Prediction of antitumour necrosis factor clinical efficacy by real-time visualisation of apoptosis in patients with Crohn’s disease

Jan M H Van den Brande, Tamara C Koehler, Zuzana Zelinkova, Roelof J Bennink, Anje A te Velde, Fibo J W ten Cate, Sander J H van Deventer, Maikel P Peppelenbosch, Daniel W Hommes

Background: The human anti-tumour necrosis factor (TNF) antibody infliximab binds to the membrane TNF and subsequently induces apoptosis of activated lamina propria T lymphocytes in patients with Crohn’s disease in vitro.

Aim: To test whether the ability of rapid anti-TNF-induced apoptosis in the gut predicts the efficacy of anti-TNF treatment in inflammatory bowel disease.

Methods: 99mTc-technetium–annexin V single-photon emission computer tomography (SPECT) was performed in 2 models of murine experimental colitis and in 14 patients with active Crohn’s disease as assessed by the Crohn’s Disease Activity Index (CDAI) to study the effect of anti-TNF treatment on apoptosis in the intestine during active colitis. Disease activity was evaluated 2 weeks after infliximab infusion using the CDAI (definition response: drop of >100 points).

Results: Colonic uptake of 99mTc-annexin V significantly increased in 2,4,6-trinitrobenzene sulphonate-induced colitis as well as in transfer colitis on administration of anti-TNF antibodies compared with a control antibody as determined with dedicated animal pinhole SPECT. In addition, uptake of 99mTc-annexin V significantly increased in patients with active Crohn’s disease responding to infliximab treatment. Colonic 99mTc-annexin V uptake ratio (mean (SEM)) increased from 0.24 (0.03) to 0.41 (0.07) (p<0.01), 24 h after infliximab infusion (5 mg/kg). A mean increase of 98.7% in colonic uptake of 99mTc-annexin V could be detected in 10 of the 14 responding patients (CDAI >100 points at week 2) compared with 15.2% in non-responding patients (p=0.03). Analysis of the mucosal biopsy specimens identified lamina propria T cells as target cells undergoing apoptosis.

Conclusions: These in vivo observations support the notion that colonic uptake of 99mTc-annexin V correlates with clinical benefit of anti-TNF treatment and might be predictive of therapeutic success.
METHODS
Animal models
The animal studies ethics committee of the University of Amsterdam, Amsterdam, The Netherlands, approved of all experiments. BALB/c mice (Harlan Sprague Dawley, Horst, The Netherlands) and CB17 severe combined immunodeficient (SCID) mice (Charles River, Someren, The Netherlands) were used. The BALB/c mice were housed under standard conditions, whereas the SCID mice were housed in filter-top cages under specific pathogen-free conditions at the animal care facility. The experiments were conducted in 8- and 10-week-old female BALB/c mice and in 7–10-week-old female CB17 SCID mice.

2,4,6-Trinitrobenzene sulphonate colitis
Acute colitis was induced as described previously. Two doses of 1 mg of 2,4,6-trinitrobenzene sulphonic acid (TNBS; Sigma Chemical, St Louis, Missouri, USA) dissolved in 40% ethanol (Merck, Darmstadt, Germany) were rectally administrated (separated by a 7-day interval, ie, on days 0 and 7) using a vinyl catheter that was positioned 3 cm from the anus. During the instillation, the mice were anaesthetised using isoflurane (1-chloro-2,2,2-trifluoroethanol-isoflurane-diluromethyl-ether; Abbott Laboratories, Queenborough, Kent, UK). They were kept vertically after the instillation for 60 s. In total, 30 mice were used, which were divided into three groups.

