Colesevelam for the treatment of bile acid malabsorption-associated diarrhea in patients with Crohn's disease: A randomized, double-blind, placebo-controlled study

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Abstract

Background and Aims: Bile acid malabsorption (BAM)-associated diarrhea is an important clinical issue in patients with Crohn's disease (CD). We analyzed the efficacy and safety of the bile acid sequestrant colesevelam for treatment of BAM-associated diarrhea in CD patients in a randomized, double-blind, placebo-controlled study.

Methods: The primary endpoint was the proportion of patients with ≥30% reduction of liquid stools/day from baseline to termination visit at week 4. Secondary endpoints were reduction of the number of liquid stools/day, improvement of stool consistency and quality of life.

Results: 26 patients were analyzed in the intention-to-treat (ITT) analysis. The primary endpoint was reached by 10 patients (69.7%) in the colesevelam group compared to 3 patients (27.3%) in the placebo group (risk difference RD = .394, 95%CI:[0.012; 0.706]; P = .0566). In
1. Introduction

Bile acid malabsorption (BAM) and BAM-associated diarrhea are important clinical issues in patients with Crohn’s disease (CD), since up to 60% of CD patients require small bowel resection due to stenosis. Subsequently, reabsorption of bile acids in the ileum and the enterohepatic bile acid circulation is disrupted resulting in watery diarrhea. Fibroblast growth factor 19 (FGF19) is known to inhibit hepatic bile acid synthesis and impaired ileal production of FGF19 after resection or functional loss may be one reason for bile acid-induced diarrhea. Moreover, bile acid malabsorption has been linked to the deactivation of pregnane-X receptor (PXR) in CD, and PXR downregulation is associated with the susceptibility and exacerbation of CD. Recognition and diagnosis of BAM in patients with CD in remission is challenging, since symptoms are similar to those of active inflammatory disease. To date, three diagnostic tests are available to confirm the diagnosis of BAM: the (75)SeHCAT (TauroH-23-α-hydroxy-4-chol esten-3-one (cholestenone) test) and fecal bile acid measurement. However, these tests are not routinely available in all countries. SeHCAT is absorbed from the gut and excreted into the bile at the same rate as cholic acid and can therefore serve as marker for bile acid loss in the stool. The (75)SeHCAT test is inconvenient due to its cumbersome radioactive test principle. Testing for cholestenone in blood serum, which is an indirect test for bile acid loss by detection of increased bile acid production, is more convenient but not widely available. Therefore, in most of the suspected cases of BAM, empiric treatment with a bile acid sequestrant like cholestyramine, which is approved for the use in BAM, is conducted. However, patients are often intolerant to cholestyramine given its side effects such as nausea, bloating, constipation and not all patients respond to it.

Colestevram (Cholestelag®, Genzyme, Naarden, The Netherlands) is a novel bile acid sequestrant taken orally in tablet form which is approved for the treatment of hypercholesterolemia and is currently developed in clinical studies for the treatment of diabetes mellitus type 2. Colestevram is optimized for binding bile acids by its chemical structure (hydrophilic polymer backbone with hydrophobic side chains). Also, it is well tolerated in terms of lower gastrointestinal side effects like constipation, which is often the problem with other bile acid sequestrants.

Bile acid sequestrants are also used for empirical treatment of cholestatic pruritus. Therefore, the efficacy of colestevram for amelioration of cholestatic pruritus was investigated in a placebo-controlled study which, however, did not show any significant effects on pruritus compared to placebo. There are studies that demonstrated positive effects in patients with irritable bowel syndrome (IBS) and diarrhea. Moreover, there are some small open-label studies, which are only published in abstract form and retrospective studies in which colestevram was effective in BAM-associated diarrhea of CD patients and other diseases. Therefore, the aim of this study was to analyze the efficacy and safety of colestevram on BAM in CD patients in an investigator-initiated, multicentre, double-blind, randomized, placebo-controlled trial.

2. Materials and methods

2.1. Study design

This study was an investigator-initiated, randomized, double-blind, placebo-controlled, multicentre trial. The inclusion of patients started in May 2011, the follow-up was completed in July 2013. CD patients with ≥ 3 and ≤ 15 liquid stools/day in clinical remission, defined as Crohn’s disease activity index (CDAI) ≤ 150 points and CRP-value ≤ 1 mg/dL were eligible for the study. Other inclusion criteria were age ≥ 18 years and ≤ 65 years, contraception in women, medication with either oral aminosalicylates, azathioprine, mercaptopurine, methotrexate, oral steroids (<10 mg/day), or anti-TNF alpha antibody treatment (infliximab, adalimumab) in a stable dose for at least 12 weeks prior to inclusion into the study and written informed consent. Moreover, to confirm the diagnosis of BAM, a further inclusion criterion was a cholestenone serum level of ≥ 50 ng/mL which was centrally assessed at the screening visit.

