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Research Article

Real-Life Ustekinumab Response and Blood Cytokine Profiles in Patients with Crohn's Disease

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Abstract

Background: Crohn's Disease (CD) is a chronic inflammatory bowel disease with strong impact on morbidity and quality of life. Ustekinumab (UST) has been shown to be an efficient agent for bionaive and patients with treatment failure of anti-TNF-alpha drugs in randomized controlled trials, but real-life efficacy and safety has still to be evaluated. Furthermore, due to a growing number of treatment options, personalized treatment approaches are increasingly warranted. We report the experience of the first 51 patients treated with UST and evaluated blood cytokine levels as possible predictors of treatment response.

Methods: 53 CD patients who participated in our hospitals Bio-Database program treated with UST were retrospectively identified and clinical data were collected. Baseline demographic and disease related data were correlated with treatment response according to Crohn's Disease Activity Index (CDAI) reduction. Peripheral cytokine profiles of 25 selected cytokines related to CD pathogenesis were established in a subset of patients from a pre-therapeutic blood draw and correlated with treatment response.

Results: 51 patients were included into further analysis. 96.1% of patients had received anti-TNF-alpha treatment before and UST was introduced because of secondary treatment failure in 65.2% of patients and for 26.1% for primary failure. 52% and 53.5% had a CDAI reduction of 100 or more at week 8 and 16, respectively, defining treatment response. All 18 patients who had responded at week 8 and data available for week 52 were still responding to UST after one year of treatment. Seven patients were treated for predominant Extra-Intestinal Manifestations (EIM) and all had clinical response to treatment. No clinical parameter at baseline correlated with clinical response and formerly described predictive markers could not be confirmed. Higher total cytokine levels at baseline correlated with treatment response and a higher IL-17 level was the only cytokine associated with response to UST therapy.

Conclusion: UST is highly efficient for treatment of otherwise therapy resistant CD patients in a real-life setting. Treatment response is durable and efficacy for EIM is high. Prediction of response cannot be done with certainty based on clinical characteristics, but profiling of peripheral cytokine levels, especially IL-17, is promising for further evaluation.

Keywords: Ustekinumab; Crohn's disease; Real-life data; Biomarker; Inflammatory bowel disease

Introduction

Crohn's Disease (CD) is a chronic disease that primarily affects the intestine, leading to transmural inflammation of the intestinal wall with loss of digestive function, secretory diarrhea and severe complications such as fistulas, abscesses or stenosis. Due to early onset, typically in the young, and the chronic course, CD has tremendous impact on quality of life [1]. Treatment usually involves administration of glucocorticosteroids and for patients in whom remission without systemic steroids cannot be maintained or who are refractory to this treatment, immunosuppressive therapy is introduced. While Azathioprine is the classical agent, modern therapy usually involves targeted treatment with antibody- or small molecule based inhibition of key links in pathogenic inflammatory processes. Ustekinumab (UST), a monoclonal antibody neutralizing interleukins 12 and 23 (IL-12, IL-23) by targeting the shared p40 subunit, has proven efficacy in treatment of CD patients after failure of former Tumor Necrosis Factor alpha (TNFα) targeted treatment (REF) [2,3]. The molecular basis of UST efficacy is likely attributable to its effects on IL-23 rather than IL-12, targeting an important agent in the development of auto-inflammatory subsets of T-helper cells (Th17) [4]. Subsequently, this new agent is now in broad use. Additionally, due to proven efficacy and approval in other entities such as psoriasis, Ustekinumab is a promising agent against Extraintestinal Manifestations (EIM) of CD. Real-life-data of smaller patient cohorts already show good clinical efficacy [5,6]. Due to progressive differentiation and a rapidly growing arsenal of agents available in treatment of CD, personalization of therapy is desirable, but to date, valid markers for personalized decision making are lacking. Promising attempts have been made based on drug levels or clinical characteristics [5,7]. Furthermore, according to the specific mode of action of UST and observed therapeutic efficacy in only a subset of patients, it is a tempting hypothesis that predominance of an IL-23 dependent inflammatory pathway in these patients is the basis for response. Therefore, we aimed to cross-validate the clinical efficacy of UST and previously published predictive patient characteristics on our tertiary referral center for CD and evaluate peripheral blood cytokine profiles as a predictor of therapy response.

