Impact of the increasing use of immunosuppressants in Crohn’s disease on the need for intestinal surgery

J Cosnes, I Nion-Larmurier, L Beaugerie, P Afchain, E Tiret, J-P Gendre

Background/Aim: Immunosuppressants are now used much earlier in the course of Crohn’s disease; however their effect on the natural history of the disease, especially on the need for surgery, is not known. The aim of this study was to assess the evolution of the need for surgery in Crohn’s disease during the last 25 years.

Patients and Methods: The medical charts of 2573 patients were reviewed retrospectively. The use of immunosuppressants (azathioprine or methotrexate), the need for intestinal resection, and the occurrence of intestinal complications were assessed using Kaplan-Meier analysis in five consecutive cohorts of patients defined by the date of diagnosis of Crohn’s disease (1978–82; 1983–87; 1988–92; 1993–97; 1998–2002).

Results: In 565 patients seen in the authors’ unit within the first three months after diagnosis, characteristics of Crohn’s disease at diagnosis did not differ from one cohort to another. The five year cumulative probability to receive immunosuppressants increased from 0 in the 1978–82 cohort to 0.13, 0.25, 0.25, and 0.56 in the 1983–87, 1988–92, 1993–97, and 1998–2002 cohorts, respectively (p<0.001). Concomitantly, the cumulative risk of intestinal resection remained unchanged (from 0.35 to 0.34 at five years; p=0.81). The cumulative risk of developing a strictureing or a penetrating intestinal complication remained also unchanged. Similar results were obtained in the 2008 patients seen during the same period who were referred to us more than three months after diagnosis.

Conclusion: Although immunosuppressants have been used more frequently over the last 25 years, there was no significant decrease of the need for surgery, or of intestinal complications of Crohn’s disease.
diarrhoea following intestinal resection. Intestinal complications of CD were defined according to the Vienna classification: intestinal strictures as the occurrence of constant luminal narrowing demonstrated by radiological, endoscopic, or surgical examination combined with prestenotic dilatation and/or obstructive signs or symptoms but without evidence of penetrating disease. Perforations were intra-abdominal fistulas, inflammatory masses, and/or abscesses. First morphological demonstration of narrowing or penetrating complication was used to date the occurrence of the complication.

Treatment of Crohn’s disease

Our treatment policy has been described elsewhere. Flare up episodes were treated with mesalazine (3–4 g daily) or prednisolone (1 mg/kg per day, progressively tapered after four weeks), according to their clinical severity. When steroid therapy failed, patients seen before 1999 were given a three week course of enteral or parenteral nutrition; those seen after June 1999 (when infliximab became available in France) received infliximab 5 mg/kg.

As maintenance treatment, we used aminosalicylates (sulphasalazine, olsalazine, or mesalazine, 2–3 g daily) for asymptomatic or moderately active forms of the disease, and immunosuppressive drugs for severe forms (patients who were steroid dependent or poorly responsive to steroids). Azathioprine 2 mg/kg per day was used as the first line immunosuppressive drug. In case of repeated flare-ups or chronic active disease in a patient receiving azathioprine, its dosage was increased to 2.5–3 mg/kg per day. Intramuscular methotrexate (20–25 mg weekly) was used in patients unresponsive or intolerant to azathioprine. Its dosage was tapered progressively to 10–15 mg, and re-augmented in case of clinical relapse.

Although the overall strategy remained mostly unchanged, over time there was a clear tendency to initiate immunosuppressants earlier in the disease course.

Surgery was reserved for stenotic and extraparietal complications, or intractable forms after a well conducted medical management.

Statistical analysis

Continuous data are expressed as median (interquartile range), and differences between cohorts were tested for significance by ANOVA. Discrete data are given as percentages. Continuous data are expressed as median (interquartile range), and differences between cohorts were tested for significance by ANOVA. Discrete data are given as percentages.

Table 1 Characteristics at diagnosis of the five consecutive chronological cohorts of patients seen in our unit within the three months following diagnosis (group 1)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patients (n)</th>
<th>Male (%)</th>
<th>Median age (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978–82</td>
<td>34</td>
<td>15 (44)</td>
<td>25 (21–35)</td>
</tr>
<tr>
<td>1983–87</td>
<td>46</td>
<td>15 (41)</td>
<td>27 (21–38)</td>
</tr>
<tr>
<td>1988–92</td>
<td>102</td>
<td>21 (40)</td>
<td>27 (20–37)</td>
</tr>
<tr>
<td>1993–97</td>
<td>176</td>
<td>27 (40)</td>
<td>29 (22–38)</td>
</tr>
<tr>
<td>1998–2003</td>
<td>207</td>
<td>31 (48)</td>
<td>27 (20–36)</td>
</tr>
</tbody>
</table>

Disease location

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Ileocolonic</td>
<td>13 (38)</td>
<td>19 (41)</td>
<td>41 (40)</td>
<td>60 (34)</td>
<td>72 (35)</td>
</tr>
<tr>
<td>Small bowel</td>
<td>11 (32)</td>
<td>16 (35)</td>
<td>32 (31)</td>
<td>59 (34)</td>
<td>70 (34)</td>
</tr>
<tr>
<td>Colon</td>
<td>7 (21)</td>
<td>10 (22)</td>
<td>27 (26)</td>
<td>52 (30)</td>
<td>60 (29)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (9)</td>
<td>1 (2)</td>
<td>2 (2)</td>
<td>5 (3)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>13 (38)</td>
<td>20 (43)</td>
<td>35 (34)</td>
<td>49 (28)</td>
<td>63 (30)</td>
</tr>
<tr>
<td>Familial history</td>
<td>4 (12)</td>
<td>13 (28)</td>
<td>10 (10)</td>
<td>30 (17)</td>
<td>33 (16)</td>
</tr>
<tr>
<td>Smokers</td>
<td>18 (53)</td>
<td>31 (67)</td>
<td>51 (50)</td>
<td>86 (49)</td>
<td>100 (48)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>30 (88)</td>
<td>40 (87)</td>
<td>93 (91)</td>
<td>153 (87)</td>
<td>167 (81)</td>
</tr>
<tr>
<td>North-African non-Jewish ancestry</td>
<td>2 (6)</td>
<td>6 (13)</td>
<td>8 (8)</td>
<td>16 (9)</td>
<td>26 (13)</td>
</tr>
<tr>
<td>Living outside Paris area</td>
<td>13 (38)</td>
<td>19 (41)</td>
<td>26 (25)</td>
<td>57 (32)</td>
<td>65 (31)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages. Intergroup comparisons showed no significant differences.

Results

The characteristics of CD at diagnosis in the five cohorts of group 1 are given in table 1. Patients were very similar at diagnosis from one cohort to another, with a predominance of females, a mean age about 30 years, a large proportion of smokers (half the patients), and a similar disease location. Table 2 gives the cumulative characteristics of the disease at the end of 2003. No attempt was made to contact the patients at that time and 31% of them had been lost to follow up. Because patients from the oldest cohorts had a longer disease duration, they developed more stricturing or penetrating complications and were classified so according to Vienna classification. Similarly, the respective proportions of patients needing steroids or immunosuppressants, and operated on, should be interpreted in relation to different durations of follow up. Azathioprine was maintained for a prolonged period in most cases but had to be stopped within the first month because of adverse events in 16 patients (11%). It was switched to methotrexate in eight of those latter patients.