Exclusion criteria were allergy, hypersensitivity or intolerance to any of the components of colestevram, participation at another clinical trial within a period of 4 weeks before the screening visit, presence of any addiction, alcohol abuse or specific disease that would not allow the patient to understand the essence and requirements and potential consequences of the participation in the clinical trial, signs suggestive of the patient being unable to follow the visit schedule as required, pregnancy and lactation, infectious diseases (HIV, hepatitis B, hepatitis C, tuberculosis,
listeriosis, *Clostridium difficile* toxin in feces), systemic lupus erythematosus, multiple sclerosis, intraabdominal abscess, cholestatic liver disease, bowel or biliary obstruction, dysphagia, known malignancy or history of malignancy, a history of intestinal surgery within 6 months from screening, resection of more than 100 cm small intestine, short bowel syndrome, planned gastrectomy, ileostomy or colectomy, treatment with cyclosporine or tacrolimus 12 weeks before study inclusion, treatment with antibiotics 3 weeks before study inclusion, topical treatments with steroid or aminosalicylates 3 weeks before study inclusion, treatment with a bile acid sequestrant 6 weeks before study inclusion.

Patients received either colestevam (3 × 2 tablets a day) or identically shaped placebo (3 × 2 tablets/day). The total individual treatment period was 4 weeks. Randomization was centralized with serial-numbered envelopes containing a specific kit-code prepared by the trial unblinded pharmacist. The unblinded pharmacist, who was a pharmacist from our hospital pharmacy, had no contact to the patients during the treatment period. Participants were assigned to one of the two arms according to a standard randomization schedule.

### 2.2. Measurement of cholestenone serum levels

Measurement of cholestenone in serum samples of screened patients was performed as previously described.  

### 2.3. Ethical statement and study approval

The study was approved by the ethical committee of the Ludwig-Maximilians-University Munich and by the German ministry for pharmaceutical products (Bundesministerium für Arzneimittel und Medizinprodukte), registered at the ClinicalTrials.gov database (ClinicalTrials.gov Identifier: NCT01203254) and conducted, recorded, and reported in compliance with International Conference on Harmonisation Good Clinical Practice and national regulations. All authors had access to the study data and had reviewed and approved the final manuscript.

### 2.4. Study visits and endpoint parameters

Participants visited the outpatient clinic or practice of the study centers three times: the first visit (day – 28 until – 7 to baseline) for screening, the second time (day 0) for baseline visit (start of treatment), and the last visit (day 28+/– 2 from baseline) for study termination at the end of treatment. At all visits, the CDAI was calculated and the CRP value was measured. The cholestenone value in serum was measured at the screening visit. At each visit, liquid stool frequency was calculated from a patient’s diary and the mean count of liquid stools of the last 7 days was recorded. The level of stool consistency using the Bristol stool chart was recorded from the day of each visit. At baseline and termination visits, each patient completed a SF-36v2 questionnaire for quality of life evaluation. Laboratory investigations, including measurements of blood count, CRP, fibrinogen, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, bilirubin, lipase, sodium, potassium, glucose, creatinine, cholesterol, high-density lipoprotein, low-density lipoprotein and triglycerides were performed at each visit. All patients were monitored regarding potential side effects. Stool examinations for pathogenic specimens were performed at screening. A clinical examination was performed at each visit.

The primary endpoint was defined as proportion of patients with >30% reduction of liquid stools/day from baseline to termination visit at week 4. Secondary endpoint parameters were reduction of liquid stools/day and improvement of stool consistency using Bristol stool chart and improvement of quality of life using the SF-36v2 questionnaire, which includes the analysis of eight health and social modalities (physical functioning, role physical functioning, bodily pain, general health, vitality, social functioning, emotional functioning and mental health).

### 2.5. Statistical analysis

The power calculation was based on expected primary outcome in at least 80% of patients treated with colestevam and in 30% of those treated with placebo. The null-hypothesis was $H_0$: RD = $p_1 - p_0 = 0$. Therefore, Barnard’s combinatorial exact test was applied. To detect this difference with a significance level of 5% and a power of 90% (β = 10%), 23 participants had to be included in each treatment arm.

Comparison of demographic and clinical characteristics of the study populations was performed using the $\chi^2$-test for categorical variables. If not stated otherwise, all other statistical analyses were performed by using the two-tailed Student's t-test. $P$ values $< 0.05$ were considered as statistically significant.