Transfer colitis
Chronic CD45RBlow transfer colitis was induced as described previously. BALB/c splenocytes were first enriched for CD4 cells by red-cell lysis and negative selection by using the following rat anti-mouse monoclonal antibodies (mAbs): B220 (clone RA3-6B2), Mac-1 (clone M1/70) and CD8α (clone 53-6.7) (gift from Dr R Mebius, Vrije Universiteit Medical Center, Amsterdam, The Netherlands). mAb-stained cells were removed in a magnetic field by using sheep anti-rat IgG-coated magnetic beads (Dynal, Hamburg, Germany). The resulting CD4 cells were stained with cytochrome-conjugated CD4 and fluorescein isothiocyanate-conjugated CD45RB (both BD Biosciences-Pharmingen, San Diego, California, USA) mAbs. Subpopulations of CD4 cells were generated by two-colour sorting on the FACS Vantage (BD Biosciences). Populations were found to be >95% pure on reanalysis. To induce colitis, only CD45RBlow CD4 cells (1–x 10^6) were transferred to two groups of CB17 SCID mice. In all, 13 mice were used, divided into 2 groups. Animals were either injected with a chimaeric murine/rat monoclonal anti-TNF-α antibody (clone cV1q; gift from D Shealy, Centocor, Horsham, Pennsylvania, USA) as a single intraperitoneal injection (1 mg/mouse), or with a chimaeric murine/rat monoclonal cVαM negative control antibody, or a vehicle, 64 days after the transfer model or 1 day after the second TNBS administration. For injection and scintigraphy, mice were sedated by a single intraperitoneal administration of 0.5 ml/10 g with FFDM mix, consisting of 0.1 ml fentanyl/fluanisone (Hynpnom; Vetapharma, Leeds, UK), 0.1 ml domperidum (5 mg/ml, Roche Nederland BV, Woerden, The Netherlands) and 1.5 ml NaCl 0.9% (Fresenius Kabi Nederland BV, Hertogenbosch, The Netherlands). After 24 h of treatment, mice were injected intravenously with 50 MBq 99mTc-hydrazinonicotinamide (99mTc-HYNIC) annexin V component is renally fully excreted with neither hepatic nor intestinal clearance.

Assessment of inflammation
Mice with chronic colitis (transfer model) were weighed twice a week and mice with acute colitis (TNBS model) were weighed daily. Weight loss (percentage of baseline body weight) of 25% in transfer colitis was considered adequate. After killing the mouse, the colon weight was used as an index of disease-related intestinal wall thickening. The longitudinally divided colons were rolled up and fixed in 4% buffered formalin. Fixed tissues were embedded in paraffin wax, and 4-μm sections were stained with H&E for histological grading. In the transfer model, inflammation was scored on a scale of 0–4, representing no inflammation to severe inflammation. A different scoring system was used for the TNBS colitis model by using the following parameters: (1) percentage of colon involved, (2) fibrosis, (3) oedema, (4) erosions and ulcerations, (5) crypt loss, (6) infiltration of mononuclear cells or (7) polymorphonuclear cells. The total score ranged from 0 (normal colon) to a maximum of 20 points (most severe inflammation). Tissue slices were analysed by a pathologist blind to the study, and scored accordingly.

Treatments and radiolabelling
For injection and scintigraphy, mice were sedated. A chimaeric rat/mouse monoclonal anti-TNF-α antibody (clone cV1q; gift from D Shealy, Centocor) was administered as a single intraperitoneal injection (1 mg/mouse). As negative control groups, age-matched mice were treated with either intraperitoneal 1 ml saline or injection of an irrelevant IgG1 isotype chimaeric antibody (Vam (1 mg/mouse; gift from D Shealy, Centocor)). 99mTc-HYNIC annexin V in kit formulation was prepared according to the guidelines by the manufacturer (Theseus Imaging). After 24 h of treatment, mice were injected intravenously with 50 MBq of the radiopharmaceutical compound 99mTc-HYNIC annexin V (further referred to as 99mTc annexin V) followed by scintigraphy after 1 h. 99mTc-HYNIC annexin V component is renally fully excreted with neither hepatic nor intestinal clearance.

Patients
Patients were recruited from the clinical department and outpatient clinic of the Department of Gastroenterology and Hepatology, University of Amsterdam, Amsterdam, The Netherlands. Disease activity was determined using the Crohn’s Disease Activity Index (CDAI). Patients were required to be taking stable drugs: no initiation or change in dosages was allowed within 8 weeks for thiopurine treatment or methotrexate, and within 2 weeks for corticosteroids. Patients who received infliximab within 8 weeks before the initiation date were excluded. After written informed consent was obtained, patients underwent a baseline colonoscopy and scintigraphy. Endoscopy included assessment of disease activity and collection of biopsy specimens. Severity of endoscopic disease was categorised according to the Simple Endoscopy Score. Mucosal specimens were placed in formalin and prepared for H&E staining at the site of inflammation for confirmation of disease activity. After concluding both the endoscopic and scintigraphic procedures, patients received a 2 h intravenous infusion of infliximab (Remicade, 5 mg/kg body weight) at the day care centre. Repeated endoscopy and scintigraphy was performed 24 h after infliximab dosing. Response to infliximab was defined as a drop of >100 points in the CDAI 2 weeks after infliximab treatment. This study was approved by the local medical ethical review board of the Academic Medical Center, Amsterdam, The Netherlands.