Changes in the use of immunosuppressants over 25 years in group 1 patients

Figure 1 shows the cumulative use of immunosuppressants in the five cohorts. Data of patients for whom immunosuppressants had to be stopped early are included. As expected, immunosuppressants have been used more and more early over the last 25 years, with a five year cumulative probability
of prescription of zero in the 1978–82 cohort to 0.56 (95% CI 0.31 to 0.78) in the 1998–2002 cohort.

**Cumulative need for excisional surgery in the five group 1 cohorts**

One hundred and ninety patients (34%) were operated on at least once. Excisional surgery was performed in 41 cases before first admission in our unit and thereafter in 149 cases. Figure 2 gives the cumulative need for first excisional surgery. The curves were superimposed, with no significant difference from one curve to another (log rank, p = 0.81). Excluding the 80 patients who had had surgery within the first three months following diagnosis yielded the same result (log rank, p = 0.49), although immunosuppressants were used in that group much earlier over the years (log rank, p = 0.0001). In the whole group of 565 patients, Cox analysis confirmed that the year of diagnosis had no significant effect upon the need for surgery. Factors associated with surgery were ileal involvement (HR 2.78; 95% CI 2.19 to 3.51) and absence of rectal involvement (HR 0.34; 95% CI 0.27 to 0.43). In the cohorts 1978–82, 1983–87, 1988–92, 1993–97, and 1998–2002, the five year cumulative probabilities of having a large intestinal resection, defined by a post-surgical handicap index ≥20, were 0.29 (0.15–0.50), 0.20 (0.10–0.36), 0.24 (0.16–0.35), 0.13 (0.07–0.22), and 0.17 (0.04–0.49), respectively. The curves were not significantly different according to log rank test (p = 0.23). The five year cumulative probability of having a definitive stoma varied not significantly between 0 and 0.03 from one cohort to another (p = 0.33).

**Indications for first intestinal resection in group 1 patients**

Table 3 gives the indications for the first intestinal surgery in the five cohorts. The proportion of patients being operated on for medical failure, stricture, and perforation, respectively, did not change significantly between the five cohorts, although in the most recent cohort there was a clear reduction of operations for medical failure (13% v 22–38% in the other cohorts). Kaplan-Meier analysis of the cumulative probability of intestinal stricture and perforation did not show significant differences between cohorts. In the cohorts 1978–82, 1983–87, 1988–92, 1993–97, and 1998–2002, the five year cumulative probabilities of intestinal stricture were 0.23 (0.11–0.43), 0.14 (0.06–0.29), 0.19 (0.12–0.30), 0.17 (0.11–0.26), and 0.10 (0.02–0.42), respectively (log rank test, p = 0.16).

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**Table 2** Cumulative characteristics of the five consecutive chronologic cohorts of patients seen in our unit within the three months following diagnosis (group 1)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>34</td>
<td>46</td>
<td>102</td>
<td>176</td>
<td>207</td>
</tr>
<tr>
<td>Deceased</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow up</td>
<td>14 (41)</td>
<td>20 (43)</td>
<td>42 (41)</td>
<td>70 (40)</td>
<td>24 (12)</td>
</tr>
<tr>
<td>Median disease duration, months (interquartile range)</td>
<td>188 (23–256)</td>
<td>181 (64–207)</td>
<td>123 (43–144)</td>
<td>59 (10–91)</td>
<td>14 (5–35)</td>
</tr>
<tr>
<td>Vienna classification</td>
<td>inflammatory</td>
<td>15 (44)</td>
<td>17 (37)</td>
<td>42 (41)</td>
<td>95 (54)</td>
</tr>
<tr>
<td></td>
<td>structuring</td>
<td>9 (26)</td>
<td>4 (9)</td>
<td>17 (17)</td>
<td>22 (13)</td>
</tr>
<tr>
<td></td>
<td>penetrating</td>
<td>10 (29)</td>
<td>25 (54)</td>
<td>43 (42)</td>
<td>59 (34)</td>
</tr>
<tr>
<td>Medical treatment</td>
<td>no steroids, no IS</td>
<td>6 (18)</td>
<td>7 (15)</td>
<td>20 (20)</td>
<td>57 (32)</td>
</tr>
<tr>
<td></td>
<td>steroids, no IS</td>
<td>22 (65)</td>
<td>29 (63)</td>
<td>51 (50)</td>
<td>80 (45)</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>6 (18)</td>
<td>10 (22)</td>
<td>31 (30)</td>
<td>39 (22)</td>
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<td></td>
<td>early withdrawal of IS</td>
<td>0</td>
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<td>2 (2)</td>
<td>1 (1)</td>
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<tr>
<td></td>
<td>infliximab</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Cumulative number of intestinal resections</td>
<td>0</td>
<td>18 (53)</td>
<td>23 (50)</td>
<td>53 (52)</td>
<td>120 (68)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11 (32)</td>
<td>17 (37)</td>
<td>42 (41)</td>
<td>50 (28)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 (6)</td>
<td>5 (11)</td>
<td>5 (5)</td>
<td>6 (3)</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>3 (9)</td>
<td>1 (2)</td>
<td>2 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.

IS, immunosuppressants.

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**Table 3** Indications for the first intestinal resection in the five consecutive chronologic cohorts of patients seen in our unit within the three months following diagnosis (group 1)

<table>
<thead>
<tr>
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<td>Resections (n)</td>
<td>16</td>
<td>23</td>
<td>49</td>
<td>56</td>
<td>46</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>1 (6)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Stricture</td>
<td>6 (38)</td>
<td>3 (13)</td>
<td>11 (22)</td>
<td>8 (14)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Perforation</td>
<td>3 (19)</td>
<td>15 (65)</td>
<td>21 (43)</td>
<td>23 (45)</td>
<td>31 (67)</td>
</tr>
<tr>
<td>Failure of medical treatment</td>
<td>6 (38)</td>
<td>5 (22)</td>
<td>16 (33)</td>
<td>19 (34)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Other*</td>
<td>0</td>
<td>0</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.

*Other included mesenteric infarction, haematochezia, and small bowel adenocarcinoma, respectively.

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**Figure 1** Kaplan-Meier estimates of the cumulative risk of receiving immunosuppressants in five chronologic cohorts of patients with Crohn’s disease.
Evolution of the need for surgery 1978–2003

In the total cohort of 2573 patients from groups 1 and 2, 1070 underwent 1426 intestinal resections from January 1978 to December 2003 (22 928 patient years). Two hundred and seventeen resections (15%) were performed within the first three months following diagnosis. Except for the year 1978 (46 patients only), the percentage of patients who were operated on during the first three months following diagnosis remained less than 5% (fig 3). After the first three months, the operative rate (number of operations performed per year) fluctuated within a narrow range (3.3–7.5%), without any significant change over 26 years (fig 3).

DISCUSSION

This study shows that although immunosuppressants were initiated much earlier during the course of CD, the need for intestinal resection remained stable over 25 years. The percentage of patients requiring intestinal surgery each year remained equal. The probability of having a definitive stoma appeared also to be unaffected from 1978 to 2002. However, large intestinal resections became more unusual.

