low doses in many inflammatory disorders. In  
21 Crohn's disease, it is usually proposed after azathioprine failure or intolerance as a second-  
22 line immunosuppressant <sup>26-29</sup>. Indeed, methotrexate has been shown to have a steroid-sparing  
23 effect <sup>23</sup> and this compound could provide prolonged remission <sup>28, 30</sup>. Although the duration of  
24 treatment is not defined, two main reasons can lead to discontinue the drug. Firstly, the long-  
25 term duration of treatment with methotrexate may expose patients to side effects related to  
26 high cumulative doses leading then to withdrawal <sup>28, 30</sup>. Secondly, an increasing proportion of  
27 patients become methotrexate-refractory with time <sup>28, 30, 31</sup>. This secondary loss of response to  
28 methotrexate could be related to partial **efficacy** of the drug, characterized by symptom relief

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3 without complete mucosal healing, as suggested here. The route of administration probably  
4 cannot explain the low rate of complete endoscopic healing observed as all the included  
5 patients were treated parenterally (sub-cutaneous and/or intra-muscular). In the same way, the  
6 dosage used in the present study was the regimen proven to be effective as maintenance in  
7 Crohn's disease (15-25 mg/week) <sup>26</sup>. However, we cannot exclude that higher methotrexate  
8 doses may promote better endoscopic response.  
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10 Overall, the disappearance of all mucosal ulcerations is probably uncommon with  
11 methotrexate. However, it does not imply that methotrexate exerts no effect on gut mucosa.  
12 Considering the CDEIS, which is more quantitative and a less stringent criterion than the  
13 complete disappearance of all ulcerations, more than half of patients receiving methotrexate  
14 achieved Crohn's disease endoscopic remission. Moreover, whatever endoscopic tool used for  
15 mucosal healing assessment, the discrepancy observed between patients receiving  
16 methotrexate and those treated with azathioprine or infliximab was mostly due to the  
17 persistence of few ulcerations. Prospective studies are now required to determine if  
18 methotrexate is able to induce and maintain Crohn's disease endoscopic improvement.  
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20 Histology is an established procedure for the diagnosis of Crohn's disease. Nevertheless, the  
21 evolution of the microscopic intestinal features has been poorly investigated and there is a  
22 lack of data regarding histological response to the main medications used in Crohn's disease.  
23 Some changes have been observed, mainly on signs of mucosal injury and the presence of  
24 neutrophils <sup>32</sup>, and in a small placebo-controlled study, significant histological improvement  
25 due to the disappearance of the inflammatory infiltrate was seen after a single infliximab  
26 infusion <sup>18</sup>. Moreover, the correlation between clinical and endoscopic findings with histology  
27 is a matter of debate <sup>32</sup>. In the present study which enrolled patients in clinical remission,  
28 endoscopic and histologic activities were not correlated. Furthermore, there is no validated  
29 and standardized microscopic scoring system for Crohn's disease. Targeting mucosal  
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3 inflammation to achieve histological healing could be a greater objective than mucosal  
4 healing in Crohn's disease<sup>22</sup> and should be explored in future prospective studies.  
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8 Mucosal healing is an important issue in Crohn's disease patients and can probably predict the  
9 course of disease, as observed in the present study. Using quantitative measures such as  
10 CDEIS, methotrexate appears less effective than azathioprine or infliximab to heal the gut  
11 mucosa. However, further prospective studies have now to determine if this compound is able  
12 to induce a significant improvement of Crohn's disease bowel lesions, not only on the  
13 mucosal side but also on the entire gut wall in this transmural disease.  
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3 **Figure legends**  
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8 **Figure 1:** Mucosal healing rates (%) in Crohn's disease patients in sustained remission with  
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10 methotrexate, azathioprine and infliximab monotherapy.  
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15 **Figure 2:** Endoscopic activity in Crohn's disease patients in remission with maintenance  
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17 monotherapy considering the Crohn's Disease Endoscopic Index of Severity (CDEIS) <sup>19</sup> (A)  
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19 and the Simple Endoscopic Score for Crohn's Disease (SES-CD) <sup>20</sup> (B). Thick points indicate  
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21 values for individual patients, the wide horizontal line indicates median value.  
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27 **Figure 3:** Kaplan-Meier curves of survival without relapse according to complete mucosal  
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29 healing <sup>10</sup> at baseline. Continuous line: CDEIS<4; discontinuous line: CDEIS≥4  
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**Table 1** Microscopic Crohn's disease activity according to the Scoring System for Crohn's disease adapted from D'Haens *et al*<sup>18</sup>.

