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SAFETY AND EFFICACY OF INTRAVENOUS CYCLOPHOSPHAMIDE PULSE THERAPY IN THERAPY REFRACTORY CROHN'S DISEASE PATIENTS

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A major challenge in the management of persistently active Crohn's disease patient's refractory to treatment regimen following the current guidelines is the induction of remission, which is a prerequisite for subsequent maintenance therapy. The aim of this study was to evaluate both the clinical and endoscopic benefit of intravenous cyclophosphamide pulse therapy in patients with active and therapy refractory Crohn's disease. Nine patients with acute moderate to severe Crohn's disease, not responding to conventional as well as biological therapy regimen received 3 – 9 cycles of monthly treatments with intravenous cyclophosphamide (680 – 1000 mg) in an uncontrolled setting and were retrospectively analyzed. Eight of nine patients (88.9%) had a clinical response (measured by a decrease in the Harvey-Bradshaw index, HBI ≥ 3) and two of nine patients (22.2%) achieved clinical remission (HBI ≤ 4) at week 8 after two applications of intravenous cyclophosphamide therapy. These response and remission rates remained unchanged after individual completion of cyclophosphamide therapy. Median HBI decreased from 18 (7 – 25) at the beginning of therapy to 7 (3 – 18) at week 8. 5 of 9 patients (56%) showed endoscopic response (defined by a reduction of ulcers) and one patient (11%) reached endoscopic remission (defined by the absence of ulcers) after the last application of cyclophosphamide. Arthralgia, which was present in 4 of 9 (44%) patients, was unchanged in most patients after cyclophosphamide therapy, although one patient described a marked reduction in joint pain. Cyclophosphamide pulse therapy was well tolerated during the whole treatment course in all subjects. One patient with long-standing Crohn's disease was diagnosed with a high-grade intraepithelial neoplasia in the rectum and underwent surgical intervention, where the diagnosis of an early stage adenocarcinoma was made. We concluded that intravenous cyclophosphamide pulse therapy was well tolerated by most patients and effective for inducing clinical and endoscopic response and remission in patients with therapy refractory Crohn's disease. In patients who are unresponsive to available therapies, including available biological treatment options, cyclophosphamide therefore represents a potential option to induce therapeutic response, which must then be maintained by other treatment modalities.

Key words: *Crohn's disease, inflammatory bowel disease, arthralgia, mucosal healing, cyclophosphamide, Harvey-Bradshaw index, corticosteroids*

INTRODUCTION

Systemic corticosteroids are still the treatment of choice during acute severe flares in Crohn's disease patients. However, 20 – 30% of patients only show a partial response while 16% of patients are even refractory to treatment with systemic corticosteroids (1). Moreover, corticosteroids are not able to maintain remission or induce mucosal healing in sufficient patient numbers (2). Anti-tumor necrosis factor-antibodies and the α4β7-anti-adhesion molecule vedolizumab are effective therapies to induce and maintain remission in moderate to severe active Crohn's disease. These substances represent effective treatment options, particularly after failure of previous immunomodulatory treatment. In spite of the increasing therapeutic options in Crohn's disease, there is still a pronounced need for a rapidly acting and long lasting alternative treatment in patients not responding to available therapy regimen for the induction of clinical and endoscopic response and remission.

Cyclophosphamide, initially patented 1962 for oncological purposes, constitutes a central position in the treatment of inflammatory disorders including systemic lupus erythematodes, systemic vasculitis, granulomatosis with polyangiitis and distinct forms of arthritis (3, 4). Cyclophosphamide is an alkylating molecule with direct cytotoxic effects of its metabolite chloracetaldehyde upon resting and activated lymphocytes, which leads to its immunosuppressive effect (5). It has been shown that cyclophosphamide suppresses the function of T-helper cells in patients with rheumatoid arthritis, which causes a reduction of activated T-cells ($CD25^{+}HLA^{-}DR^{+}$) by 30 – 40%. Moreover, it leads to the marked reduction of CD19+ B-lymphocytes and CD16+CD65+ natural killer cells (6).

In the context of therapy refractory Crohn's disease, the application of intravenous cyclophosphamide represents a

possible treatment option to induce rapid therapeutic response. Constantly increasing data is available regarding its well-known efficacy in rheumatology (3-13), but only very little data exist with respect to the treatment of Crohn's disease patients (2, 14-16).

PATIENTS AND METHODS

Patients

Between April 2013 and July 2016, nine patients (5 female and 4 male) with moderate to severe Crohn's disease (measured by the Harvey-Bradshaw index (HBI) as a simple index of Crohn's disease activity (17), median at baseline was 18, range 7 – 25) and a mean duration of disease of 6 years (range 1 - 32 years), received 3 – 9 (median 5) monthly treatments with intravenous cyclophosphamide (mean single dose 900 mg, range 680 – 1000 mg,) in an uncontrolled setting and were retrospectively analyzed. Patients total number of treatments was based on clinical and endoscopical response. The respective dose, administered all 4 weeks, was calculated according to body weight (10 mg/kg) in 36% of all administrations (18/50), 64% of all cyclophosphamide infusions (32/50) were administered as a fixed dose of 1000 mg. Two patients received their last two infusions of intravenous cyclophosphamide as an additional treatment starting at week 49 and 51, respectively, as they experienced a clinical relapse. *Table 1* shows an overview of all 9 patients, describing their individual number of cumulative cyclophosphamide infusions, the mean single dose, the dosing range and calculated cumulative dose.

The data was collected at the Department of Internal Medicine I, University Hospital of Erlangen. No external financial support or funding was needed to perform this study, there are no conflicts of interest to declare. Our research complies with the guidelines for human studies. Since we performed a retrospective analysis, no statement from the ethics committee was needed.

Research data

Diagnosis of Crohn's disease was made in all patients according to standard criteria, including prior clinical assessment, and endoscopy, histology and radiology findings. In addition to physical examination, standard laboratory tests and stool cultures were used to rule out concomitant infections. CMV-associated colitis was excluded by immunohistochemical staining of intestinal tissue samples obtained from endoscopic evaluation prior to the initiation of the cyclophosphamide pulse therapy.

At week 0, when the first application of intravenous cyclophosphamide took place, 5 of 9 patients (55.6%) received concomitant medication with prednisolone (2.5 – 50mg/d, mean dosage 30.5 mg/d). In all 5 patients, the prednisolone dosage was tapered after beginning of the cyclophosphamide therapy, reaching a mean dosage of 4 mg/d (0 – 0mg/d) at week 20 (*Fig. 1*).