This study has some limitations. Firstly, the retrospective nature of the study may have led to bias in the interpretation of the data—however, it was necessary to obtain an observation period long enough to ascertain the long term effect on surgery of changes in the medical strategy of CD. In addition, intestinal resection can be considered as an unbiased and solid criterion, even retrospectively, as it is performed only when necessary. Besides, during a period of 20–25 years, many factors other than the treatment strategy may have influenced the indications for surgery. However, all patients seen from the beginning were followed up in the same unit by the same small group of physicians, who used homogeneous guidelines and took collegial decisions. Moreover, comparison of cohorts at inclusion showed that compared with the first group of patients, a higher proportion of referred patients had had surgery before admission in our unit (p<0.001). Otherwise, results observed were similar to those of group 1 regarding an increased use of immunosuppressants but a stable need for excisional surgery over the years (table 4). The risk of having a definitive stoma remained also stable. However, in that group, the risk of having a large intestinal resection decreased significantly with time.

### Table 4 Five year cumulative rates (95% CI) of therapeutic intervention in the patients referred to our unit more than three months after diagnosis of Crohn’s disease (group 2, n = 2008)

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>218</td>
<td>342</td>
<td>486</td>
<td>563</td>
<td>399</td>
<td></td>
</tr>
<tr>
<td>IS therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative probability (95% CI)</td>
<td>0.04 (0.02–0.08)</td>
<td>0.14 (0.10–0.18)</td>
<td>0.27 (0.23–0.32)</td>
<td>0.45 (0.40–0.50)</td>
<td>0.63 (0.49–0.76)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Patients at risk (n)</td>
<td>195</td>
<td>278</td>
<td>296</td>
<td>203</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Large intestinal resection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative probability (95% CI)</td>
<td>0.36 (0.29–0.48)</td>
<td>0.30 (0.25–0.35)</td>
<td>0.32 (0.28–0.37)</td>
<td>0.31 (0.27–0.36)</td>
<td>0.36 (0.22–0.53)</td>
<td>0.528</td>
</tr>
<tr>
<td>Patients at risk (n)</td>
<td>137</td>
<td>227</td>
<td>274</td>
<td>263</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Definitive stoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative probability (95% CI)</td>
<td>0.01 (0.00–0.03)</td>
<td>0.02 (0.01–0.04)</td>
<td>0.02 (0.01–0.04)</td>
<td>0.02 (0.01–0.04)</td>
<td>0.02 (0.01–0.03)</td>
<td>0.72</td>
</tr>
<tr>
<td>Patients at risk (n)</td>
<td>202</td>
<td>315</td>
<td>387</td>
<td>355</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3  Evolution of the annual rate of intestinal resections from 1978–2003 in 2573 patients with Crohn’s disease. The numbers above the columns indicate the number of patients at risk for intestinal resection at the beginning of the year.
they were very similar regarding demographic characteristics and disease location. In particular there is no reason to believe that CD became more severe with time while other disease characteristics did not change. Secondly, it should be noted that a relatively large proportion of patients were lost to follow up. We made no attempt to contact the patients or physicians to update the data. We do not believe this may have minimised the need for surgery of the oldest cohorts because patients who are lost to follow up are usually those doing well and not requiring further surgery. The cumulative probability of surgery in our patients was very similar to those reported in two unbiased and complete series of the literature, the NCCDS and the Copenhagen County cohort study. Finally, our unit is a tertiary referral centre and referral bias is unavoidable. To limit this bias, we restricted the analysis to patients seen during the first three months of the disease course. This precaution was not sufficient to eliminate such a referral bias because an important proportion of these patients came to surgery during that period. However, when we excluded these latter patients, analyses gave similar results and, in particular, the discrepancy between an increased use of immunosuppressants and a stable need for surgery remained unchanged. These results were confirmed in a second large group of patients.

The occurrence of strictureing and perforative complications was the main reason for excisional surgery. The frequency of these complications did not change significantly from one cohort to another. This is a disappointing result because it could be expected that immunosuppressants could have an ameliorative effect and prevent these complications. Indeed, D’Haens et al reported that in 74% of patients with colonic or ileocolonic disease who were clinically responders to azathioprine, endoscopic lesions had healed completely or nearly completely after a mean of two years of treatment. Histologically there was disappearance of the inflammatory infiltrate, with only a degree of architectural disturbance remaining. In the present study there was, over the most recent years, a clear decrease of intestinal resection for medical failure, which represented only 13% of surgical indications in the 1998–2002 cohort. This result may be related to immunosuppressants, but also to infliximab even though it was used sparingly in very few patients. In any case, the absence of a decrease in the need for surgery over the last 25 years questions the efficiency of our medical strategy. In fact, there is the possibility that in this study immunosuppressants had no impact on complications and the need for surgery because they were given too late during the course of the disease. Supporting this hypothesis, a large majority of patients came to surgery while not having received immunosuppressants, or were operated on early (within the first three months), while the mean time of response to purine analogues is three months. By contrast, if we make the hypothesis that immunosuppressants are able to change the natural history of CD, nearly half the patients who were operated on more than three months after diagnosis could have avoided surgery. Thus we can extrapolate that in our series immunosuppressants were given too late and too scarcely to have a significant impact on the need for surgery. Such an assertion is not in accordance with the anatomic effect observed by D’Haens et al, that immunosuppressants, even when given early, may have no preventive effect on the occurrence of strictureting and penetrating complications.

An interesting and more encouraging result of our study was the decrease of the probability of having a large intestinal resection over the last 25 years in the group of referred patients. A similar trend, although not significant, was observed in patients who were seen early after diagnosis and were, for the most part, operated on in our surgical unit. The reason for such a decrease, from 29% to 12% five years after diagnosis, may be linked to a greater use of immunosuppressants, but may also be a change in the surgical strategy favouring segmental and limited resections in the most recent years. In summary, this study shows that immunosuppressants have been used increasingly over the years. However, this evolving therapeutic strategy was not associated with a decrease in the need for surgery or in a decrease of the occurrence of intestinal complications. This result does not question the efficacy of immunosuppressants for achieving and maintaining remission, sparing steroids, and improving quality of life, but it does questions the timing of starting immunosuppressants in patients with moderate to severe Crohn’s disease.

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REFERENCES
Efficiency and strategy of pneumatic dilatation in achalasia

We read with interest the article by Eckardt et al. regarding the long term results of pneumatic dilatation in achalasia (Gut 2004;53:629–33). Fifty four patients were followed up for a median of 14 years after a single pneumatic dilatation using the Browne-McHardy dilator. Five and 10 year remission rates were 40% and 36%, respectively, and repeated dilatations only mildly improved the clinical response. Most of the relapses occurred within one year of dilatation. Patients with post-dilatation lower oesophageal sphincter pressures of < 10 mm Hg had a significantly better outcome. The authors suggest that failure to respond to the first dilatation should lead to consideration of alternative therapy.

We disagree with this conclusion and we would like to bring to your attention a recent prospective study on the long term effects of pneumatic dilatation in 11 patients with achalasia.1 A different approach was chosen—that is, treatment consisted of one or more pneumatic dilatations under conscious sedation in order to achieve stable clinical remission, defined as persisting one year after dilatation. To this end, close follow up was performed in the first year after dilatation (scheduled assessments at three and 12 months). Thereafter, clinical and manometric assessments were performed yearly for six years. The clinical score was according to Eckardt et al. Five patients needed one (30 mm diameter Rigiflex dilator) and six needed two (30 and 35 mm diameter) dilatations. No complications occurred. All patients remained in clinical remission and their lower oesophageal sphincter pressure decreased to < 10 mm Hg and remained unchanged over time.