Histological variable	Grading
Epithelial damage	0 = normal; 1 = focal; 2 = extensive
Architectural changes	0 = normal; 1 = moderate; 2 = severe
Mononuclear cells in lamina propria	0 = normal; 1 = moderate increase; 2 = severe increase
Polymorphonuclear cells in lamina propria	0 = normal; 1 = moderate increase; 2 = severe increase
Neutrophils in epithelium	1 = surface epithelium; 1 = cryptitis; 2 = crypt abscess
Erosion or ulceration	0 = no; 1 = yes
Granuloma	0 = no; 1 = yes



**Table 2** Main characteristics of the 51 patients included in the study according to the maintenance therapy (methotrexate, azathioprine, or infliximab).

	Methotrexate	Azathioprine	Infliximab
n	18	18	15
Median age, years [range]	40 [21-56]	48 [21-89]	46 [30-79]
Male gender, n (%)	3 (17)	6 (33)	4 (27)
Median disease duration, years [range]	9.1 [1.1-29.5]	12.9 [6.5-27.7]	10.8 [5.8-29.8]
Disease location, n (%)			
ileal	1 (5)	2 (11)	0
colonic	7 (39)	10 (56)	7 (47)
ileo-colonic	10 (56)	4 (33)	8 (53)
Perianal disease, n (%)	8 (44)	11 (73)	11 (61)
Current smoker, n (%)	7 (39)	3 (18)	7 (47)
Previous intestinal resection, n (%)	9 (50)	8 (44)	5 (33)
Previous treatments, n (%)			
AZA/6-MP	14 (78)	NA	11 (73)
Methotrexate	NA	0	7 (47)
IFX	15 (83)	11 (61)	NA
Median duration of current treatment, months [range]	24 [7-92]	57 [19-115]*	28 [12-90]
Median CRP level, mg/L [range]	0 [0-87]	0 [0-13]	0 [0-27]
Indication for colonoscopy, n (%)			
Screening for dysplasia	12 (67)	14 (78)	15 (100)
Suspected stenosis	6 (33)	4 (22)	0

\*: p=0.014 as compared to the methotrexate group

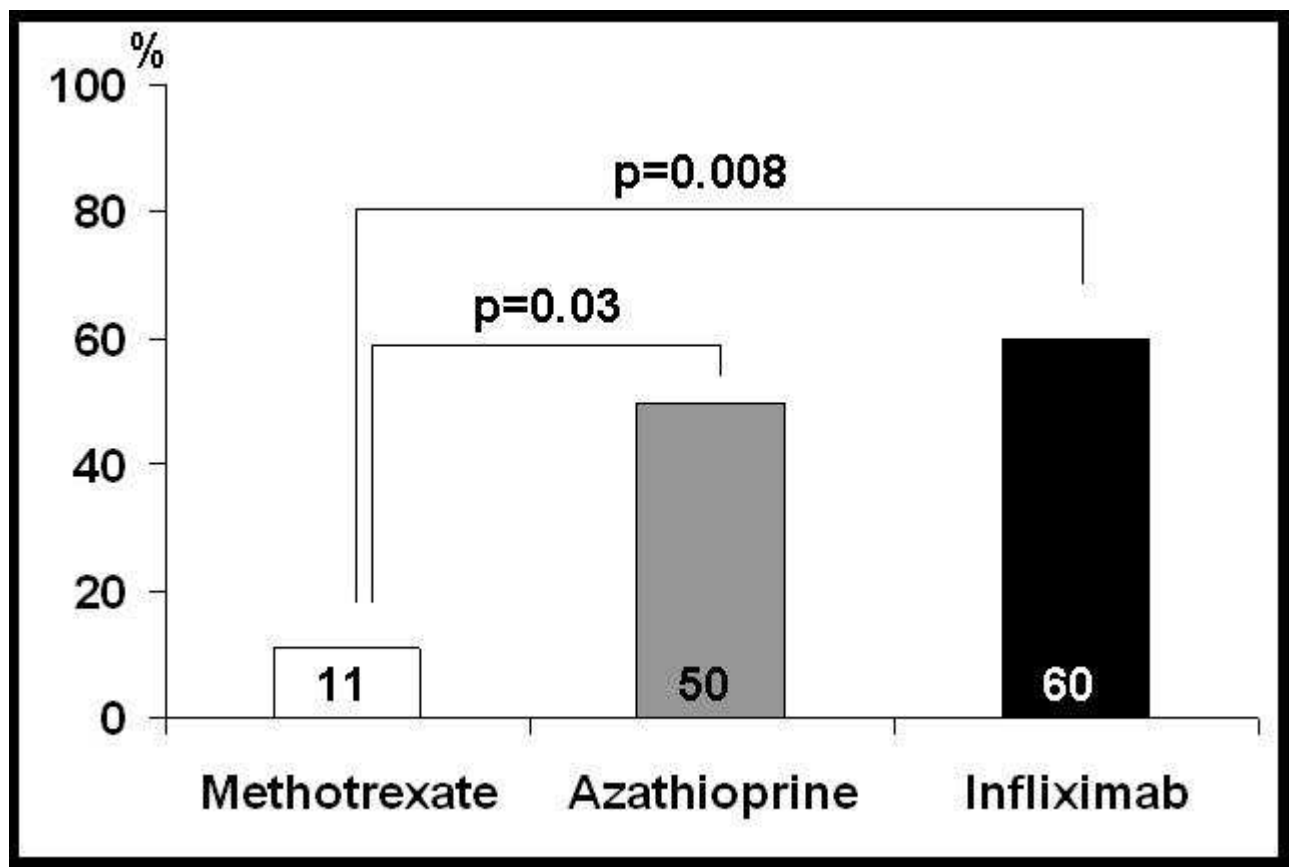
Other differences between the methotrexate group and the others are not significant.