No corticoid therapy was initiated or increased during the course of cyclophosphamide treatment in any of the patients.

Pre-existing therapy with budesonide was continued in two patients (22%) at a dose of 3 and 9 mg/d respectively, throughout the duration of the cyclophosphamide therapy. One of these patients additionally self-administered budesonide topically in

Table 1. Individual dosing regimen of patients receiving intravenous cyclophosphamide pulse therapy.

Patient No	Number of cumulative cyclophosphamide infusions	Mean single dose in mg	Range single dose in mg	Calculated cumulative dose in mg
1	9	708	680 – 750	6370
2	4	720	700 – 750	2880
3	5	900	750 – 1000	4500
4	6	875	750 – 1000	5250
5	6	1000	1000	6000
6	8	1000	1000	8000
7	4	1000	1000	4000
8	5	1000	1000	5000
9	3	1000	1000	3000

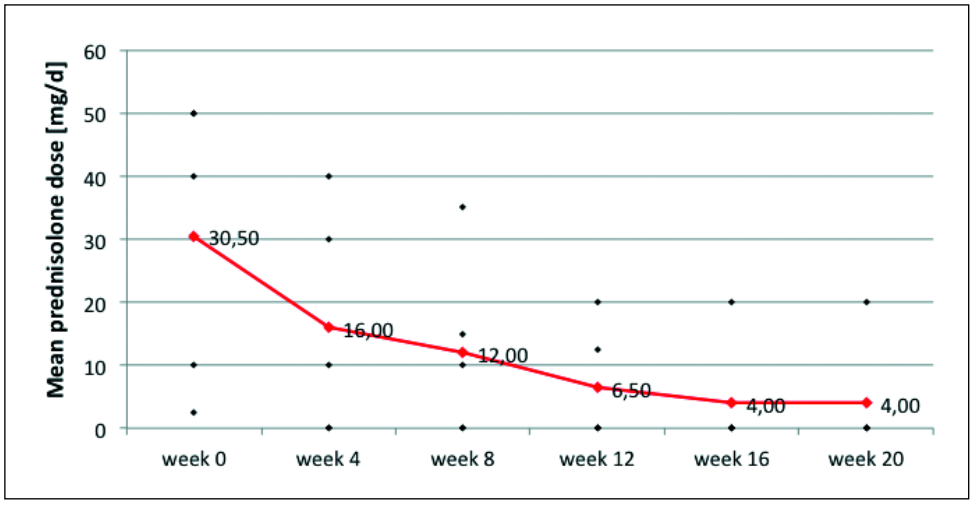

Fig. 1. Concomitant medication of the Crohn's disease patients with prednisolone during the course of the cyclophosphamide pulse therapy.

Table 2. Clinical characteristics of Crohn's disease patients receiving intravenous cyclophosphamide pulse therapy.

Patient No	Age/sex	Duration of CD	Previous surgery	HBI before tratment	EIM	Localization of Crohn's disease	Prior medication	Smoking status
1	40/F	6	none	18	arthralgia	I, C, R	AZA, 6MP, MTX, IFX, ADA, Tac, CicA	non-smoker
2	35/M	1	none	24	none	duodenum, jejunum, I, C	AZA, IFX	non-smoker
3	35/F	4.5	none	7	none	stomach, duodenum, I	AZA, MTX, IFX, ADA	former-smoker (stop 2002)
4	53/M	32	2	15	arthralgia	I, C	AZA, MTX, IFX, ADA	active-smoker (30py)
5	38/F	20.1	2	19	arthralgia	I, C, R	AZA, IFX, ADA	former-smoker (stop 2004)
6	25/F	6	none	25	arthralgia	esophagus, stomach, I	AZA, MTX, IFX, ADA	non-smoker
7	49/F	25	none	12	none	I, C	AZA, ADA	non-smoker
8	58/M	6	none	19	none	C, R	AZA, IFX, ADA, VDZ, CicA	former-smoker (stop 1985)
9	32/M	20	none	14	none	I, C, R	AZA, IFX, VDZ, CicA	non-smoker

form of a rectal foam during the whole cyclophosphamide course. These two patients did not receive any other specific medication for their Crohn's disease. Two patients (22%) received concomitant medication with immunmodulators. Among them, a pre-existing therapy with azathioprine was continued in one patient, in another patient 6MP was started after the 6th administration of cyclophosphamide.

All patients treated with cyclophosphamide had been refractory to conventional treatments and anti-tumor necrosis factor (TNF) antibody therapy. In particular, all nine patients had been refractory to treatment with azathioprine, 4 of 9 patients (44.4%) did not respond adequately to a treatment with methotrexate. All nine patients were refractory to a therapy with at least one anti-TNF-antibody. 8 of 9 patients (88.9%) were refractory to infliximab treatment and 6 of 9 patients (66.7%) did not respond sufficiently to subsequent treatments with adalimumab or infliximab. Two out of the nine patients (22.2 %) had unsuccessful therapy with vedolizumab and 3 out of 9 (33.3%) were refractory to previous calcineurin inhibitor (cyclosporine A, tacrolimus) treatment.

One out of the nine patients (11.1%) did not receive infliximab but was refractory to therapy with the anti-TNF-antibody adalimumab after failing treatment with azathioprine. Proposed infliximab therapy was rejected by the patient. 33.3% (3 of 9) of the patients received a combination therapy with an anti-TNF-antibody and azathioprine or 6-mercaptopurine without sufficient improvement of the disease before starting intravenous cyclophosphamide pulse therapy. 44.4% (4 of 9) of the patients had extraintestinal manifestations (EIM), consisting of arthritis. None of the patients had any other EIM. Two out of the nine patients (22.2%) already underwent repeated surgery due to their Crohn's disease prior to the first intravenous application of cyclophosphamide, additionally emphasizing the disease severity of these patients. A summary of patient characteristics is shown in *Table 2*.

Cyclophosphamide was intravenously applied during an inpatient hospital stay combined with an intravenous rate of fluids at a rate known to produce an urine output of 2 ml/kg/h. Four hours after the application of cyclophosphamide, all patients received 2-mercaptoethansulphonic acid (mesna) in a dosage equivalent to 50% of the cyclophosphamide dose to prevent hemorrhagic cystitis. Follow-up for each administration of cyclophosphamide included a differential blood count on post-cyclophosphamide days 10, 14 and 21 to detect WBC nadir and rebound levels.