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Materials and Methods

Patients and blood samples

The first 53 patients who were treated with UST for CD in our tertiary referral center and consented to participate in our Biobank and database program (Ethics approval 159/19) were included. Peripheral blood was collected prior to every administration of UST. Serum and plasma were separated by standard centrifugation and stored at -80°C until analysis. First patient started treatment in December 2016, the last patient in March 2019. Clinical data was analyzed retrospectively. Data available before February 2020 was included in the analysis. Therapeutic response was evaluated using the Crohn's Disease Activity Index (CDAI) with response defined as a reduction of 100 or 70 points from baseline at week 8 or 16 (as indicated), remission was defined as a CDAI of less than 150 points.

Cytokine detection

Cytokine detection was performed using the Invitrogen™ eBioscience™ ProcartaPlex Human Cytokine Panel 1b according to standard protocol using a Luminex® 200™ plate reader. The assay simultaneously estimates the concentration of 25 human cytokines (GM-CSF, IFN-alpha, IFN-gamma, IL-1-RA, Interleukins 1-alpha, 1-beta, 2, 4, 5, 6, 7, 9, 10, 12p70, 13, 15, 17a, 18, 21, 22, 23, 27, 31, TNF-alpha, TNF-beta) on a single sample using fluorescent beads and labelled detection antibodies. The panel was chosen for its broad representation of cytokines involved in CD [8-18]. No sample dilution was performed. In short, all reagents were prepared according to the manual. Plasma-samples were thawed on ice and vortexed rigorously. Prepared magnetic beads were added to the pre-wet 96-well plate and washed. Standards in a dilution row and plasma samples were added and incubated, followed by a washing step. Detection antibody and subsequently Streptavidin-PE was added with washing steps in between. Then the beads were resuspended in reading buffer and analyzed on a Luminex® 200™ plate reader. Measurements were done in duplicates. Standard curves were interpolated and the mean observed concentration was calculated for every sample.

Statistical analysis

Descriptive statistical analysis was performed using standard methods (mean, standard deviation). Mann-Whitey-U-test, Chi-Square- and t-test for dependent and independent samples were used for alpha error estimation, dependent on the distribution of data. Normal distribution of data was evaluated visually on a histogram and by Shapiro-Wilk-Test.

Logistic regression and univariate statistical tests were performed using R version 3.6.0. Figure 1 was created using Microsoft™ Excel® 2016.

Results

Baseline characteristics

53 patients were included in the retrospective analysis. The baseline characteristics are shown in Table 1. Two patients were excluded from further analysis; in detail, an uncharacteristic perianal dermatitis that was initially diagnosed as an EIM was reevaluated as not CD related in one case and another patient was lost to follow-up already at week four. All patients had a chronic active course. The reason for therapy change was secondary treatment failure to anti-TNF agents in 65.2% or primary non-response in 26.1% and occurrence of side effects of prior therapy in 8.7%. Colonoscopy was done at baseline in 28 patients. 23 (82.1%) had macroscopically highly active disease and 4 (14.3%) were classified as moderately active. Ultrasound imaging at baseline was available for 40 patients, and 36 (90%) had signs of active CD. Mean thickness of inflamed bowel wall was 6.4 mm (± SD 2.07) and vascularization pattern was described as Limberg II to III (2.36 ± SD 1.11).