Mucosal T cell isolation and analysis
Mucosal biopsy specimens were collected from three patients during endoscopy and immediately placed in ice-cold phosphate-buffered saline (PBS). To isolate lamina propria mononuclear cells (LPMNCs), biopsy specimens were mechanically destroyed using an automated mechanical tissue-disaggregation device (Medimachine System, Dako, Glostrup, Denmark).
The obtained cell suspensions were washed twice in PBS. To prevent changes in the studied surface markers, no collagenase step was used during isolation. Cell suspensions were centrifuged with Ficoll (Pharmacia, Uppsala, Sweden) and the mononuclear cells were subsequently passed through a 40 μM filter cell strainer (Becton/Dickinson Labware, Franklin Lakes, New Jersey, USA). Cells were then washed with FACS buffer (PBS, containing 0.5% bovine serum albumin, 0.3 mmol/l EDTA and 0.01% sodium azide) and were kept on ice for the rest of the procedure. After washing, LPMNCs were placed in FACS buffer and stained with CD4 PE antibody for 30 min on ice. Subsequently, LPMNCs were washed twice with PBS and placed in annexin-binding buffer and incubated 15 min with annexin V—fluorescein isothiocyanate, and then with 7-aminoactinomycin (7AAD, Via-Probe BD Bioscience) for 5 min, both on ice in the dark, and analysed by flow cytometry (FACS Calibur). 7AAD was used to exclude necrotic cells. The LPMNC population was gated according to the localisation on the forward/side scatter, and further analysis of the percentage of CD4 apoptotic cells was performed in the 7AAD-negative subpopulation—that is, necrotic cells. Data shown are representative for three patients.

Scintigraphy

Scintigraphy in animals was performed using a dedicated animal pinhole γ-camera SPECT as described earlier. A tungsten pinhole insert of 3 mm aperture was used. All studies were acquired with a 15% energy window on the 140 keV 99mTc photo peak. An operating workstation (Hermes, Nuclear Diagnostics, Stockholm, Sweden) was used to control both the camera and the step motor. The SPECT acquisition was performed 1 h after intravenous injection of 50 MBq 99mTc-annexin. In all, 50 projections of 30 s in a 64×64 matrix were acquired in a 360° orbit. SPECT reconstruction was performed using an application program (Hermes, Nuclear Diagnostics) adapted to pinhole SPECT, using filtered back-projection. A Butterworth post reconstruction filter (order, 5; 0.8 cycle/cm) was applied.

Images were interpreted and scored by an experienced nuclear medicine physician, blinded to the cytological data. A colon uptake ratio (CUR) was determined semiquantitatively based on an uptake hierarchical supervisor-guided protocol. The CUR was expressed as a background-corrected ratio to bone marrow (CUR). Pre- and post-therapeutic scans were assessed in the same segments of colon or ileum.

Statistical analysis

Results are expressed as median (range) or as mean (SEM) where appropriate. All statistical tests were performed using SPSS v 2.01 for Windows. A p value of <0.05 was considered to represent a significant difference. Differences in mice colorectal 99mTc uptake between the various treatment groups (NaCl, control antiserum and m2TNF) were analysed by the heteroscedastic one-tailed t test. Differences between treatment groups in time were tested by analysis of variance for repeated measures. Correlations between change in CDAI and CRP were analysed using Spearman’s correlation coefficient (r).