Endoscopic examination was performed within 1 week before starting cyclophosphamide pulse therapy in 8 out of 9 patients, one patient received endoscopy 4 weeks before starting cyclophosphamide treatment. In In the time period between endoscopy and beginning the time of endoscopy and beginning of cyclophosphamide application, no new Crohn's disease therapy was initiated, nor was an existing therapy regimen changed. Each patient received at least 1 endoscopic re-evaluation during the course of the cyclophosphamide pulse therapy.

The Harvey-Bradshaw index (HBI) has been chosen as a simple score to clinically assess Crohn's disease activity and was calculated each time when cyclophosphamide was administered. Clinical response to treatment was defined as a HBI-decrease ≥ 3 points from baseline. Clinical remission was defined by a HBI score ≤ 4 (17, 18).

All patients were evaluated for adverse and serious adverse events according to the patients reports, medical history, physical examination and laboratory findings throughout the duration of the cyclophosphamide application.

C-reactive protein (CRP) serum levels were controlled before the first cyclophosphamide application took place and at each time when cyclophosphamide was administered throughout the study.

Statistical analysis

All results are described as median, mean and range. Statistical differences were calculated using the Wilcoxon rank test, wherein significance was considered at a P-value of ≤ 0.05 using SPSSwin.

RESULTS

Patients response to cyclophosphamide treatment

Four weeks after the first application of cyclophosphamide, 88.9% (8 of 9) of the patients showed a clinical response, defined by a reduction of the HBI ≥ 3 compared to baseline HBI (range of reduction from 3 to 15). Two patients achieved remission at week 4 with a decrease of the HBI score by 4 and 15 points, resulting in a HBI score of 3 and 4, respectively (*Fig. 3*). Only one patient did not clinically respond to the first cyclophosphamide application at week 4, as he had an unchanged HBI score of 18. Looking at the time of the third application of cyclophosphamide at week 8, rates of clinical response and clinical remission remained unchanged compared to week 4.

The median HBI of all patients decreased to almost one third of its initial value of 18 at week 0 to 6.5 at week 12. Reduction of the median HBI score was greatest between week 0 and 4 (7 points), followed by a further reduction from week 4 to 8 (4 points) and week 8 to 12 (0.5 points). Subsequently, the median

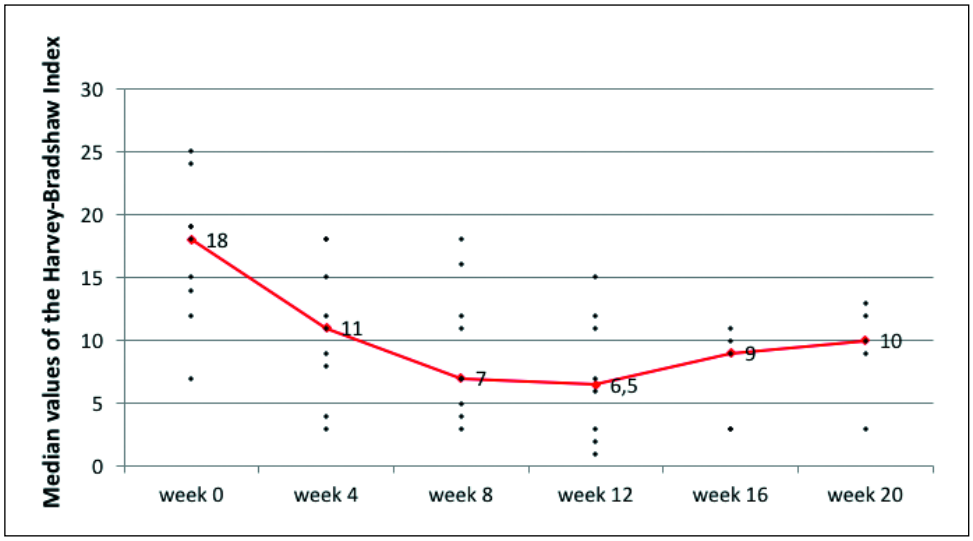

Fig. 2. Median Harvey-Bradshaw index (HBI) values of the Crohn's disease patients receiving cyclophosphamide pulse therapy.

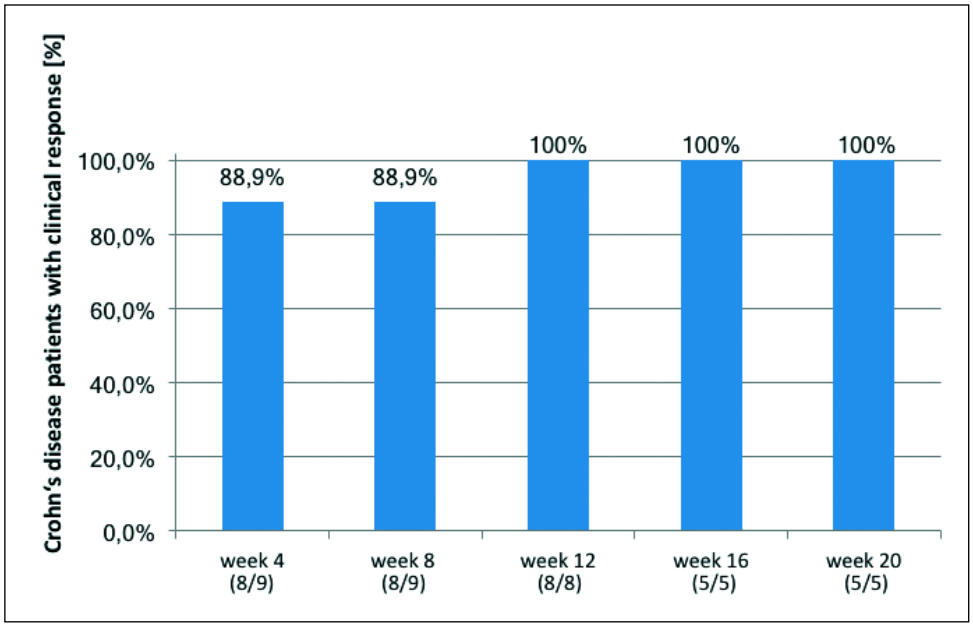

Fig. 3. Portion of Crohn's disease patients receiving cyclophosphamide pulse therapy with clinical response, defined as a decrease in the Harvey-Bradshaw index (HBI) of ≥ 3, from 4 to 20 week.