Disease activity and therapy response

Therapy response is shown in Figure 1. Clinical follow-up data were available at week 8 (7.9 ± 0.75 weeks) for 50 (98%) patients, week 16 (16.2 ± 1.08 weeks) for 46 (90.2%) patients, week 32 (31 ± 1.8 weeks) for 41 (80.4%) patients and week 52 (51.9 ± 3.5 weeks) for 33 (64.7%) patients. Mean CDAI Score at baseline was 243.2 (SD ± 92.7). Seven patients had a CDAI score of less than 150 and therefore no active CD according to the score. All of these patients received UST for EIM or persisting fistulas; response in these patients was evaluated separately. For the remaining patients, overall treatment response was a reduction in CDAI of 95.4 points ($p=4.04 \times 10^{-7}$) for week 8 and 101.9 points ($p=3.56 \times 10^{-6}$) for week 16. 60.5% of patients had a reduction in CDAI of 100 points or more at week 8, 41.9% had a reduction in CDAI of 100 or more and reached clinical remission (CDAI <150). At week 16, 59% had a reduction in CDAI of 100 or more and 48.7% were in clinical remission. All 18 (100%) patients who had clinical response at week 8 and data available at week 52 had persistent response after one year; the same was likewise true for 17 of 19 (86.7%) patients at week 16.

Seven patients who were treated with UST despite missing CD activity according to CDAI, there was clinical response in all seven

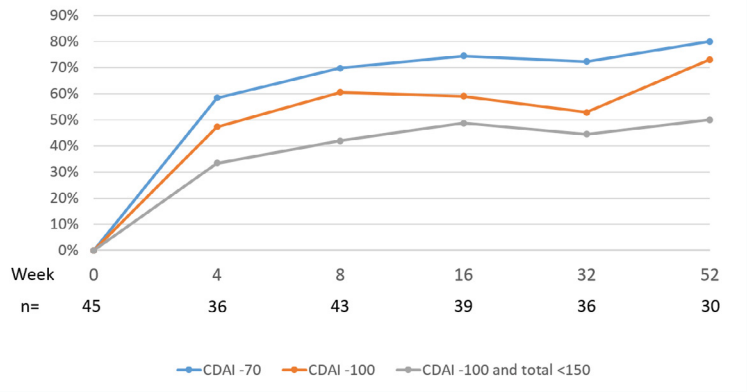

Figure 1: Response rate to UST over one year of treatment according to different definitions of response: Reduction of CDAI of at least 70 (blue) or 100 (red) points or reduction of at least 100 points and total CDAI of less than 150 points (grey). n: number of patients.

Table 1: Baseline clinical characteristics of total cohort, UST responders and non-responders. Response was defined as CDAI reduction of at least 100 points at week 16. Values are given ± standard deviation. P-values are given for group differences between responders and non-responders.