RESULTS

Experimental colitis

For the experiments in TNBS colitis, a total of 30 mice were used in 4 separate experiments. For the transfer colitis, CD45RBhigh CD4 cells (1–4×10⁶) were transferred to two groups of CBI7 SCID mice. In this experiment, a total of 13 mice were used, in two separate experiments. Both models were effective in inducing colitis: in the transfer model weight loss was observed 3 weeks after the transfer, whereas in the TNBS model, colitis was evident with mice demonstrating diarrhea and rectal blood loss. BALB/mice had a baseline histopathological score of 0.3 (0.5), and normal SCID mice had a score of 0.2 (0.2). In TNBS-induced colitis, the histopathological score increased to 10.6 (2.8), whereas in the transfer colitis the histopathological score increased to 1 (0.6). Hence, all mice had colitis at time of collection of the colons, and this was confirmed by decreasing body weight curves and increasing colon weights (data not shown). In mice with either transfer or TNBS colitis treated with NaCl, 99mTc–annexin V was hardly detectable in the colon (figs 1 and 2A,B). The average colorectal 99mTc uptake was 0.53 (0.27) and 0.31 (0.23), respectively. On administration of negative control chimaeric antibody cVaM, the average colorectal 99mTc uptake was 0.44 (0.19) and 0.32 (0.32) for the transfer and TNBS models, respectively. Thus, chimaeric antibodies are not proapoptotic as such. Injection of the chimaeric murine/rat monoclonal C1V1q caused an average colorectal 99mTc uptake of 1.11 (0.48) for the transfer model and 1.04 (0.63) for the TNBS model (fig 1 and 2A,B). The average colorectal 99mTc uptake ratio differed significantly between NaCl and anti-murine TNF antibodies in the transfer and TNBS colitis models, p = 0.016 and p = 0.01, respectively. Likewise, the average colorectal 99mTc uptake ratio differed significantly between control chimaeric antibodies and anti-murine TNF antibodies in the transfer and TNBS colitis models, p = 0.04 and p = 0.02, respectively. Healthy rodents show little annexin V binding in the mucosa of the intestine (not shown), demonstrating that such high annexin V binding is not a characteristic of the uninflamed mucosa. On the basis of the annexin uptake ratio in experimental colitis, we concluded that anti-TNF antibodies cause a rapid increase in apoptosis in the murine colon.

Patients with Crohn’s disease

Fourteen patients with active Crohn’s disease consented to participate in this study. Table 1 summarises the patients’ characteristics. The mean CDAI was 381 (range 229–587) points, the mean CRP 55.9 (3–154) mg/l, and all except one patient had active distal disease accessible for endoscopy. One patient had a severe ileitis, and did not undergo endoscopy. All patients received corticosteroids and/or immunomodulators at...
baseline (table 1). Three patients received episodic infliximab infusions before inclusion. These patients had shown a clinical response to their last infusion. Infliximab treatment did not result in any serious adverse event. All patients underwent uncomplicated dual SPECT scanning (at baseline and within 24 h after infliximab treatment).

Clinical response to infliximab

Figure 3A,D shows the clinical responses of the 14 included patients. The mean CDAI at baseline for all patients was 381 points (range 220–587), which decreased to 198 at week 2 (range 36–394, p<0.01). At week 2, the CDAI decreased >100 points in 10 patients (mean decrease 226 points, range 113–359), demonstrating a predefined clinical response. Four patients failed to show a response to infliximab, the mean change in CDAI was 12 points. In addition, responses were confirmed by changes in CRP. CRP levels decreased significantly on infliximab treatment (at baseline and within 24 h after infliximab treatment).

The mean levels decreased from 50.4 l to 7.5 mg/l (fig 3B, p = 0.04). No significant correlation was observed between change in CDAI and change in CRP (r = −0.19, p = 0.317; −0.097%, p = 0.0742).

99mTc Annexin V uptake

Baseline SPECT scanning displayed limited 99mTc–annexin V accumulation in the colon, and the CUR was 0.24 (0.03). An increase in CUR to 0.41 (0.07) was observed (p<0.01) 24 h after receiving 5 mg/kg infliximab (fig 3C; table 2). Figure 5 shows representative SPECT-CT images of the sigmoid colon before and after infliximab infusion. Subsequently, we investigated whether the accumulation of 99mTc–annexin V was different in patients exhibiting a favourable response to infliximab from those who did not display an objective response. The CUR increased in both responders and non-responders, but interestingly the increase in CUR was markedly higher in the group of responders (fig 3D). In 10 responding
patients, the mean increase in CUR was 98.6%, compared with 15.2% in the 4 non-responding patients (p = 0.03; fig 3D). Endoscopic investigation of the patients involved, before experimentation, had shown that disease was present in the colon or both in the colon and small intestine. The scintigraphic signals obtained corresponded with these diseased regions, at least at this macroscopic level (table 2), especially in the colon. The correlation with disease localisation and increased annexin V uptake was striking and held true for 13 of 14 patients (1 did not undergo endoscopy). These data suggest that higher levels of apoptosis correlate with clinical response to infliximab. 99mTc–annexin V uptake was analysed before and after infliximab treatment in the subgroup of seven patients using azathioprine (AZA; an apoptosis-inducing agent27) at baseline. Co-treatment with infliximab did not result in higher CUR in this subgroup receiving AZA (fig 3E). Importantly, a volunteer not displaying colitis was infused with 99mTc–annexin V, and on analysis showed little annexin V binding in the intestine (not shown), demonstrating that the high annexin V binding is not a characteristic of the uninflamed mucosa, but is apparently an active representation of the clinical healing process.