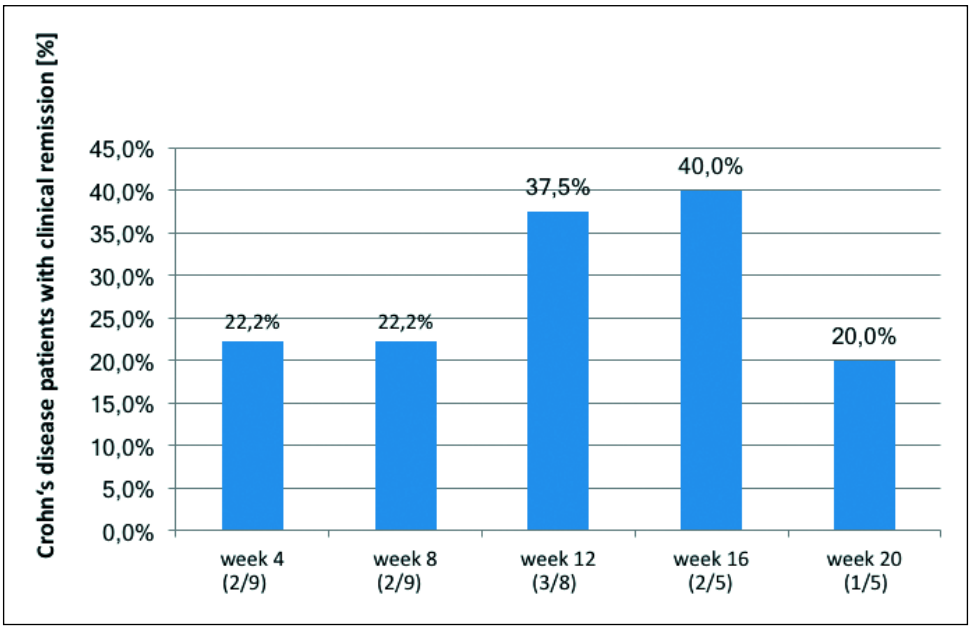

Fig. 4. Portion of Crohn's disease patients receiving cyclophosphamide pulse therapy with clinical remission, defined as a Harvey-Bradshaw index (HBI) score ≤ 4, from 4 to 20 week.

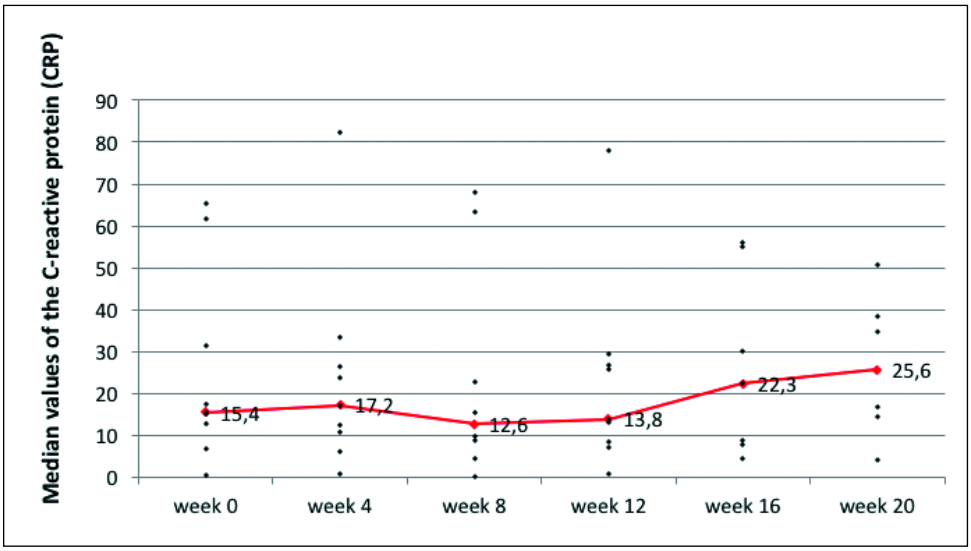

Fig. 5. Median C-reactive protein (CRP) values of all Crohn's disease patients treated with cyclophosphamide pulse therapy from week 0 to week 20.

HBI showed a discrete increase between week 12 and 16 (2.5 points) and from week 16 to 20 (1 point), resulting in a median HBI value of 10 at week 20 (*Fig. 2*).

Regarding week 12, when the 4th application of cyclophosphamide took place, all 8 patients (100%) showed a clinical response with a mean reduction of the HBI by 11.5 (range 3 to 23) points, clinical remission was observed in 37.5% (3 of 8) of all treated patients (*Figs. 3* and *4*). At week 16 and 20, when the 5th and 6th cyclophosphamide administration took place, 5 patients were still receiving cyclophosphamide. One patient was switched to maintenance therapy with adalimumab after reaching clinical and endoscopic remission, marked by a reduction of the HBI score from 24 at week 0 to 7 at week 12 and signs of mucosal healing in the follow-up endoscopy, which was performed at the time of the last cyclophosphamide application. The cyclophosphamide pulse therapy was stopped in another patient after week 8 due to the diagnosis of a high-grade intraepithelial neoplasia in the rectum, which turned out to be an early-stage adenocarcinoma. The last patient not receiving another cyclophosphamide pulse therapy at week 16 was postponed to week 20 due to clinical worsening while reducing the concomitant medication with prednisolone. The rate of patients in clinical response at week 16 and 20 was 100% (5/5 patients at week 16 and 20), clinical remission was observed in 40% and 20% of patients (2/5 and 1/5 patients) at week 16 and 20, respectively. One patient did not receive the cyclophosphamide infusion at week 20, as the patient showed persistent active disease and unchanged endoscopic signs of mucosal inflammation. Only two patients received further administration of cyclophosphamide pulse therapy after week 20. One of them reached prior clincal remission at week 8, lasting until week 20 during the first course of intravenous cyclophosphamide pulse therapy. The patient then experienced a relapse of disease at week 51, which was treated by re-initiation of cyclophosphamide therapy. This led to clinical improvement (HBI reduction from 21 to 13). After week 51 no further cyclophosphamide infusions were performed. The other patient received three more applications of cyclophosphamide at week 24, 49 and 53, which led to clinical improvement. HBI remained stable during the treatment (12 at week 24, 10 at week 49 and 13