There are similarities in the results of the two studies. However, the outcome of our 11 patients was comparable with that of the eight patients of Eckardt et al with a lower oesophageal sphincter pressure of < 10 mm Hg who had a remission rate of 77% after 3 years; and (2) the observation that the six patients in our series who needed a second dilatation all relapsed within one year of the first dilatation agrees with the data by Eckardt et al, showing that most relapses occurred in the first 12 months. However, our dilatations were more successful and, importantly, a second dilatation led to a sustained remission in all patients. We do not know the reasons for this difference but we believe it may be at least partly related to our use of the non-compliant Rigiflex dilator, which is currently considered the best choice, although there are no adequately powered comparisons with the Browne-McHardy dilator in the literature.2 Similarly to our result, a recent paper has shown very good efficacy of a second dilatation with the Rigiflex dilator in patients who had relapsed.3 Another possible reason is the use of conscious sedation during the procedure which allowed us to complete all dilatations; Eckardt et al., who used topical anaesthesia only, had to prematurely terminate 17% of the procedures.

In conclusion, our published experience and our current clinical practice, involving treatment and follow up of 10–15 new achalasia patients each year, suggest that performance of one or two dilatations until stable clinical remission is a valuable strategy, and that pneumatic dilatation under conscious sedation with the Rigiflex dilator is an effective long term treatment in most patients with achalasia.

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Conflict of interest: None declared.

References

Authors’ reply
Penagini and Cantù should be congratulated for the remarkable results they were able to obtain in 11 patients with achalasia treated by pneumatic dilatation. To my knowledge, not a single study has so far produced similar results. A review of prospective studies in achalasia reported the successful application of the oral route for probiotic attenuation of colitis.1

We agree with the corresponding commentary of Ghosh et al (Gut 2004;53:620–2) regarding the need to study mechanisms underlying probiotic interactions. Recently, we further standardised a method to compare the anti-inflammatory potential of orally administered lactic acid bacteria (LAB) in a murine model of acute 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis.2 This model allowed us to discriminate “protective” strains, showing between 30% and 70% reduction of inflammatory score, from strains which did not significantly attenuate experimental colitis. We could select highly performing strains of Lactobacillus salivarius and Lactobacillus rhamnosus that consistently lowered colitis.3 In comparison, strains of Lactobacillus acidophilus, Lactococcus lactis, and Streptococcus gordonii never showed any improvement. For all five strains, we investigated the protective effect of a single intraperitoneal injection of 5 × 10^8 live microorganisms, 24 hours prior to induction of colitis. Surprisingly, protection by the LAB strains via this systemic route closely matched the efficiency of the oral route (fig 1).

When evaluating both routes simultaneously, the anti-inflammatory effect was of comparable magnitude. Moreover, the prophylactic impact of the intraperitoneal Lactobacillus salivarius strain was established up to two hours before TNBS administration. It is noteworthy that two delayed injections could lead to protection but caused marked weight loss (25% (p = 0.05) v 15% and 11%, respectively, for control and oral route groups) with alleged fever.3

Our findings clearly confirm those of Sheil et al (Gut 2004;53:694–700) showing strain specific in vivo probiotic effects and interferon in serum of intraperitoneal injected Lactobacillus bulgaricus while Streptococcus thermophilus did not. Recent

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Probiotics in IBD: mucosal and systemic routes of administration may promote similar effects

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that the maximal non-lethal quantity of probiotics with other well known anti-inflammatory or immune cells, showing effects comparable with strain and viability status. Both pro- and hypothesised that toxicity may also differ reduction of mean macroscopic inflammation of the TNBS-induced colitis model to evaluate the oral or intraperitoneal route of LAB administration on reduction of TNBS-induced colitis in mice. Results are expressed as per cent reduction of mean macroscopic inflammation* of mice treated with LAB, in relation to the mean score of non-treated mice. Colitis index was assessed 48 hours after TNBS administration. Each bar represents an independent experiment of control (n = 10) and LAB treated mice (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001, significantly different from the corresponding TNBS control group (Mann-Whitney U test). CFU, colony forming unit; d, day.

observations showed that heat treatment of an orally administered probiotic cocktail abolished the colitis protection in a DSS model, while irradiation improved it. Consequently, cellular integrity appears to be necessary to explain at least some part of the effect, although cell walls and peptido-glycans of killed bacteria cannot be considered as passive. Possibly both “good” and “bad” signals are given out by LAB, and the immune system is integrating all of them. Those “mixed” signals will no doubt be specific for each strain as well as dose dependent. Differences in physicochemical status could explain the mortality seen by Shell et al., especially when using heat treated bacteria. Pereyra and colleagues established that the maximal non-lethal quantity of injected live LAB was $5 \times 10^6$ but it can be hypothesised that toxicity may also differ with strain and viability status. Both pro- and anti-inflammatory components of probiotics have been reported to interact with systemic immune cells, showing effects comparable with other well known anti-inflammatory or therapeutic molecules. It is therefore most probable that systemic delivery of specific live or killed probiotics may influence the delicate balance between TH1 and TH2 immunity, and subsequently have an impact on local immunity. Clear relationships, however, are not obvious. A first example is the case of subcutaneous CpG DNA that promoted a TH1 response and was able to alleviate some symptoms of DSS colitis but caused inflammation when administered after the onset of colitis. Secondly, different experimental models of colitis support a potential benefit of probiotic DNA, although it seems very premature to restrict this probiotic effect to nucleic acids only.

As emphasised by Gosh and colleagues (Gut 2004;53:620–2), approaches involving fractional studies are essential tools to complete the knowledge obtained from in vitro and ex vivo models and assist in understanding the interactions between LAB and the immune system. These studies may reveal common mechanisms active in inflammation, tolerance, and allergy models. Even if this study confirms the importance of the systemic route for certain probiotic activity, we cannot neglect the possible influence of local and innate immunity, the general status of the gut flora, and the role of epithelial cells in the cross talk between both.

Acknowledgements

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References


Mutations in anionic trypsino gene are not associated with tropical calcific pancreatitis

Pancreatitis is considered to be an autodigestive disease due to premature activation of trypsigen inside the pancreas. Its genetic basis has recently been established with the identification of causal mutations in cationic trypsigen gene (PRSS1) in patients with hereditary and non-hereditary pancreatitis. Mutations in other genes such as SPINK1 (encoding pancreatic secretory trypsin inhibitor) and cystic fibrosis transmembrane conductance regulator (CFTR) genes have also been associated with the disease. Tropical calcific pancreatitis is a type of idiopathic pancreatitis, reported particularly in the tropics. Recently, we and others demonstrated absence of PRSS1 mutations but significant prevalence of the N34S mutation in the SPINK1 gene in these patients. However, our study raised two important questions: firstly, the exact role of SPINK1 mutations in disease causation as cationic trypsigen is normal with an intact auto-lysis site; and secondly, the cause of the disease in the remaining patients negative for both PRSS1 and SPINK1 mutations.

Of the nine members of the human trypsigen family, only PRSS1, PRSS2, and PRSS3 are functional genes coding for cationic, anionic, and meso-trypsins, respectively. The finding that mutations in the anionic trypsino gene may contribute to the pathogenesis of tropical calcific pancreatitis is supported by the results of our study. The absence of mutations reported to date in the PRSS1 gene, 17 are clustered in exons 2 and 3 only. The remaining three are in the promoter region but reported in isolated patients. Hence we initially screened exons 2 and 3 of the anionic trypsigen gene in 68 well characterised Indian patients with tropical calcific pancreatitis. Subsequently, we also sequenced the promoter, complete coding region, and the flanking region in an attempt to look for any novel mutation.