### 3. Results

#### 3.1. Patients’ characteristics

Overall, 55 patients were screened within the study period. Twenty-one patients were screening failures, mostly due to elevated CDAI or CRP serum levels or cholestenone values of $<50$ ng/mL. Accordingly, 34 patients were randomized.

Out of the 34 randomized patients, 3 patients terminated the study prematurely due to AES; one patient did not take the study medication according to the study protocol. In detail, three patients receiving placebo stopped due to nausea, constipation and lack of improvement of the diarrhea, respectively. Six patients, who had ≥ 3 liquid stools/day at screening visit, did not fulfill this inclusion criterion at baseline and must therefore be treated as protocol deviation and had to be excluded from analysis. Similarly, two patients who had CDAI values $<150$ at screening had CDAI values $>150$ at baseline. Accordingly, the intention-to-treat analysis comprised 26 patients and 22 patients could be analyzed in the per-protocol analysis (Fig. 1). Demographic data of the intention-to-treat population are provided in Table 1. There were no significant differences between both groups at baseline regarding age, weight, disease duration, thiopurine treatment, anti-TNF treatment, CRP, cholestenone, CDAI, liquid stools/day and history of small bowel resection. However, there were significant more male patients in the colestevam group ($P = 0.01$).
3.2. Primary outcome parameter: >30% reduction of liquid stools/day from baseline to termination visit at week 4

Regarding the primary outcome parameter, which was a >30% reduction of liquid stools/day from baseline to termination visit at week 4, there was a trend towards significant difference in the primary intention-to-treat (ITT) analysis (risk difference RD = 0.394, 95%-CI: [-0.012; 0.706], p(H0: RD = 0) = 0.0566 (Barnard's exact test)) between the colesevelam (10/15 patients; 66.7%) and placebo groups (3/11 patients; 27.3%). In the per-protocol (PP) analysis, there was a statistical significant risk difference (RD = 0.470, 95%-CI: [0.018; 0.788], p(H0: RD = 0) = 0.0364; NNT = 2.1, 95%-CI: [1.3; 54.7]) between the colesevelam (9/13 patients; 69.2%) and placebo group (2/9 patients; 22.2%) regarding the primary outcome parameter (Fig. 2A and B).

3.3. Secondary outcome parameters

Analyzing the ITT-population, the secondary outcome parameter “reduction of liquid stools/day from baseline to termination visit at week 4” showed a non-significant effect with a risk difference of 0.394 (95%-CI: [-0.012; 0.706], p(H0: RD = 0) = 0.0566 (Barnard's exact test)).

Table 1: Demographic and clinical characteristics of the two study groups (intention-to-treat population; n = 26) at baseline.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Colesevelam group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Patients (n=)</td>
<td>15</td>
<td>11</td>
<td>N/A</td>
</tr>
<tr>
<td>Age (years) [Range]</td>
<td>47.5 [27–64]</td>
<td>44.6 [26–65]</td>
<td>NS</td>
</tr>
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<td>Disease duration (yrs) [Range]</td>
<td>15.1 [5–27]</td>
<td>18.2 [9–38]</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg) [Range]</td>
<td>77.3 [61–112]</td>
<td>68.4 [51–108]</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>9 (60.0)</td>
<td>1 (9.0)</td>
<td>.01</td>
</tr>
<tr>
<td>Thiopurine treatment (%)</td>
<td>4 (26.7)</td>
<td>1 (9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-TNF treatment (%)</td>
<td>10 (66.7)</td>
<td>4 (36.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Ileocolic resection (%)</td>
<td>8 (53.3)</td>
<td>5 (45.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Ileal involvement (%)</td>
<td>15 (100)</td>
<td>15 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Ileocolonic involvement (%)</td>
<td>11 (73.3)</td>
<td>7 (63.6)</td>
<td>NS</td>
</tr>
<tr>
<td>CDAI [Range]</td>
<td>115.3 [38–143]</td>
<td>101.8 [38–149]</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/dL) [Range]</td>
<td>0.36 [0.1–0.6]</td>
<td>0.24 [0.1–0.5]</td>
<td>NS</td>
</tr>
<tr>
<td>C4 (mg/dL) [Range]</td>
<td>232.2 [60.4–410.3]</td>
<td>198.3 [77.1–371.8]</td>
<td>NS</td>
</tr>
<tr>
<td>Frequency of liquid stools/day</td>
<td>4.7 [3.0–6.2]</td>
<td>4.2 [3.0–6.3]</td>
<td>NS</td>
</tr>
</tbody>
</table>