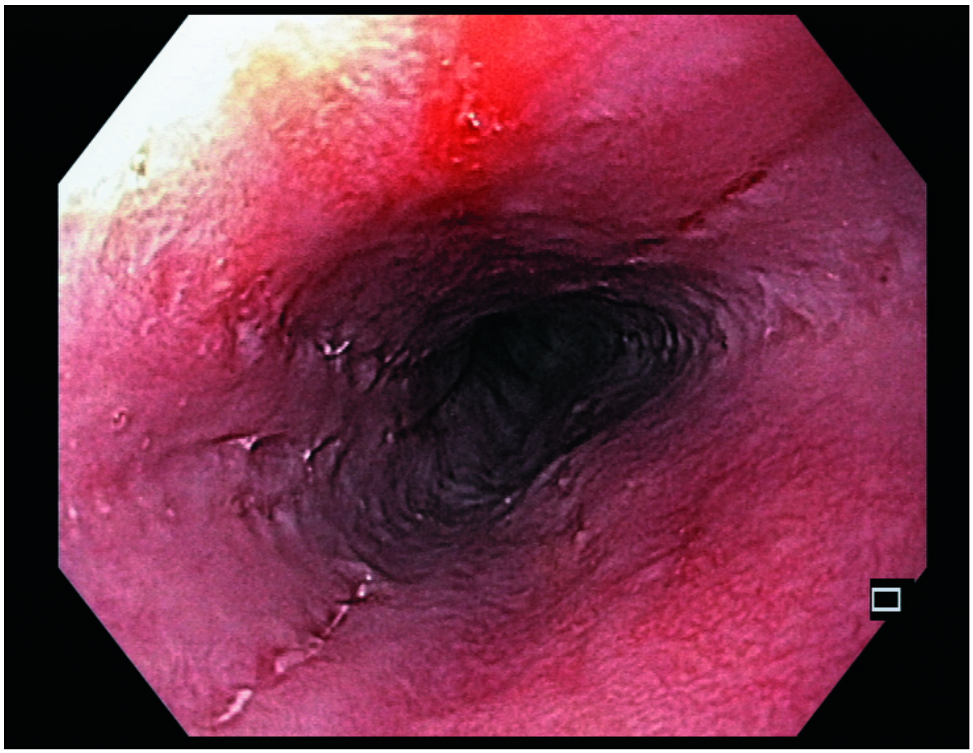

Fig. 6. Endoscopically active Crohn's esophagitis, that was histologically confirmed in a patient prior to the initiation of cyclophosphamide therapy.

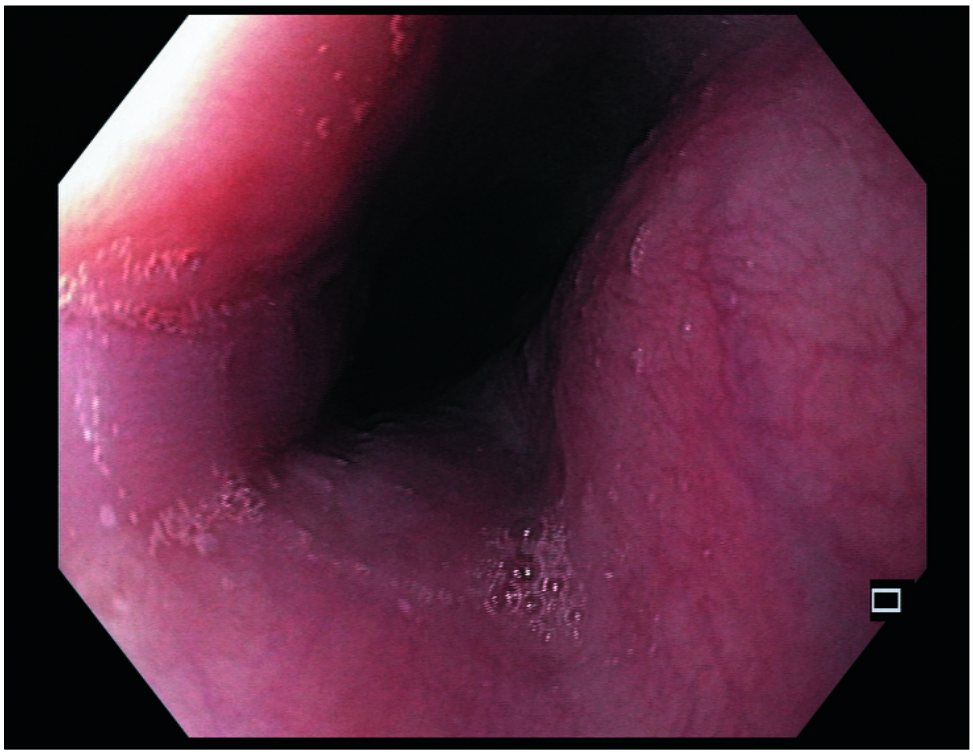

Fig. 7. Mucosal healing with no endoscopic signs of erosions or ulcers in the esophagus after 6 administrations of intravenous cyclophosphamide pulse therapy. In the histological examination, only signs of discrete inflammation were reported.

at week 53). No further applications of cyclophosphamide were performed afterwards, as the patient had reached a cumulative dose of 6 g cyclophosphamide.

All results for clinical response until week 12 have been calculated using the signed Wilcoxon rank test and showed significance with a P-value of 0.008.

Eight of nine patients (88.9%) showed clinical response at week 4 and week 8, and at ween 4 and week 8. At week 12, 16 and 20, all remaining patients maintained clinical response. Two of nine patients (22.2%) showed clinical remission at week 4 and 8, while 3 of 8 patients (37.5%) and two of five patients (40%) showed clinical remission at week 12 and 16, respectively. At week 20 one out of five treated patients (20%) was in clinical remission.

Concerning median concentration of C-reactive protein, no significant decrease could be observed from week 0 to week 20. The initial median CRP value of 15.4 mg/l (range 0.3 – 65.2) at

week 0 did not markedly differ at week 4 – 20 (12.6 – 25.6 mg/l, range 0.2 – 82.2). The exact results for each time-point are shown in *Fig. 5*.

Extraintestinal manifestations

Four of nine patients (44.4%) initially suffered from arthirtis, assessed as IBD-associated spondylarthropathy. No other types of extraintestinal manifestations were reported.

During the cyclophosphamide pulse treatment 1 of 4 patients (25%) became symptom-free regarding joint involvement at week 8, synchronized with the onset of clinical remission regarding the patients Crohn's disease. This effect persisted throughout the full course of the cyclophosphamide pulse treatment with the last administration performed at week 16. The patient was in clinical remission regarding both Crohn's disease and accompanying joint involvement until week 51, when a relapse of both occurred.