Owing to the extremely high sequence homology between PRSS1 and PRSS2, a nested polymerase chain reaction (PCR) was used to ensure specificity. The primers were selected from the published study of Chen and colleagues and all of the exons of PRSS2 were PCR amplified, purified, and sequenced on both alleles using both primers and the Big Dye terminator cycle sequencing approach. However, we did not find any of the reported or any novel mutations in the coding region or in the splice site junctions, except a synonymous polymorphism A90A (GCA→GCC) in exon 3 of the anionic trypsigen gene. This variation was observed in both the heterozygous
and homozgyous states with a mutant allele frequency of 0.58 (9 AA, 20 GG, and 39 AG) and was comparable with 0.61 in 50 controls (7 AA, 18 GG and 25 AG) analysed.

Our results thus exclude any association of mutations in the anionic trypsinogen gene in tropical calcific pancreatitis and suggest a role for other genetic or non-genetic factors in the pathogenesis of the disease. Screening of genes such as CPT1A may explain the disease in the remaining patients. It also affirms the importance of the N34S mutation in SPINK1 as the major genetic factor for this type of pancreatitis.

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References


Elevated plasma protein C levels correlate with the presence of fatty liver (NASH and NAFLD)

The clinical implications of non-alcoholic fatty liver disease (NAFLD) are derived mostly from its common occurrence in the general population and the potential of the condition to progress to fibrosis and cirrhosis. Markers that help in making an early diagnosis and treatment are warranted. Protein C is a vitamin K dependent glycoprotein that functions as a circulating anticoagulant through proteolytic cleavage and inactivation of the coagulation factors Va and VIII. Whether protein C levels increase in patients with NAFLDs has not been assessed.

We measured protein C levels in 44 patients (28 men and 16 women; mean ages 45 (11) and 49 (12) years, respectively); 15 patients with fatty liver (FL), 15 with non-alcoholic steatohepatitis (NASH), and 14 with chronic viral hepatitis B+C (CH). All were diagnosed by histology and liver technetium scan or ultrasound. Ten healthy subjects were used as controls. Obesity, hyperlipidaemia, and diabetes were present in 60%, 73%, and 25% of cases, respectively; 64% of patients had elevated liver enzyme tests (alanine aminotransferase 45 (21) IU/l in FL and 43 (18) IU/l in NASH). Mean protein C levels were significantly increased in patients with NAFLD (n = 30) compared with those with chronic viral hepatitis (140 (36) % 101 (24); p <0.0009) and healthy individuals (140 (36) % 120 (12); p <0.04).

No significant difference in protein C levels was noted between simple fatty liver and NASH (149 (34) % 130 (37); respectively; p = 0.07)). A significant correlation was found between protein C and extent of fatty infiltration (r = 0.63; p <0.001) (fig 1), insulin resistance index (r = 0.3; p <0.01), and triglyceride levels (r = 0.45, p <0.001). Protein C could discriminate correctly between NAFLDs and chronic viral hepatitis in 82% of cases. No significant association was found between protein C and aminotransferase levels.

In conclusion, protein C was elevated in patients with NAFLD. The underlying mechanism remains unknown. Agewall et al suggested an increase in hepatic synthesis of protein C due to increased hepatic insulin resistance. Increased levels have been reported in patients with diabetes, hypertriglyceridaemia, and nephrotic syndrome, with the use of anabolic steroids, oral contraceptives, and alcohol, and with increasing age. Diabetes and hypertriglyceridaemia are predisposing conditions to fatty liver and were present in 23% and 73% of cases, respectively. The remaining conditions were excluded by clinical and biochemical findings. Although more studies are needed, these preliminary findings suggest that elevated protein C levels together with elevated liver enzymes may be used as markers for NAFLD and may obviate the need for liver biopsy.

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References


Coexistent chronic idiopathic intestinal pseudo obstruction and inflammatory bowel disease

Chronic idiopathic intestinal pseudo-obstruction (CIIP) is a severe condition presenting with abdominal pain and dysmotility. Inflammatory or degenerative changes of the autonomic nervous system or of the muscles of the bowel have been observed in CIIP. As patients with inflammatory bowel disease (IBD) may show clinical and histological signs of autonomic neuropathy and dysmotility, the aim of this study was to examine whether there is an association between CIIP and IBD.

Six patients at our hospital presenting with signs and symptoms of intestinal dysmotility were diagnosed with CIIP based on clinical features, antroduodenoejunul manometry, and full thickness biopsies (table 1). Patient No 1 had an acute erosive colitis some years previously with bloody diarrhoea and an enhanced sedimentation rate, which was treated with steroids, and patient No 2 had relapsing proctitis treated with 5-amino-salicylic acid (5-ASA). Patient No 3 was
Table 1  Summary of the findings in our patients

<table>
<thead>
<tr>
<th>Patient No:</th>
<th>Debut age/CIIP diagnosis age (y)</th>
<th>Main symptoms</th>
<th>Clinical diagnosis</th>
<th>Endoscopic pathology</th>
<th>Histopathology</th>
<th>Antroduodenal manometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23/F 16/22</td>
<td>Pain, bloody diarrhea</td>
<td>Crohn’s disease, CIIP</td>
<td>Small and large bowel</td>
<td>Degenerative neuropathy: Suspected</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>26/F 15/25</td>
<td>Pain, vomiting</td>
<td>Proctitis, CIIP</td>
<td>Rectum</td>
<td>Degenerative neuropathy: Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>3</td>
<td>35/F Teenage/29</td>
<td>Constipation, dyspepsia</td>
<td>Suspected Crohn’s disease, CIIP</td>
<td>Small and large bowel</td>
<td>Ganglioneuronitis: Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>4</td>
<td>44/F 35/39</td>
<td>Constipation, pain</td>
<td>Suspected Crohn’s disease, CIIP</td>
<td>Small bowel</td>
<td>Ganglioneuronitis: Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>5</td>
<td>55/M 39/41</td>
<td>GORD, later pain and diarrhoea</td>
<td>Suspected Crohn’s disease, CIIP</td>
<td>Normal</td>
<td>Ganglioneuronitis: Crohn’s disease</td>
<td>Abnormal</td>
</tr>
<tr>
<td>6</td>
<td>67/M 61/64</td>
<td>Pain, weight loss</td>
<td>Crohn’s disease, CIIP</td>
<td>Large bowel</td>
<td>Ganglioneuronitis: Crohn’s disease</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

CIIP, Chronic idiopathic intestinal pseudoobstruction; GORD, gastro-oesophageal reflux disease.

totally and patient No 4 partially colectomised because of slow transit constipation. Patient No 6 was proctocolectomised because of slow transit constipation. The patients were further investigated with magnetic resonance (MR) enterography and video capsule enteroscopy to establish whether there were any signs of IBD. If these examinations showed any pathology, push enteroscopy and ileocolonoscopy were also performed. All biopsies collected over the years were re-evaluated.

MR enterography did not reveal any pathological changes in any of the subjects. In three patients (Nos 1, 3, and 4), video capsule enteroscopy revealed Crohn-like ulcerations/erosions in the stomach and small intestine. Further examination of patient No 1 by push enteroscopy confirmed the erosions in the stomach and one third of the proximal small intestine. In patient No 3, capsule enteroscopy showed aphthous ulcers typical of Crohn’s disease throughout the distal jejunum and ileum (fig 1A). Ileocolonoscopy showed the same picture in the ileum and ileorectal anastomosis.