Mucosal T cell analysis
To test whether lamina propria T cells were indeed target cells for annexin V binding, LPMNCs were isolated from fresh biopsy specimens and the percentage of apoptotic cells in the CD4 positive subpopulation was analysed in three patients. The analysis of 7AAD-negative cells showed marked increase in apoptosis of CD4 cells 24 h after infliximab administration (fig 4). This suggests that apoptosis of lamina propria T cells contribute to the increased level of apoptosis in the intestine.

DISCUSSION
Infliximab has become the mainstay for the treatment of steroid-refractory Crohn’s disease; however, the molecular mechanism underlying its beneficial effects remains unclear and it is difficult to predict the clinical success of this expensive treatment in individual patients. Recently, it was shown that in vitro infliximab can bind to membrane-bound TNF and cause apoptosis in activated T cells15 possibly through reverse signalling,16 but the in vivo clinical relevance of these findings remained unclear. In this study, we showed in vivo that apoptosis in the intestine is induced on chimaeric anti-TNF treatment in both experimental colitis and in human Crohn’s disease.

Apoptosis is a distinct mode of cell death and represents a crucial regulatory mechanism to remove redundant cells in many physiological events. Recently, the imaging of drug-induced apoptosis has generated enormous interest both in experimental and clinical research.24 Annexin V has been validated for in vivo scintigraphic imaging of apoptosis both in animals20–22 and in humans.23 24 In the management of malignant disorders, this has proved a valuable tool for the accurate monitoring of apoptotic tumour response and chemosensitivity,25–27 as well as the early prediction of chemoresistance in response to various pro-apoptotic interventions.28 As further evidence that the chronicity of inflammation in Crohn’s disease is largely due to apoptotic-resistant cells in affected

### Table 1 Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>14</td>
</tr>
<tr>
<td>Male/Female</td>
<td>9/5</td>
</tr>
<tr>
<td>Age, years (mean (range))</td>
<td>34 (21–47)</td>
</tr>
<tr>
<td>Baseline CDAI, points (mean (range))</td>
<td>381 (229–387)</td>
</tr>
<tr>
<td>Baseline CRP, mg/l (mean (range))</td>
<td>55.9 (3–154) mg/l</td>
</tr>
<tr>
<td>Baseline endoscopy score [SES (24)]</td>
<td>4.9 (0.5–1.5)</td>
</tr>
<tr>
<td>Disease duration, years (mean (range))</td>
<td>4.9 (0.5–1.5)</td>
</tr>
<tr>
<td>Localisation of Crohn’s disease</td>
<td>Large intestine 9, Combination large and small intestine 5, Fistulising/non-fistulising disease 6/8</td>
</tr>
<tr>
<td>Co-medications</td>
<td>5-AZA 7, Corticosteroids 9, Azathioprine 7, 6-Thioguanine 1, Methotrexate 5</td>
</tr>
</tbody>
</table>

AZA, azathioprine; CDAI, Crohn’s Disease Activity Index; CRP, C reactive protein; SES, Simple Endoscopy Score.
Crohn’s mucosa, we have previously shown that infliximab exerts a pro-apoptotic effect on lamina propria or peripheral T cells from patients with active Crohn’s disease, which was subsequently shown by Di Sabatino et al. to be caspase-dependent phenomenon. The increased accumulation of radioactivity after infusion of infliximab in the colonic region of patients with Crohn’s disease is certainly in agreement with this hypothesis, but this interpretation of our data critically depends on the assumption that the colonic scintigraphic signals obtained are derived from annexin binding to apoptotic T cells. To confirm this, T cells were isolated from biopsy specimens of three study patients and investigated with FACS for the presence of annexin V and the apoptosis status in the T cell compartment by co-staining with CD3 antibodies and 7AAD. 7AAD only enters membrane-compromised cells and binds to DNA. When the 7AAD-negative compartment was investigated (thus excluding necrosis), an increase in annexin V-positive cells was observed after infliximab infusion. Thus, the increased scintigraphic signals seem to include apoptosis in the T cell compartment by co-staining with CD3 antibodies and 7AAD. 7AAD only enters membrane-compromised cells and binds to DNA. When the 7AAD-negative compartment was investigated (thus excluding necrosis), an increase in annexin V-positive cells was observed after infliximab infusion. Thus, the increased scintigraphic signals seem to include apoptosis in the T cell compartment and provide, to a certain extent, a bona fide representation of in vivo apoptosis. This observation correlates well with studies in which scintigraphic imaging was used for examining in vivo apoptosis, providing evidence that the signals obtained are truly generated by annexin V binding to apoptotic cells. The observation that absolute levels of apoptosis correlate well with the clinical response in patients has important implications. Non-responders do not seem to have an intrinsic deficiency in their capacity to react to infliximab with apoptosis; rather, the levels of apoptosis induced in non-responders seem to be insufficient. To further support this notion, both in responders as well as non-responders, CRP levels are reduced in response to infliximab treatment hence, it seems that the magnitude of the infliximab-induced apoptosis is important, and non-responders’ reaction to the treatment by increasing apoptosis in the intestine is not sufficiently large to