Two of four patients with arthralgia (50%) likewise reported joint involvement during the complete course of cyclophosphamide pulse therapy, although reaching clinical response concerning the underlying Crohn's disease at week 4 and 12, respectively.

Safety

In general, cyclophosphamide pulse therapy was well tolerated by all patients, regardless of the duration of the therapy.

One third of the patients (33%, 3 out of 9) complained about newly occurred nausea after beginning of cyclophosphamide pulse therapy. However, this side effect was well controlled in all patients using ondansetron as an antiemetic therapy.

Other reported adverse events were dry, scaly palms in one patient, which was successfully treated topically. Another patient complained about pronounced fatigue directly after the third administration of cyclophosphamide, lasting for four days. However, this only occurred once.

One patient showed a complicated urinary tract infection during the second planned hospital stay for administration of cyclophosphamide, which led to the postponement of cyclophosphamide therapy for another four weeks. There was proof of *Candida albicans* in the urine and in the blood culture taken from the port-catheter in this patient. The patient received the anti-mycoticum caspofungin and the port system was surgically removed. The taken blood culture was also indicative of *Klebsiella pneumonia*, *Enterococcus faecalis* and *Enterococcus faecium*, which led to a therapy with the antibiotics vancomycin and meropenem. Three days later the patient demonstrated acute kidney failure with a creatinine level of 2.55 mg/dl, probably due to heightened vancomycin blood levels. After adjusting the dosage of vancomycin, all kidney parameters normalized within two weeks. The patient also showed a drop in the hemoglobin level from 12 g/dl, to 7.6 g/dl 2 weeks later, which was most likely associated with the persistent inflammation and transient renal failure, since the patient showed no signs of bleeding. The patient received two erythrocyte concentrates and recovered quickly. It has to be noted, that this patient showed clinical and endoscopic remission after completing the cyclophosphamide pulse therapy.

One patient received a surgical intervention due to a perineal abscess with extension to the left edge of the vagina, infiltrating the labie after the fifth administration of cyclophosphamide pulse therapy. Another patient with an extended infestation of his colon and disease duration of 20 years showed a high grade intraepithelial neoplasia in a tissue sample of the rectum in a follow-up endoscopy at the time of the third administration of intravenous cyclophosphamide. This patient underwent surgical intervention, where the diagnosis of an early-stage adenocarcinoma was made. The development of this adenocarcinoma of the rectum from our point is rather the result of a 20-years uncontrolled chronic-active inflammatory disease course of the patient, than the result of two cyclophosphamide applications.

No patient showed significant laboratory changes related to the cyclophosphamide pulse therapy, concerning renal and liver parameters, WBC, hemoglobin or thrombocyte count. None of the patients died during the application of cyclophosphamide pulse therapy. Regarding safety considerations, no unexpected AE's or SAE's occurred.

Due to insufficient clinical and endoscopic improvement, two patients did not receive the 6th cyclophosphamide administration at week 20. Two other patients with previous clinical response (one of them with clinical remission) received two more administrations of cyclophosphamide pulse therapy around week 50. However, after these two additional administrations, cyclophosphamide therapy was stopped due to insufficient effectiveness and to prevent excessive cumulative doses of cyclophosphamide. A cumulative life time dose of 6 g was not exceeded in our studies, as this dosage has been described to increase the risk of cancer (19). However, other studies with the same questioning and partly other entities investigating Caucasian patients, describe no substantial risk of late-occuring, serious malignancies for cumulative lifetime dosages lower than 20 g of cyclophosphamide (20).

In patients without clinical remission, a trend could be observed towards a longer duration of the disease and a more intense use of immunosuppressive agents prior to cyclophosphamide pulse therapy. Moreover, no patient with previous surgical intervention (n = 2) and no active smoker (n = 1) entered clinical remission. With respect to the localization of the disease, we observed, that the two patients, who reached clinical remission at week 8 and the only patient reaching endoscopic remission, had an upper gastrointestinal involvement. No patient with colonic or ileocolonic disease reached clinical remission.

No clear distinctive features could be further discovered in our investigated, small group of patients regarding response or non-response to the cyclophosphamide therapy. Other criteria such as higher baseline HBI values or higher baseline CRP levels were not suitable to distinguish between responders and non-responders to cyclophosphamide treatment.

Endoscopic changes in inflammation

All included patients had endoscopically active inflammation at baseline. Five out of nine patients (56%) showed endoscopic response (defined by a reduction of ulcers) to the treatment with cyclophosphamide, all 5 patients showed clinical response during endoscopic examination, which was performed in all patients at the time of the last cyclophosphamide infusion. One of nine patients (11.1%) received early endoscopy at week 6 due to an extensive disease with initially endoscopic signs of high inflammation in the upper gastrointestinal tract including the duodenum. This patient showed early endoscopic response as well as the second patient who received early endoscopy at week 8 due to a known inflammatory stenosis in the neoterminal ileum and persisting abdominal pain.

Despite the high rate of patients with clinical response and more than half of the patients with endoscopic improvement of the lesions, only one patient reached complete endoscopic remission with disappearance of ulcers. In this 35-year-old, male patient, the last endoscopic assessment of the upper gastrointestinal tract including the jejunum, at the time of the last administration of

cyclophosphamide at week 13, no longer showed active signs of inflammation, indicating an effectiveness of the cyclophosphamide pulse therapy regardless of the infestation pattern. This patient was also suitable to fulfill the criteria of mucosal healing, with the resolution of visible ulcers in the endoscopic assessment (21).

However, a second patient showed significant endoscopic improvement, when assessed after the sixth administration of intravenous cyclophosphamide. This 25-year-old woman had extended Crohn's disease affecting the terminal ileum and the upper gastrointestinal tract including active inflammation of the esophagus (*Fig. 6*), the stomach and the duodenum.

In the endoscopic reassessment after six administrations of intravenous cyclophosphamide, the patient only showed mild signs of inflammation in the upper gastrointestinal tract, which is shown in *Fig. 7*. Moreover, this patient also showed clinical remission at week 8.

DISCUSSION

Biological treatment represents the current standard therapy in patients with active refractory Crohn's disease, not adequately responding to steroids or immunmodulatory treatment. Despite the high effectiveness of anti-TNF antibody treatment in moderate to severe active Crohn's disease, up to 30% of the patients are primary non-responders and up to 46% of the patients lose response over time (22).