	Total	Responder	Non-responder	p value
Age (years)	38.1 ± 13.2	38.8 ± 11.6	36 ± 14.8	0.185
Sex female	64.70%	60.70%	69.60%	0.716
Sex male	35.30%	39.50%	33.30%	0.716
Body weight (kg)	72.6 ± 17.5	74.4 ± 17.5	70.3 ± 17.7	0.368
BMI (kg/m²)	24.4 ± 5.2	24.9 ± 5.6	23.9 ± 4.8	0.589
Disease duration (years)	12 ± 8.5	14.1 ± 9.4	9.3 ± 6.7	0.034
Location of disease				
Colitis	23.50%	21.40%	26.10%	0.953
Ileocolitis	68.60%	67.90%	69.60%	1
Ileitis terminalis	7.80%	10.70%	4.30%	0.75
Upper GI (additional)	2%			
Perianal disease	33.30%	32.10%	34.80%	1
Fistulizing disease	51%	42.90%	60.10%	0.318
Stricturing disease	27.50%	35.70%	21.70%	0.435
EIM total	54.90%	57.10%	52.10%	0.94
EIM joints	33.30%	35.70%	30.40%	0.582
EIM eyes	5.90%	3.60%	8.70%	0.5
EIM skin	21.60%	14.30%	30.40%	0.292
Prior therapy				
Anti-TNF:	96.10%	96.40%	95.60%	1
Anti-Integrin (Vedolizumab)	43.10%	46.40%	39.10%	0.811
Azathioprin:	70.60%	64.30%	78.30%	0.435
Current systemic glucocorticosteroids	52.90%	50%	56.50%	0.855
Blood tests				
White blood cells (G/l)	11 ± 3.7	11.4 ± 4.0	10.4 ± 3.5	0.47
Hemoglobin (g/dl)	12.9±1.5	12.7 ± 1.4	13.0 ± 1.5	0.476
Thr (1/nl)	346.9 ± 125.2	364 ±147	328 ± 95	0.477
CRP (mg/l)	26.2 ± 37.9	26.9 ± 34.9	25.4 ± 31.3	0.654
Ferritin (ng/ml)	122.9 ± 126.0	121.3 ± 122.6	124.5 ± 133.3	0.763
Albumin (g/l)	40.7 ± 5.2	40.8 ± 5.3	40.7 ± 5.3	0.749
Fecal calprotectin (ug/mg)	532 ± 308.6	566.1 ± 311.9	472.3 ± 339.6	0.774
Sodium (mmol/l)	140 ± 2.6	139.9 ± 3.9	140.1 ± 2.8	0.602
Potassium (mmol/l)	3.8 ± 0.4	3.9 ± 0.5	3.8 ± 0.4	0.413
GOT (U/l)	24.1 ± 10.9	25.9 ± 11	22.4 ± 10.7	0.222
GPT (U/l)	31.3 ± 27.4	35.6 ± 30.1	26.9 ± 24.1	0.334
Last therapy				
Adalimumab	14	28.10%	26.10%	1
Vedolizumab	12	21.40%	26.10%	0.953
None	10	21.40%	17.40%	0.995
Infliximab	7	14.30%	13%	1
Golimumab	3	7.10%	4.30%	1
Filgotinib	3	3.60%	8.70%	0.86
MTX	2	0%	4.30%	0.921
Prior operation	35.30%	39.30%	30.40%	0.716

EIM: Extraintestinal Manifestations; GI: Gastrointestinal Tract; BMI: Body Mass Index; TNF: Tumor Necrosis Factor alpha; CRP: Capsid Reactive Protein; MTX: Methotrexate

patients. In detail, three patients treated for perianal fistulas and did not respond to anti-TNF-alpha treatment recovered completely. Two patients who suffered from cutaneous manifestations reached clinical remission. One patient had predominantly systemic symptoms and a severely reduced general condition and weight loss with few CDAI relevant symptoms and recovered under UST treatment, and one patient had active sacroiliitis that was alleviated under UST.

Side effects

Since clear differentiation of UST side effects and CD symptoms is not possible, every new or worsened symptom was considered a possible side effect. Possible side effects are summarized in Table 2. The most common new symptoms were nausea, headache, abdominal pain and nasopharyngitis, occurring in about one third of patients. Seven patients were hospitalized during observation period. Two patients had recurrent subileus and were managed without change of therapy. One patient had a rash after first exposure to UST, so it was administered on ward but rash was not induced again. One patient had an add-on steroid trial in hospital on week 4 and remission was induced successfully. One case of CD flare and one patient with perianal abscess were admitted to hospital, and one patient had diverticulitis. One case of pneumonia was recorded at week 27. Three patients underwent surgery, two for small intestinal fistula and one for perianal abscess.

Baseline predictors of clinical response

Baseline characteristics of patients were screened for their role as predictors for therapy response using univariate analysis of group differences (Table 1). Among the parameters tested, only a longer duration of illness (p=0.034) and a higher CDAI (p=0.001) at baseline were associated with a better treatment response. Both were included in multivariate analysis using a logistic regression model, where only a higher baseline CDAI retained a statistical significant association with response to UST therapy (p=0.014), whereas disease duration only showed a trend (p=0.07). Since initiation of UST treatment for pure EIM was associated with lower baseline CDAI, those patients who had no CDAI score of 150 or more were removed from cohort for a second analysis of the data reducing the cohort to n=44. As expected, no further association with baseline CDAI was observed (p=0.442), nor did any other marker improve its prognostic value relevantly.