**Table 3:** Factors associated with mucosal healing in multivariate analysis (logistic regression)

	OR	95% CI	p
Infliximab <i>vs.</i> methotrexate	19.02	2.49-145.52	0.005
Azathioprine <i>vs.</i> methotrexate	7.13	1.11-45.68	0.038
Ileal location <i>vs.</i> ileocolonic	5.77	1.27-26.28	0.024

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Figure 1



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Figure 2A

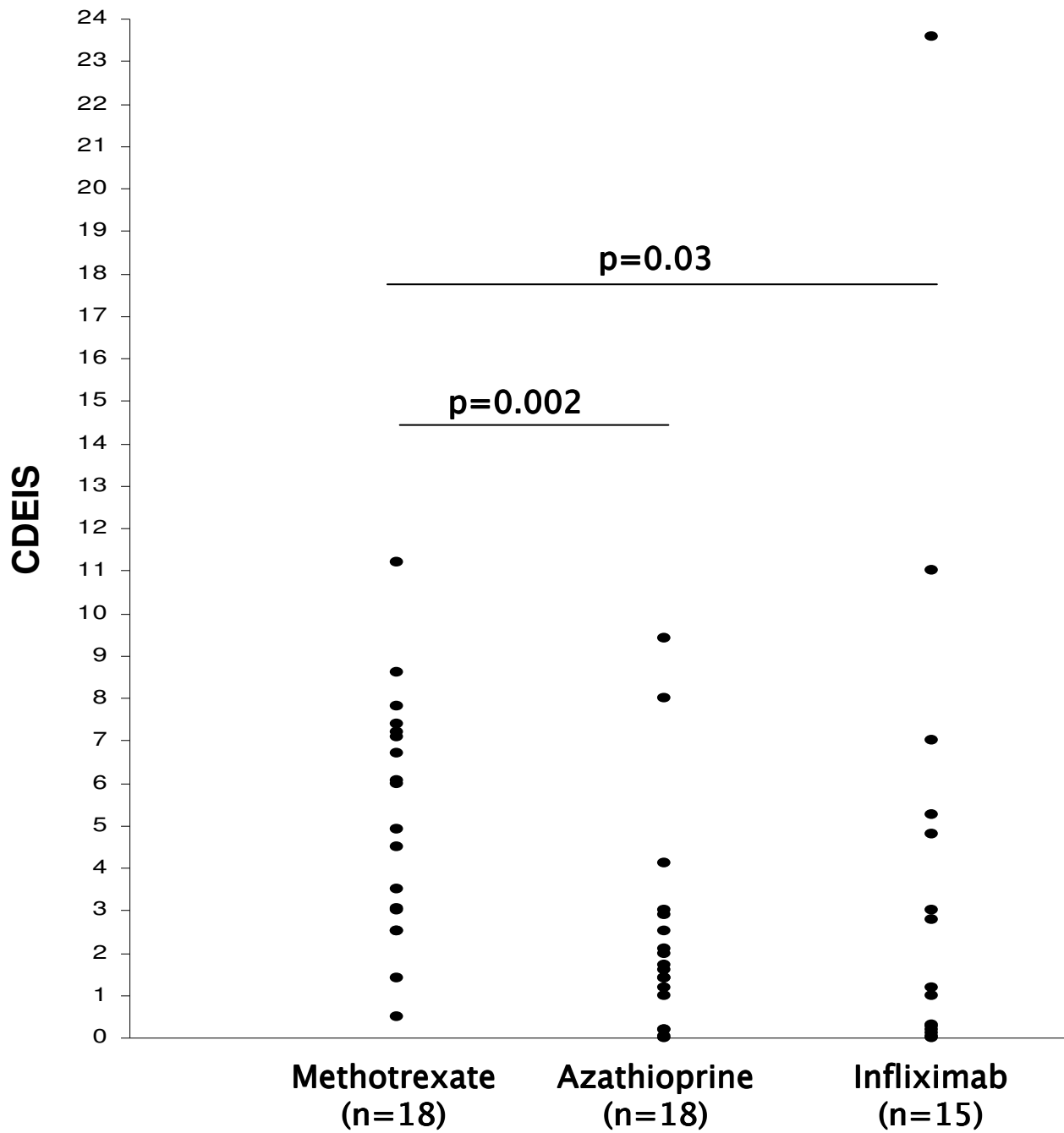


Figure 2B

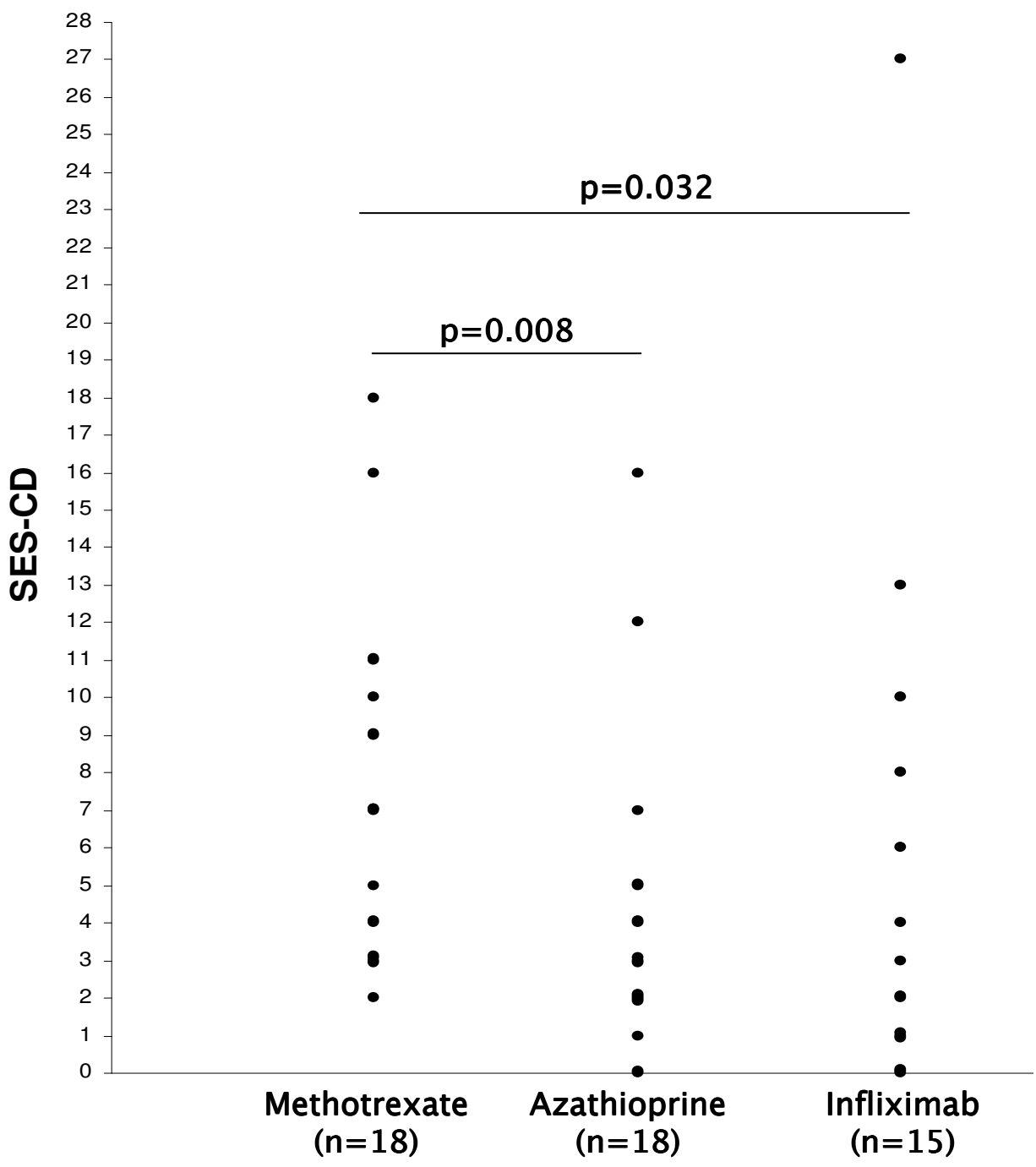


Figure 3