Histopathological examination of the full thickness biopsies from patient Nos 1 and 2 showed visceral degenerative neuropathy, combined with vellusisation of the intestinal cells of Cajal (ICC). In patient No 3 and 6, lymphocytic ganglioneuronitis was found in both neural plexa of the resected colon and ileum (fig 1B), with signs of neurone degeneration, and 50% and 80% reduction of ICCs in the perinervicillary ICC-plexus and deep muscular plexus of the circular muscle layer, respectively. Patient No 4 had a normal biopsy, and patient No 5 was not biopsied. Examination of mucosal biopsies from patient No 1 revealed focal active inflammation in the duodenum and caecum, and chronic inflammation in the rectum; patient No 5 had multifocal mild antral cryptitis, and both patients were diagnosed with suspected Crohn’s disease. Colon biopsies from patient No 6 revealed epitheloid cell granulomas and multinucleated giant cells, as well as multifocal transmural lymphoid hyperplasia consistent with Crohn’s disease.

In three patients (Nos 1, 3, and 4), dysmotility preceded the mucosal changes. In patients Nos 2 and 5, these two entities occurred simultaneously, while in patient No 6, dysmotility developed after proctocolectomy. Ganglionitis in patient No 3 could have been caused by Crohn’s disease before other symptoms of the disease developed. Treatment with 5-ASA has reduced her abdominal pain. The normal histology of the sigmoidum in patient No 4 does not exclude the possibility of ganglioneuritis in other parts of the bowel due to the known patchy involvement of the gut in Crohn’s disease.

The present observations indicate that apart from inflammation, even purely degenerative neuronal and ICC changes seen in CIP can occur in patients who also have IBD/IBD-like condition. At present, it is not known whether the observed abnormalities are part of IBD or independent of each other. This small patient sample prevents us from drawing any definite conclusion regarding this question. Further observations are needed to establish whether or not this connection is causal.

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References
Cannabinoid hyperemesis: not just a problem in Adelaide Hills

We read the article by Allen and colleagues (Gut 2004;53:1566–70) with interest and would like to report a case of probable cannabinoid hyperemesis seen in a district general hospital in the UK.

A 21 year old chef was admitted to our hospital on seven occasions over a two year period (April 2001 to December 2002) with profuse vomiting. Apart from a history of migraine as a child, he was fit and well. He smoked cannabis. Physical examination was unremarkable. The observation that the patient wanted to take regular baths because he had found that bathing eased the sickness was documented in the nursing notes but its significance was not appreciated. Investigations during attacks disclosed neutrophilia but blood urea, electrolytes, liver biochemistry, and serum amylase were normal. Abdominal x ray was also normal. Upper gastrointestinal endoscopy showed grade I oesophagitis and gastritis. Gastric biopsies were histologically normal. An abdominal ultrasound scan and small bowel barium follow through examination were normal. Additional normal or negative investigations included: autoantibodies and immunoglobulins, C reactive protein, and urinary porphyrin screen. Computed tomography scan of the brain was also normal.

During his last admission, the patient’s girlfriend showed us an article published in an Australian newsletter which she had obtained via the internet, in which Dr JH Allen had raised the possibility of a link between recurrent vomiting and cannabis abuse. With the aid of the internet we traced and contacted Dr Allen who shared his experience of this condition with us.

Reviewing the patient’s history, he freely admitted to smoking cannabis and experiencing the compulsive desire to bathe during bouts of vomiting. Following his last admission in December 2002, our patient stopped smoking cannabis and has remained free of symptoms. The clinical presentation was almost identical to the cases described by Allen et al, together with the response to cessation of smoking cannabis, supports the view that our patient was suffering from cannabinoid hyperemesis and that this condition is international.

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Inflammatory bowel disease stimulates formation of carcinogenic N-nitroso compounds

In patients with inflammatory bowel disease (IBD), the incidence of colorectal cancer is observed. Although severe inflammatory conditions per se represent a risk factor for neoplasia, we would like to draw attention to the possible role of increased activity of inducible nitric oxide synthase (iNOS), as found in IBD patients, in the endogenous formation of carcinogenic N-nitroso compounds (NOC). In healthy individuals, relatively small amounts of NOC are formed by the interaction between NOC precursors (NOCPs), present in dietary items such as meat and fish, and nitrosating agents derived from dietary nitrate. It has been proposed that endogenous formation of NOC may explain the link between meat consumption and colon cancer risk found in epidemiological studies. We hypothesised that as a result of chronic inflammatory conditions in the large intestine, increased colonic iNOS activity may produce an excess of NO, nitrogen oxides, and nitrite, which in turn react with NOC present in the colon to produce relatively high levels of NOC. Increased formation of NOC in IBD patients may thus contribute to the relatively high incidence of colorectal cancer associated with this disease.

A recent population based case control study showed that in cases with a history of IBD, increased exposure to drinking water nitrate was associated with an increased risk of colon cancer whereas no such association was found in the overall population. This clearly indicates that the risk of colon cancer in IBD patients is not only determined by the disease itself but dietary factors known to influence the endogenous formation of NOC are also associated with an increased risk in these patients. Although both the increased formation of NOC found in mice with chemically induced colitis and increased levels of NO and nitrite found in the colonic lumen of patients with ulcerative colitis support this hypothesis, NOC levels have never been investigated in IBD patients.

Therefore, we collected faecal samples from 17 patients diagnosed with ulcerative colitis and 17 healthy controls, and determined levels of N-nitrosodimethylamine (NDMA), a predominant carcinogenic NOC, using gas chromatography-mass spectrometry, as previously described. The study was approved by the medical ethics committee of the Maasland Hospital, Sittard, the Netherlands, and all patients gave their consent. In 41% of patients, we found levels of NDMA above the detection limit of 1 ng/g faeces, compared with 35% of controls. Comparison of concentrations in NDMA positive samples showed that the average concentration in patients was significantly higher than that in the control group (table 1). When IBD patients were subdivided into hospitalised and non-hospitalised cases, the difference between the non-hospitalised group and controls was even more pronounced, whereas NDMA concentrations in hospitalised patients and controls were similar. All hospitalised patients received only liquid nutrition (Nutren; Nutricia, UK) without additional intake of NOCP rich dietary foods, these results confirm that the combination of high dietary NOCP intake and inflammation may present a risk factor.

Most research on endogenous NOC exposure has focused predominantly on the intragastric formation of these compounds in relation to the gastric cancer risk. However, we now report that faecal NDMA levels in IBD patients are considerably higher than those we reported previously in gastric juice (0.25 (0.3) ng/g), which indicates that NOC exposure may be even more relevant in colon carcinogenesis.

Based on these results, we conclude that the colon of IBD patients is exposed to relatively high concentrations of this carcinogenic compound, probably as a direct consequence of continuous NO production by the inflammatory process. As this exposure may strongly contribute to the increased colon cancer risk associated with IBD, dietary recommendations for IBD patients, avoiding high NOCP intake, seem warranted.