Cytokine profiles and therapy response

Pre-therapeutic plasma samples were available for 31 patients of the cohort. When mean levels of each cytokine measured were compared between responders and non-responders, higher cytokine levels were associated with therapy response. This was true for response at week 8 and week 16, and for cut-off values for CDAI reduction of 100 and 70 points (~1.3 fold mean cytokine level, p values between 5.63×10^{-12} to 1.77×10^{-7}). Removal of patients with a baseline CDAI of less than 150 did not change this association (n=25, $p=4.77 \times 10^{-13}$) with UST responders and non-responders having 1.52 and 0.48 times the mean cytokine level of each cytokine measured. The cytokine test results are shown in Figure 1. On univariate analysis, the only cytokine associated

Table 2: Adverse events of UST during observation period. All new or aggravated symptoms were considered possible side effects.

Adverse events during observation period	Frequency	Average week of occurence
Hospitalization	13.70%	20.1
Surgery	5.90%	35.7
Infection	2.00%	27
Headache	29.40%	19.7
Nausea	31.40%	23
Fever	9.80%	33
Nasopharyngitis	33.30%	20.2
Abdominal pain	29.40%	15.1
CD Flare	15.70%	13.4
Fatigue	51.00%	15.7
Dry skin	5.90%	27.3
Depression	3.90%	25.1
Hair loss	2.00%	12