The antibody against the integrin α4β7, vedolizumab, represents a further treatment option, particularly in patient's refractory to anti-TNF-antibody therapy. Its efficacy however, is limited to subgroups of patients again. Considering vedolizumab as a treatment in Crohn's disease patients, the response to the treatment with vedolizumab seems to be slower compared to anti-TNF-antibody therapy and reduced in anti-TNF refractory patients (23). According to the GEMINI II trial, 14.5% of the patients receiving vedolizumab were in clinical remission at week 6, compared to 6.8% of the patients in the placebo group ($P = 0.02$) (24).

By analyzing the subgroup of patients with Crohn's disease, in whom tumor necrosis factor antagonist treatment failed, Sands *et al.* showed that patients treated with vedolizumab showed no statistically significant difference in reaching clinical remission at week 6 compared to patients treated with placebo (15.2% of the patients treated with vedolizumab versus 12.1% of those given placebo, $P = 0.433$). However, 4 weeks later, at week 10, significantly more patients treated with vedolizumab (26.6%) than patients receiving placebo (12.1%) were in clinical remission ($P = 0.001$) (25).

Patients with higher clinical Crohn's disease activity (CDAI > 330) showed impaired clinical response at week 6, but not at week 52 in the GEMINI II trial (26, 27). This data is relevant when deciding about treatment options aiming at induction and maintenance of remission in patients with severe Crohn's disease, who are refractory to anti-TNF agents.

Since anti-TNF-antibodies and meanwhile also the α4β7-integrin antibody vedolizumab represent well established therapies for patients with active Crohn's disease, who are not adequately responding to standard immunmodulatory therapy, the clinical challenge has shifted to the group of patients with a disease course, that is therapy-refractory to both available biological substance classes and now also to those that are also refractory to the third approved biological substance class consisting of the interleukin-12/interleukin-23 antibody ustekinumab.

Furthermore, several novel substances are currently tested in clinical trials, which might change the therapeutic spectrum regarding Crohn's disease. Future therapeutic options are expected to refer to more various mechanisms of action, accompanying a better understanding of the pathogenesis of Crohn's disease including the different role of the respective immune cells. For example, Hagel *et al.* suggested by measuring TNF-α and histamine plasma levels, that the proportion of TNF-α secreted from mast cells in the plasma in patients with inflammatory bowel disease is less important. In this context, the finding, that histamine and TNF-α are jointly released from mast cells within minutes, is of great importance (28).

In our study, cyclophosphamide pulse therapy was administered to patients with active Crohn's disease, not adequately responding to conventional therapy regimen. All patients were refractory to at least one anti-TNF-antibody treatment. Six of those patients were even refractory to two anti-TNF antibodies. As the third anti-TNF agent, certolizumab is not approved for treatment of Crohn's disease patients in Germany, three patients were subsequently unsuccessfully treated with a calcineurin inhibitor. After approval of vedolizumab, this therapy was initiated in one patient without clinical efficacy. All other patients were treated with cyclophosphamide prior to the approval of vedolizumab for the treatment of Crohn's disease in Germany. At the time of our study, ustekinumab had not yet been approved in Germany, but would now offer a valid alternative therapeutic option for the patients described.

Although we could find a high rate of clinical response (100%) at week 8, only 2 of 9 patients (22%) at week 8 and 3 of 8 patients (37.5%) at week 12 reached clinical remission. Five out of nine patients showed endoscopic improvement during the cyclophosphamide treatment, but only one patient reached complete endoscopic remission.

Mucosal healing evolved into a key treatment goal in IBD in the last years, since it was associated with lower rates of hospitalization and surgery, lower steroid use, higher remission rates, a decreased risk of colorectal cancer, higher clinical remission rates and improved quality of life (29, 30). Thus, while managing the treatment with cyclophosphamide pulse therapy, we also gave great importance to the endoscopic evaluation during cyclophosphamide therapy.

Admittedly, an existing weakness of our study is the lack of endoscopic assessment using validated endoscopic scoring systems. We performed a standard semiquantitiative endoscopic evaluation to assess current status and changes in inflammation. No endoscopic evaluation using SES-CD or CDEIS score (31) were performed to quantify endoscopic changes. This was due to the retrospective setting of our study.

Considering possible further studies, using cyclophosphamide as a 'rescue therapy' in therapy refractory Crohn's disease, the assessment of the SES-CD or CDEIS would represent considerable additional information, since all existing data regarding intravenous cyclophosphamide pulse therapy in patients with Crohn's disease is lacking the use of validated endoscopic scoring systems. This is also a limitation of our study, as no scores of endoscopic mucosal inflammation were recorded.

Also, due to the retrospective character of our analysis, we have not been able to analyze fecal inflammation markers throughout the patient's individual course of intravenous cyclophosphamide therapy. In particular, the measurement of fecal calprotectin as a non-invasive parameter meanwhile represents a well-established diagnostic instrument with a strong correlation to the extent of intestinal inflammation in inflammatory bowel diseases (32, 33).

A hitherto not yet established but very exciting approach to discover novel plasma parameters aiming a reproducible a precise correlation to the acitivity of Crohn's disease might be the measurement of superoxide dismutase activity. This enzyme is involved in the protection of cells from the harmful effects of reactive oxygen species, which are considered to contribute to

the intestinal damage in Crohn's disease. Recent data shows a significant inverse correlation between superoxide dismutase activity and the 'Crohn's Disease Activity Index' (34).

Moreover, in a prospective setting, currently it would be essential to correlate treatment response with a quality of life questionnaire, such as SF-36 (35) or IBDQ (36).

Another limitation of our study is the use of the Harvey-Bradshaw index as a simple score to clinically assess Crohn's disease activity instead of the CDAI (Crohn's Disease Activity Index) (37), which is mostly used in clinical trials, or the new patient reported outcomes based on abdominal pain and stool frequency (PRO-2) (38). This is also due to the retrospective setting of our study, which only allowed us to calculate the Harvey-Bradshaw index.

Comparing our study with existing literature regarding the use of cyclophosphamide - without stem cell transplantation - in inflammatory bowel diseases, our findings concerning clinical response are similar, but differ regarding clinical remission. Stallmach *et al.* reported a clinical response in all 7 patients (100%) with Crohn's disease after two monthly administered intravenous pulses of cyclophosphamide (750 mg) and 6 of 7 patients (85.7%) reached clinical remission in that study (15).