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References


Table 1 Faecal N-nitrosodimethylamine (NDMA) concentrations in patients with inflammatory bowel disease (IBD) and in healthy controls

<table>
<thead>
<tr>
<th>NDMA (ng/g)</th>
<th>Controls (n = 17)</th>
<th>All IBD cases (n = 17)</th>
<th>Non-hospitalised cases (n = 10)</th>
<th>Hospitalised cases (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA positive</td>
<td>35</td>
<td>41</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td>NDMA (ng/g)*</td>
<td>1.4</td>
<td>10.9†</td>
<td>14.3†</td>
<td>2.4‡</td>
</tr>
</tbody>
</table>

*Average concentration of NDMA positive samples. t_p<0.05, t_p=0.01: significantly higher compared with the control group (Mann-Whitney U test). t_p<0.05: significantly lower compared with non hospitalised cases (Mann-Whitney U test).
Hepatocellular carcinoma occurring after successful treatment of childhood cancer with high dose chemotherapy and radiation

Hepatocellular carcinoma (HCC) is one of the world’s most common malignancies and accounts for more than 90% of all primary liver cancers. A number of different risk factors have been identified for the development of HCC. Hepatitis B carrier state, environmental toxins, chronic hepatitis C virus infection, hereditary haemochromatosis, and liver cirrhosis of almost any cause are well known risk factors for HCC. In addition, environmental toxins such as aflatoxins and contaminated drinking water may contribute to the pathogenesis of HCC, especially in Asia and underdeveloped countries. Finally, a number of HCC cases have occurred after the use of thorotrast for diagnostic procedures, and survivors of the atomic bomb of Hiroshima were also at higher risk for HCC development, indicating that radiation might also induce the development of HCC.

Herein we describe a rare case of HCC occurring in a patient 17 years after successful treatment of peripheral neuroectodermal tumour (PNET).

A 32 year old female presented with pain in the right upper quadrant of her abdomen. Seventeen years prior to presentation in our hospital this patient was treated for a PNET with a combination of high dose chemotherapy (vincristine, Adriamycin, ifosfamide, and actinomycin D) and surgical removal of the 10 x 5 cm tumour from her right chest followed by combined radiation (60 Gy) and chemotherapy. There were no signs of any recurrence of the tumour observed on her last check up 12 month earlier. Physical examination of the patient in our clinic showed typical signs of late radiation damage (erythema of the skin and an underdeveloped right breast) (fig 1). A firm 3–5 cm mass was palpable at the lower edge of the liver. Laboratory tests showed elevated α-fetoprotein (41881 μg/l). Hepatitis serology was negative and there was no evidence of any other liver disease. Magnetic resonance imaging revealed multiple intrahepatic masses up to 6.5 cm. A biopsy from the hepatic tumour was taken and confirmed the clinical diagnosis of HCC. The patient died three months after the diagnosis was made.

To the best of our knowledge, secondary HCC following high dose chemotherapy has never been described and therefore we searched the German Childhood Cancer Registry, which started to register all cases of malignancies in children (<15 years) in 1980. This database also collects data from secondary malignancies following chemotherapy. In this database we were able to detect a total of four more cases of secondary HCC, which are summarised in table 1. Interestingly one patient was hepatitis B surface antigen positive.

Radiotherapy has been shown to be associated with an increased risk of solid tumours 10–15 years after treatment and later. There is one report in the literature of a radiation induced hepatoma in a patient with a non-malignant haemangioendothelioma, occurred 20 years after radiation of the liver with 28.5 Gy. To date, the molecular mechanism of hepatocarcinogenesis is not completely understood. The main causative agents—hepatitis B virus, hepatitis C virus, and aflatoxin B1—have been identified, which together are responsible for approximately 80% of all HCC in humans. This series of cases clearly supports the notion that secondary HCC can follow not only radiation therapy of children but also high dose chemotherapy, and may prompt careful follow up examinations of the liver in patients with a possible risk for the development of HCC.

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Table 1 Details of five cases of secondary hepatocellular carcinoma

<table>
<thead>
<tr>
<th>First malignancy</th>
<th>Age (y)</th>
<th>Treatment</th>
<th>Age when HCC was diagnosed (y)</th>
<th>Time from first to second malignancy (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>4</td>
<td>Ctx*</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>4</td>
<td>Ctx, RTx</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>na</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PNET</td>
<td>15</td>
<td>Ctx, RTx</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Teratoma</td>
<td>2</td>
<td>na</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>ALL, acute lymphocytic leukaemia</td>
<td>PNET, peripheral neuroectodermal tumour; Ctx, chemotherapy; RTx, radiation therapy.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*This patient was hepatitis B surface antigen positive.


Biologics in inflammatory disease: infliximab associated risk of lymphoma development

In their excellent overview of currently available biologic compounds that are in use or under investigation for Crohn’s disease (CD), Sandborn and Faubion (Gut 2004;53:1366–73) reconfirm the unique standing of infliximab. They also note the ongoing discussion concerning the increased risk of lymphoproliferative disorders in patients who received infliximab.

Recently, we followed a 61 year old patient with a 31 year history of relapsing CD. Initial treatment was with steroids but after 10 years his CD was uncontrolled. There were no signs of any secondary neoplasms. He was treated with infliximab during his last admission. Total infliximab therapy included three doses of 400 mg (5 mg/kg) within two months and resulted in a marked reduction of CD activity (azathioprine was maintained). On admission 10 months after the last infliximab infusion, the patient relapsed again with ulcerations and multiple rectal fistulas. Biopsies showed a polymorphous tumour infiltrate. Tumour cells were positive for CD30 and negative for T and B cell markers as well as the anaplastic large cell lymphoma (ALK) and Epstein-Barr virus (EBV) associated proteins. A multiplex polymerase chain reaction approach revealed a clonal T cell population and an oligoclonal B cell population. Based on these results, the diagnosis was ALK negative anaplastic large cell lymphoma with null/T cell phenotype. Clinical stage was IAE.

CHOP-chemotherapy resulted in complete clinical and histological remission, which was evidenced by computer tomography, positron emission tomography, and negative rectal histology. Polymerase chain reaction analysis of the rectal biopsies revealed no T cell receptor rearrangement.

Three months later, the patient presented with postobstructive pneumonia. Bronchial biopsies showed a diffuse large B cell lymphoma. In contrast with the preceding rectal biopsies, bronchial tumour cells were positive for CD20, EBER, EBNA2, and LMP-1, indicating EBV infection of latency type III, were detected in tumour cells.
However, tumour cells were negative for CD30 and ALK protein. Molecular analysis demonstrated a monoclonal immunoglobulin heavy chain rearrangement in the absence of a T cell receptor rearrangement, confirming the diagnosis. The tumour was neither a T cell receptor rearrangement, confirming heavy chain rearrangement in the absence of CD30 and ALK protein. Molecular analysis of the tumour showed a negative immunohistochemical staining for CD30 and ALK, which ruled out anaplastic large cell lymphoma (ALCL). Furthermore, tumour cells were negative for CD30 and ALK, which rules out ALCL.

Genotypes 677TT and 677CT+1298AC of methylenetetrahydrofolate reductase are associated with the severity of ulcerative colitis in central China

Increased blood levels of homocysteine have been found to be associated with inflammatory bowel disease (IBD) in several studies.1 The main genetic determinant associated with elevated plasma levels of homocysteine (t-Hcy) is the MTHFR 677 TT variant.2 The MTHFR 677 TT allele has been found to be associated with an increased risk of ulcerative colitis, with an odds ratio of 3.4 compared to the wild-type genotype. The association of IBD with MTHFR 677 TT variant has been studied in several European populations.3,4 Double heterozygosity MTHFR 677TT+1298AC also reduces the enzyme activity and increases t-Hcy, but its association with IBD has never been studied.