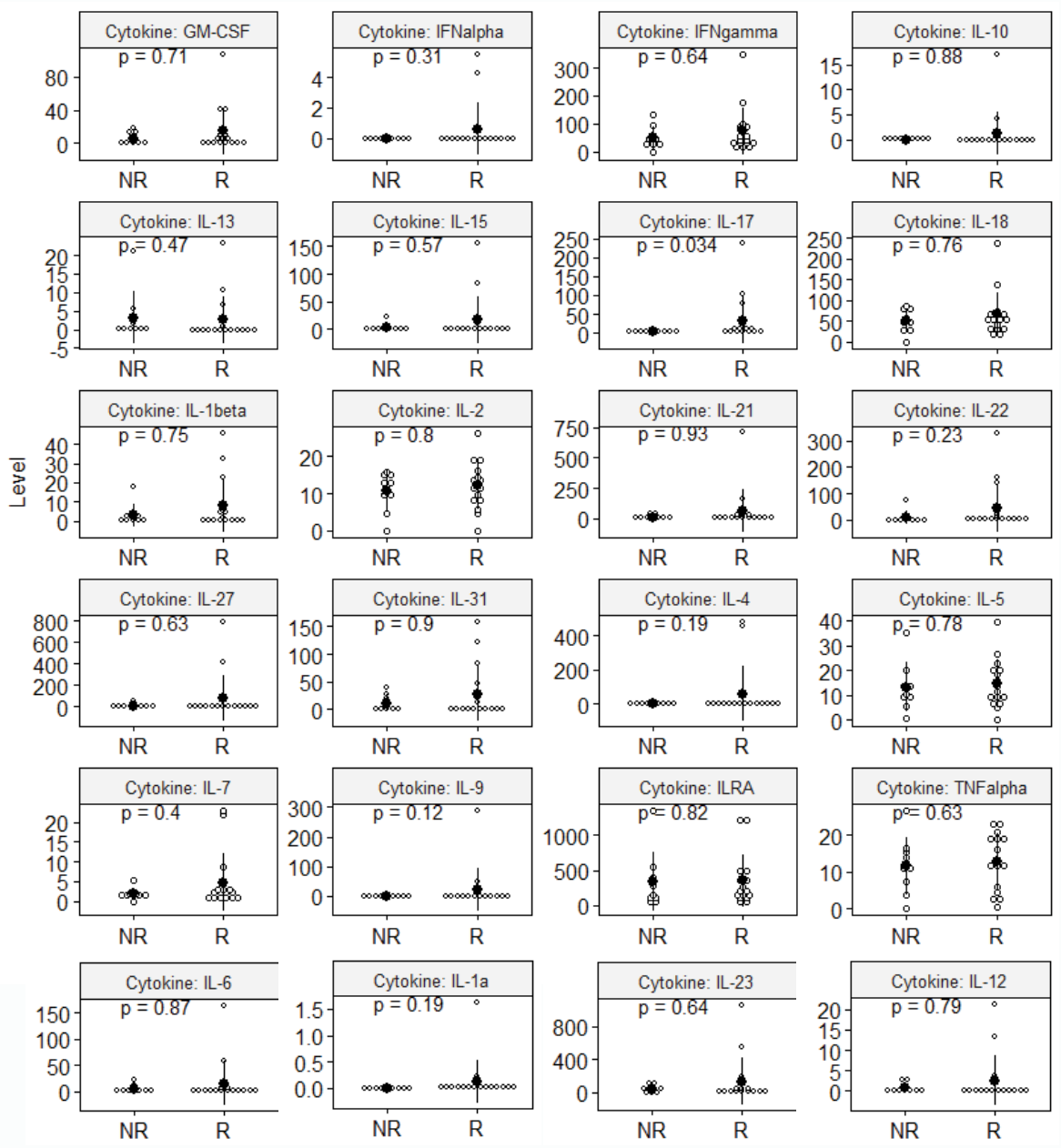

Figure 2: Cytokine levels of UST Non-Responders (NR) and Responders (R). Levels (pg/ml) of 24 cytokines measured before initiation of UST treatment. TNF-beta was not detectable in any patient and levels are not shown. Solid black dots: mean value.

with therapy response was IL-17 (44 *vs.* 3.87 pg/ml for responders and non-responders, respectively, p=0.034), while none of the other 24 cytokines analyzed showed a group difference with a p-value of less than 0.1; in particular, other UST and Th17 key cytokines IL-12 and IL-23 did not show statistically significant group differences (IL-12 2.52 and 0.59 pg/ml, p=0.79; IL-23 131.47 and 48.36 pg/ml, p=0.64 for responders and non-responders, respectively). Since no further promising discriminators could be identified, no multivariate analysis was performed.

Discussion

Due to the rapidly widening horizon of therapeutic options for treatment of CD, individualized approaches are highly warranted for appropriate choice of therapy. We and others have analyzed baseline demographic characteristics of single center cohorts of patients treated with UST and could confirm the good therapeutic efficacy of UST in intensely pre-treated CD patients and observed an excellent treatment response even after one year in these challenging patients [5,6,19]. In a few years, data will be available to demonstrate response and side-effects in the long term treatment, but already, reaching one year of persistent response in these otherwise therapy resistant patients with only few side effects is a success for the individual patient. On the other hand, prediction of response is still challenging. Previously described predictive patient characteristics, i.e. male sex, EIM or use of steroids[5], could not predict outcome in our cohort. Several reasons could have contributed to this. In our study, we defined treatment response by CDAI decrease at week 8 and 16, while the predictors mentioned above were described for steroid free clinical response at week 24 according to Harvey Bradshaw Index (HBI). In line with our own observations, HBI and CDAI do not perfectly match and can lead

to different classifications of patients. CDAI is preferred for clinical trials, but more time consuming to use [20]. Since both observations are from single center cohorts, the observed differences could also be due to differences in study populations. This is especially important for CD, since these patients are highly heterogeneous with regard to different disease localizations, courses and complications.