Figure 3  Quantification of annexin V single-photon emission computer tomography (SPECT) before and after infliximab treatment of patients with Crohn’s disease. (A) Clinical response to infliximab as measured by Crohn’s Disease Activity Index (CDAI). (B) Plasma C reactive protein (CRP) levels at baseline and after infliximab treatment. (C) Apoptosis as measured by annexin V SPECT [just before (baseline) and 24 h after infliximab infusion, expressed as colonic uptake ratio (CUR; see Methods section)]. (D) Responders to infliximab have a higher CUR of 99mTc-annexin V (98.6%) than non-responders (15.2%, p = 0.03). The results show a correlation between the occurrence of high amounts of apoptosis in the intestine 24 h after infliximab infusion and clinical response to infliximab, determined 2 weeks after infliximab treatment. (E) Influence of co-medication on infliximab-induced CUR responses. Azathioprine (AZA) did not influence the incorporation of 99mTc-annexin V in the gut mucosa in patients with Crohn’s disease receiving maintenance treatment. NS, not significant.
allow clinical recovery. A logical consequence of this notion is that when these patients are given apoptosis-inducing drugs along with infliximab, clinical responses may become apparent. We recently observed that methotrexate might act synergistically with infliximab by inducing apoptotic responses in activated T cells (unpublished observations). Also, AZA is known to induce apoptosis as a consequence of Rac1 inhibition and is thus an interesting candidate for co-application with infliximab. Interestingly, no significant increase was seen in baseline CUR in patients with AZA co-medication compared with the AZA-negative group. A possible explanation for this observation is that the low level of apoptosis might not be detectable in the maintenance setting. Finally, the observation that apoptosis levels in non-responders are lower than in responders requires validation in a larger cohort of patients, but, if confirmed, would provide a tool that can predict the response to infliximab and increase cost effectiveness.

In conclusion, infliximab rapidly induces apoptosis in the intestine of patients with Crohn’s disease. This is probably a key initiating event that triggers mucosal healing in patients. Further studies are warranted to study differences in the regulation of apoptosis in patients with Crohn’s disease and the possibilities of personalising biological treatment.