In this study, we have evaluated the association of two common genotypes (TCN1 776G→C, a genetic determinant that influences transcobalam in B12, the cofactor of the methionine synthase with transcobalamin in central China who gave informed consent. This series was compared with 111 age and sex-matched controls. The research protocol was approved by the local appointed committee. Extraction of DNA and determination of polymorphisms were performed as described previously by us.5 A continuation corrected χ2 test and an ANOVA test were used, respectively, to assess differences in categorical and continuous variables between groups. Odds ratios of independent categorical variables

| Table 1 Patients with infliximab therapy and development of lymphoma |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Case | Age (y), sex | Disease | Dose | No. of infusions | Lymphoma | EBV |
| 1 | 77, M, NR | Burkitt lymphoma | NR | 10 | NR |
| 2 | 77, M, NR | Hodgkin lymphoma | NR | 16 | NR |
| 3 | 43, F, NR | B cell NHL | NR | 20 | NR |
| 4 | 34, M, NR | DLBL | NR | 30 | NR |
| 5 | 70, M, NR | DLBL | Positive | 40 | NR |
| 6 | 29, M, CD | 5 mg/kg, 3 | Hodgkin lymphoma | NR | 50 |
| 7 | 68, F, NR | B cell NHL | NR | 60 | NR |
| 8 | 62, M, NR | DLBL | NR | 70 | NR |
| 9 | 73, M, NR | Multiple | Hodgkin lymphoma | NR | 80 |
| 10 | 74, F, RA | 10 mg/kg, 8 | B cell NHL | NR | 90 |
| 11 | 48, M, RA | 10 mg/kg, 2 | B cell NHL | NR | 100 |
| 12 | 59, F, RA | 3 mg/kg, 5 | Multiple myeloma | NR | 110 |
| 13 | 61, M, RA | 1 mg/kg, 1 | Hodgkin lymphoma | NR | 120 |
| 14 | 66, M, CD, HIV | 10 mg/kg | B cell NHL | NR | 130 |
| 15 | 62, M, CD | 10 mg/kg, 1 | Intravascular B-NHL | NR | 140 |
| 16 | 48, F, DM | 5 mg/kg, 3 | DLBL | NR | 150 |
| 17 | 47, M | 6 mg/kg, 3 | DLBL | NR | 160 |

ALCL, anaplastic large cell lymphoma; CD, Crohn’s disease; DLBL, diffuse large B cell lymphoma; DM, dermatomyositis; NHL, non-Hodgkin lymphoma; NR, not reported; RA, rheumatoid arthritis.

References
that differed significantly between patients and controls were determined by logistic regression analysis. A p value <0.05 was considered to indicate statistical significance.

The main clinical characteristics are summarised in table 1. Most of the cases were recently diagnosed. None had any thrombotic manifestations. TCN1 776G allele frequency was approximately 1.5-fold higher compared with Caucasians, and we failed to find any association with the risk of UC or severity of disease. MTHFR 677T allele frequency in our control group was close to that reported in Caucasians. The only difference in frequency between patients and controls was at the limit of significance and this could be related to the limited size of our patient series (table 1). Secondly, these genotypes were associated with an increased risk of extensive UC (whole colon) (table 1), with an odds ratio of 4.92 (95% confidence interval 1.3–18.3; p = 0.017), after adjustment for age and sex.

In conclusion, our study showed that the genotypes of MTHFR, associated with a decrease in enzyme activity, seemed to be more significantly associated with extension of disease than with the primary risk, at least in central China.

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Conflict of interest: None declared.

References


CORRECTION
doi: 10.1136/gut.2004.045299corr1

The original article by Cosnes et al (Impact of the increasing use of immunosuppressants in Crohn’s disease on the need for intestinal surgery. Gut 2005;54:237–41), published in the February 2005 issue was incomplete. Figure 2 was missing from the proof. A corrected version of the pdf can be viewed at http://gut.bmjournals.com/cgi/data/54/2/237/DC1/1, and the missing figure can be seen here.

Figure 2

Table 1 Clinical characteristics and methylenetetrahydrofolate reductase (MTHFR) and transcobalamin (TCN) polymorphisms in 72 patients with ulcerative colitis (UC) and 111 controls from central China

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ulcerative colitis</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>72</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>35/37</td>
<td>58/60</td>
<td>0.9423</td>
</tr>
<tr>
<td>Age (y) (mean (SD))</td>
<td>41 (15)</td>
<td>40 (13)</td>
<td>0.4859</td>
</tr>
<tr>
<td>Age of onset (y) (mean (SD))</td>
<td>38 (14)</td>
<td>38 (14)</td>
<td></td>
</tr>
<tr>
<td>Current smoker (n (%))</td>
<td>1 (1.4)</td>
<td>1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Extent of UC (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>40 (55.6)</td>
<td>40 (55.6)</td>
<td></td>
</tr>
<tr>
<td>Left colitis</td>
<td>15 (20.8)</td>
<td>15 (20.8)</td>
<td></td>
</tr>
<tr>
<td>Right colonic</td>
<td>1 (1.4)</td>
<td>1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Total colon</td>
<td>17 (23.6)</td>
<td>17 (23.6)</td>
<td></td>
</tr>
<tr>
<td>Treatment (n (%))</td>
<td>5 (7.1)</td>
<td>5 (7.1)</td>
<td></td>
</tr>
<tr>
<td>5-ASA/SASP</td>
<td>53 (73.6)</td>
<td>53 (73.6)</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>15 (20.8)</td>
<td>15 (20.8)</td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>13 (18.6)</td>
<td>13 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Intestinal resections (n (%))</td>
<td>1 (1.4)</td>
<td>1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Genotypic polymorphisms (n (%)) [95% CI]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR 667T allele</td>
<td>50 (34.7) [27.3–42.7]</td>
<td>41 (34.7) [27.2–42.7]</td>
<td>0.2286</td>
</tr>
<tr>
<td>MTHFR 677TT</td>
<td>10 (13.9) [7.2–23.0]</td>
<td>18 (13.9) [12.6–23.0]</td>
<td>0.5707</td>
</tr>
<tr>
<td>MTHFR 677CT C allele</td>
<td>18 (25.0) [18.8–32.0]</td>
<td>41 (31.6) [26.8–36.2]</td>
<td>0.2889</td>
</tr>
<tr>
<td>MTHFR 677CT+1298AC</td>
<td>4 (6.1) [2.0–14.0]</td>
<td>15 (11.5) [7.9–22.7]</td>
<td>0.0755</td>
</tr>
<tr>
<td>MTHFR 677TT/CT+1298AC</td>
<td>14 (21.2) [12.6–32.0]</td>
<td>38 (31.6) [22.7–44.3]</td>
<td>0.0659</td>
</tr>
<tr>
<td>TCN 776CG</td>
<td>62 (85.7) [74.3–93.6]</td>
<td>89 (77.4) [69.3–84.3]</td>
<td>0.2236</td>
</tr>
<tr>
<td>TCN 776GC+GG</td>
<td>42 (58.6) [43.7–72.3]</td>
<td>71 (64.0) [53.6–66.2]</td>
<td>0.5709</td>
</tr>
</tbody>
</table>

TCN 776CG allele frequency, as previously observed in two countries with a lower allele frequency, was considered together, that corresponds to decreased catalytic activity. Firstly, the difference in frequency between patients and controls was at the limit of significance and this could be related to the limited size of our patient series (table 1). Secondly, these genotypes were associated with an increased risk of extensive UC (whole colon) (table 1), with an odds ratio of 4.92 (95% confidence interval 1.3–18.3; p = 0.017), after adjustment for age and sex.

In conclusion, our study showed that the genotypes of MTHFR, associated with a decrease in enzyme activity, seemed to be more significantly associated with extension of disease than with the primary risk, at least in central China.

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