Extra-intestinal manifestations and solely perianal activity pose a high burden on CD patients but are only insufficiently reflected by CDAI or HBI when they are the only sign of CD activity, since response of these manifestations leads only to slight changes in scoring. Here, we report treatment success in seven patients treated despite a low CDAI for EIM. All seven responded completely. This was true for psoriasis like dermatitis that two patients developed and response is not too surprising since UST is already in use for psoriasis vulgaris [21]. Furthermore, complete response could also be observed in patients with perianal disease and one case of sacroileitis, all without significant luminal disease activity, indicating efficacy for pure EIM.

Cytokine profiling in peripheral blood revealed a highly significant association of total cytokine levels and UST therapy response. Our cytokine panel was chosen because of a high representation of cytokines involved in IL-12/IL-23 signaling and Th17 response as well as CD pathogenesis [8-18], and therefore, we hypothesize, that a highly active CD specific inflammatory process could be prognostic for UST response, selectively interfering in these pathways. Strikingly, the cytokine differentiating responders from non-responders with the lowest alpha error of p=0.034 was IL-17, the main cytokine of Th17 cells, that are thought to be the main target of UST [22]. Of course, our results have to be interpreted with caution as they are purely exploratory. We did not correct for multiple testing, analyzed a limited number of patients from a single center and already demonstrated prognostic differences to other published patient cohorts. As demonstrated in Figure 2, there is a large overlap between cytokine levels of responders and non-responders, so single cytokine levels will not be of good use in the clinical setting. Our observation, that total cytokine levels are higher in patients with UST response and the pathogenic plausibility of an association of treatment response with a Th17 predominant inflammatory reaction, holds promise that an multi-parametric test could be of good use for response prediction, but our data set is still too small for valid logistic regression modeling and independent validation of such a test. Furthermore, some of the assessed cytokines are believed to have a more protective role in CD [8,18,23]. In that light, our observation could be a hint to an overall higher inflammatory activity in responders or a reactive up-regulation of protective signaling pathways. Since response to therapy is judged based on clinical parameters and not on inflammation itself, it is possible that some of the non-responders had symptoms not caused by inflammatory activity, although neither ultrasound, nor CRP levels or endoscopic findings gave a hint to that in our cohort. Furthermore, the cytokine levels observed in this current study are rather low compared to levels observed in other disease states [24] and it is likely that prior therapy with immunosuppressive agents, like TNF-alpha blockers or glucocorticoids, have an influence on peripheral cytokine levels, so the higher levels observed in treatment responders could also point to differences in previous treatment action.

Conclusion

In our cohort of UST treated MC patients, we confirmed a high therapeutic efficacy of UST in real-life treatment with high long-term response and good activity in treatment of EIM. The previously described negative predictive factors EIM, male sex and steroid use were not predictive in our cohort. To the opposite, EIM were controlled well by UST treatment. Total peripheral blood cytokine levels in a 25-plex cytokine panel covering core Th17, IL-23 and IL-12 associated cytokines were higher in UST responders and a high IL-17 level was statistically associated with UST efficacy, showing feasibility of peripheral blood cytokine profiling for further evaluation as a predictive test for UST treatment response.

Core Tip

In a retrospective analysis, we find high treatment efficacy of Ustekinumab for treatment of intestinal and extra-intestinal manifestations of 51 patients with Crohn´s Disease in this single-center study. Formerly published predictive patient characteristics were not predictive for therapy response in our cohort, but we describe higher peripheral cytokine levels and an association with Interleukin-17 levels in patients with therapy response.

Author Contributions

Schulte L: data acquisition, laboratory testing, statistical analysis and manuscript writing. Hofelich J: data acquisition. Beck A: Manuscript proof reading. Reuther H: laboratory testing. Klaus J: study design, funding, patient recruitment, supervision.

Conflict of Interest Statement

All authors exclude any conflicts of interest regarding this work.

Consent for Publication

No identifiable personal data is included in the manuscript. All patients gave written informed consent for inclusion.

Ethics Approval

The work is approved by the local ethics committee (No 159/19) and all subjects gave written informed consent.

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Data Availability

The manuscript contains the full data set.

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