ACKNOWLEDGEMENTS

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**Table 2** Summary of disease localisation, endoscopic activity, colonic uptake ratio and severity of Crohn’s disease in patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Disease localisation</th>
<th>ROI</th>
<th>Endoscopic activity</th>
<th>CUR (baseline)</th>
<th>CUR (infliximab)</th>
<th>△CDAI (R/NR)</th>
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<tbody>
<tr>
<td>1</td>
<td>Pancolitis</td>
<td>Descendens</td>
<td>Severe</td>
<td>0.180 853</td>
<td>0.328 016</td>
<td>359 (R)</td>
</tr>
<tr>
<td>2</td>
<td>Pancolitis</td>
<td>Descendens</td>
<td>Severe</td>
<td>0.145 025</td>
<td>0.337 290</td>
<td>113 (R)</td>
</tr>
<tr>
<td>3</td>
<td>Left-sided colitis</td>
<td>Descendens</td>
<td>Moderate</td>
<td>0.288 160</td>
<td>0.448 471</td>
<td>295 (R)</td>
</tr>
<tr>
<td>4</td>
<td>Left-sided colitis</td>
<td>Descendens</td>
<td>Moderate</td>
<td>0.225 481</td>
<td>0.409 617</td>
<td>282 (R)</td>
</tr>
<tr>
<td>5</td>
<td>Terminal ileitis</td>
<td>Ileum</td>
<td>Not performed</td>
<td>0.213 216</td>
<td>0.279 879</td>
<td>40 (NR)</td>
</tr>
<tr>
<td>6</td>
<td>Left-sided colitis</td>
<td>Sigmoid</td>
<td>Mild</td>
<td>0.131 881</td>
<td>0.263 881</td>
<td>193 (R)</td>
</tr>
<tr>
<td>7</td>
<td>Left-sided colitis+</td>
<td>Sigmoid</td>
<td>Moderate</td>
<td>0.541 645</td>
<td>0.119 820</td>
<td>118 (R)</td>
</tr>
<tr>
<td>8</td>
<td>Pancolitis colitis</td>
<td>Sigmoid</td>
<td>Severe</td>
<td>0.346 508</td>
<td>0.366 517</td>
<td>0 (NR)</td>
</tr>
<tr>
<td>9</td>
<td>Ileocolitis†</td>
<td>Ascendens</td>
<td>Severe</td>
<td>0.152 953</td>
<td>0.500 448</td>
<td>240 (R)</td>
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<td>10</td>
<td>Left-sided colitis</td>
<td>Descendens</td>
<td>Moderate</td>
<td>0.042 321</td>
<td>0.059 937</td>
<td>-44 (NR)</td>
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<tr>
<td>11</td>
<td>Left-sided colitis†</td>
<td>Sigmoid</td>
<td>Severe</td>
<td>0.195 094</td>
<td>0.251 746</td>
<td>271 (R)</td>
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<tr>
<td>12</td>
<td>Right-sided colitis</td>
<td>Ascendens</td>
<td>Moderate</td>
<td>0.242 579</td>
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<td>150 (R)</td>
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<tr>
<td>13</td>
<td>Left-sided colitis+</td>
<td>Descendens</td>
<td>Moderate</td>
<td>0.318 501</td>
<td>0.679 708</td>
<td>239 (R)</td>
</tr>
<tr>
<td>14</td>
<td>Ileitis/colitis</td>
<td>Sigmoid</td>
<td>Moderate</td>
<td>0.421 178</td>
<td>0.346 258</td>
<td>51 (NR)</td>
</tr>
</tbody>
</table>

CDAI, Crohn’s Disease Activity Index; CUR, colonic uptake ratio; NR, non-responders; R, responders; ROI, region of interest.

*Including transverse colon. †Including stenosis. ‡Excluding rectosigmoid.
Figure 5 Example of 99mTc–annexin V single-photon emission computer tomography (SPECT) of human Crohn’s disease. Apoptosis in the intestine of patients with Crohn’s disease was visualised just before (baseline) and 24 h after infliximab treatment. The picture shows a representative SPECT image in pseudocolours and a CT image with a superimposed SPECT image. The scintigraphic signals obtained corresponded with the diseased regions in the colon, indicating a correlation with disease localisation and increased 99mTc–annexin V uptake.

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**REFERENCES**


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survival rates range between 5% and 22% in different series.

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was performed. No histological evidence of lymphatic metastases. Surgical wide local excision with lymphadenectomy anorectal junction with two centimetric right femoral adenopathy. Thoraco-abdominal CT scan revealed mild thickening of the anorectal junction and harbouring sheets of infiltrating melanocytes, focal nesting and intra-cytoplasmic pigmentation.

Endoscopic biopsies revealed malignant melanoma (fig 1). From the question on page 508

Answer

Endoscopic biopsies revealed malignant melanoma (fig 1). A thoraco-abdominal CT scan revealed mild thickening of the anorectal junction with two centimetric right femoral adenopathies. Surgical wide local excision with lymphadenectomy was performed. No histological evidence of lymphatic metastasis was found.

Anorectal melanomas are a very small group of mucosal melanomas with unknown aetiology and poor prognosis. They represent 1% of all melanomas and are the third most common form of melanoma. Recent epidemiologic data indicate a bimodal age distribution. To date there is no information on whether infection with the human papilloma virus plays a role in tumourigenesis. The lesions can be misdiagnosed as haemorrhoids on clinical examination.

No survival advantage has been shown for abdominoperineal resection over wide local excision. The benefit of radiotherapy after the latter is still a matter of debate. The overall 5 year survival rates range between 5% and 22% in different series.