Likewise, Schmidt *et al.* (2) described in 16 patients with steroid refractory Crohn's disease, high rates of clinical response and remission (81%) at week 8, with the included patients receiving 2 – 6 monthly treatments with 750 mg of intravenous cyclophosphamide. Seven of these patients have been described in the study of Stallmach *et al.* (15) mentioned above. Subsequently, the latter study summarizes the results of both studies. Although patient's characteristics and methods, especially dosage and application scheme of intravenous cyclophosphamide were similar to those of our study, prior medication and previous surgical history differ substantially.

Only six of the sixteen patients (37.5%) with Crohn's disease, described by Schmidt *et al.* (2), received prior immunosuppressive treatment with azathioprine, 2 patients (12.5%) were pre-treated with methotrexate and three patients (19%) received prior infusions with infliximab.

In our study, all nine patients failed treatment with at least one anti-TNF-antibody (8 patients were refractory to infliximab, 1 patient did not respond to adalimumab, 6 patients failed both (infliximab and adalimumab). Also, six patients (67%) failed at least 4 therapeutic approaches with immunosuppressive or biological agents (azathioprine, 6-mercaptopurine, methotrexate, infliximab, adalimumab, tacrolimus, ciclosporine A and vedolizumab) before starting intravenous cyclophosphamide pulse therapy, indicating the marked therapy refractory course of their Crohn's disease.

In the group of patients not responding to cyclophosphamide pulse therapy, Schmidt *et al.* (2) emphasized a more intensive treatment with immunosuppressive agents before starting cyclophosphamide pulse therapy. On the other hand, in his study 9 of 16 patients (56%) underwent previous surgery and all 3 patients (18.8%) without remission received previous surgical therapy. Notwithstanding, two-thirds of the 9 surgically pre-treated patients reached clinical remission.

Also in 2006, Barta *et al.* (16) treated 8 patients with steroid refractory inflammatory bowel disease (4 with ulcerative colitis and 4 with Crohn's disease) with 6 monthly pulses of intravenous cyclophosphamide (800 mg per application). All patients with Crohn's disease were females, a pre-treatment with immunosuppressive agents is not described, instead azathioprine (for the patients with ulcerative colitis) and methothrexate (for the patients with Crohn's disease) were initiated as a maintenance therapy following intravenous cyclophosphamide pulse therapy. The results show clinical remission in all 8 patients after the second or third pulse of intravenous cyclophosphamide without describing an exact clinical score. Also, there is no information about an endoscopic examination of the patients.

Schmidt *et al.* (14) reported 15 patients with steroid-refractory (n = 13) or steroid-dependent (n = 2) Crohn's disease, receiving 2 – 6 monthly pulses of 750 mg cyclophosphamide. At week 8, 13 of 15 patients (87%) showed a clinical response and 10 of 15 patients (67%) achieved clinical remission. In this study, 8 patients (53.3%) received previous immunosuppressive therapy with azathioprine, 1 patient (6.7%) received 6-mercaptopurine and 2 patients (13.3%) received methotrexate, the respective medication was maintained during the study. Only three of fifteen patients (20%) were pre-treated with anti-TNF-antibody therapy (infliximab). In these patients, clinical remission was only found in one (6.7%) at week 8.

Comparing these findings with our data it can be noted that both of our patients, who reached clinical remission at week 8, did not receive previous surgery. Though, the comparatively low rates of clinical remission in our study indicate, that a previous treatment failure to several immunosuppressive or biological agents, in particular anti-TNF-agents, might be a negative predictive factor for achieving clinical remission with cyclophosphamide pulse therapy. This might be due to the severity of disease which is reflected by multiple inefficient previous biological therapies.

Moreover, smoking status has not been evaluated in the studies mentioned above, but it represents a relevant risk factor for a complicated course of disease (39) and increases the risk of relapse compared to nonsmokers (40). In our study, both patients reaching clinical remission and the only patient with mucosal healing in the endoscopic examination were nonsmokers. The only patient smoking in our study experienced clinical and endoscopic response at week 8, but showed a pronounced endoscopic worsening with a progressing inflammatory stenosis of the neoterminal ileum in an endoscopic re-evaluation performed 6 months after the last (6^{th}) cyclophosphamide administration at week 20.

Considering the dynamic development of next-generation therapeutics addressing new targets, including anti-cytokine agents, integrin antagonists, and small molecules (41), the use of cyclophosphamide pulse therapy to induce clinical and endoscopic remission may in particular be worth of consideration in patients with active Crohn's disease showing a refractory disease course to all currently available treatment options.

Currently, this also includes ustekinumab, a fully human immunoglobulin G1 kappa (IgG1κ) binding the p40 subunit common to the heterodimeric cytokines interleukin-12 and interleukin-23 and neutralizing their biological activities. This substance has reached approval status in the European Union in 2016. Presently existing data suggests efficacy of ustekinumab even in patients with an 'refractory' course of their Crohn's disease (failure of immunosuppressants and anti-TNF-therapy) (42-46). Thus, the potential time for use of intravenous cyclophosphamide pulse therapy is shifted to patients, who are refractory to all currently available three biological substance classes and approved immunosuppressive treatments.

Moreover, the safety profile of cyclophosphamide pulse therapy, which is characterized by extensive clinical monitoring and the risk of cancer formation after higher cumulative doses as well as the risk of inducing genetic damage in germ cells (47) prevents a more widespread use. Assuming the approval of further substances with marked effectiveness in the treatment of Crohn's disease, that also show a low risk of side effects, the use of cyclophosphamide pulse therapy in Crohn's disease will be restricted to patients that are resistant to all available and better tolerated therapies.

Abbreviations: 6-MP, 6-mercaptopurine; ADA, adalimumab; AZA, azathioprine; C, Colon; CD, Crohn's disease; CDEIS,

Crohn's Disease Endoscopic Index of Severity; CicA, ciclosporin A; CRP, C-reactive protein; EIM, extraintestinal manifestations; HBI, Harvey-Bradshaw index; I, ileum; IBD, inflammatory bowel diseases; IFX, infliximab; F, female; M, male; mesna, mercaptoethansulphonic acid; MTX, methotrexate; R, rectum; SES-CD, Simple Endoscopic Score for Crohn Disease; Tac, tacrolimus; TNF, tumor necrosis factor; VDZ, vedolizumab

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