C4 = 7α-Hydroxy-4-cholesten-3-one; CDAI = Crohn's disease activity index; CRP = C-reactive protein; and NS = not significant.
termination visit at week 4" was significantly different between the colesevelam and placebo group. In the colesevelam group, there was a reduction of liquid stools/day from median 5.0/day (mean 4.7/day) at baseline to median 2.0/day (mean 2.5/day) at termination visit (Fig. 3A; \( P = 0.01 \), Student's \( t \)-test), while in the placebo group liquid stool frequency was median 4.0/day (mean 4.2/day) at baseline and dropped to median 3.0/day (mean 3.7/day) at termination visit, which was not statistically significant (Fig. 3B; \( P = 0.42 \), Student's \( t \)-test). For the secondary outcome parameter "improvement of stool consistency", defined as improvement by at least one level in the Bristol stool chart, there was a significant difference between the colesevelam and placebo group. In the colesevelam group, 14 patients (93.3%) had improvement of at least one level in the Bristol stool chart (median 6 at baseline to median 5 at termination) compared to 4 patients (36.4%) in the placebo group with no change from level 6 at baseline to termination (\( P = 0.003 \); OR 24.5; CI 2.29–262.53, Fisher's exact test). Regarding the secondary outcome parameter "change in quality-of-life", no statistically significant changes (Student's \( t \)-test) were found within the modalities of physical functioning (\( P = 0.92 \)), role physical functioning (\( P = 0.74 \)), bodily pain (\( P = 0.16 \)), general health (\( P = 0.18 \)), vitality (\( P = 0.71 \)), social functioning (\( P = 0.97 \)), emotional functioning (\( P = 0.78 \)), and mental health (\( P = 0.69 \)), physical component summary (\( P = 0.48 \)) and mental component summary (\( P = 0.78 \)) of the Short Form 36 v2 questionnaire in the colesevelam group compared to the placebo group before and after treatment.

In the PP-population for the secondary outcome parameter "reduction of liquid stools/day from baseline to termination visit at week 4", there was a significant difference between the colesevelam and placebo group. In the colesevelam group, there was a reduction of liquid stools/day from median 5.0/day (mean 4.8/day) at baseline to median 2.0/day (mean 2.7/day) at termination visit (Fig. 4A; \( P = 0.02 \), Student's \( t \)-test), while in the placebo group liquid stool frequency was 4.0/day (mean 4.3/day) at baseline which decreased to a median of 3.4/day (mean 3.9/day) at termination visit which was not statistically significant (Fig. 4B; \( P = 0.61 \), Student's \( t \)-test). For the secondary outcome parameter "improvement of stool consistency", defined as improvement by at least one level in the Bristol stool chart, there was also a significant difference between the colesevelam and placebo group in the PP analysis. In the colesevelam group, 12 patients (92.3%) had...
improvement of at least one level in the Bristol stool chart (median 6.0 at baseline to median 5.0 at termination) compared to 3 patients (33.3%) in the placebo group with no change from level 6 at baseline to termination (P = 0.007; OR 24; CI 2.04–282.70; Fisher’s exact test). Regarding the secondary outcome parameter “change in quality-of-life”, no statistically significant changes (Student’s t-test) were found within the modalities of physical functioning (P = 0.66), role physical functioning (P = 0.61), bodily pain (P = 0.22), general health (P = 0.35), vitality (P = 0.56), social functioning (P = 0.51), emotional functioning (P = 0.86), mental health (P = 0.77), physical component summary (P = 0.31), and mental component summary (P = 0.80) of the Short Form 36 v2 questionnaire in the colesevelam group compared to the placebo group before and after treatment.

3.4. Subgroup analysis of patients with ileocecal resection

In a post-hoc analysis of a subgroup of patients with right hemicolectomy/ileocecal resection, we found in the ITT population (colesevelam n = 8, placebo n = 5) no significant difference regarding the primary endpoint (5 patients (62.5%) in the colesevelam group reached the primary endpoint vs. 2 patients (40.0%) in the placebo group; P = 0.59), but significant differences in liquid stools/day for patients treated with colesevelam (median 4.5/day (mean 4.7/day) at baseline to median 2.2/day (mean 2.1/day) at termination visit; P = 0.001), but not in the placebo group (median 4.3/day (mean 4.3/day) at baseline to median 3.4/day (mean 3.7/day) at termination visit; P = 0.42). In the colesevelam group, 8 patients (100%) had improvement of at least one level in the Bristol stool chart compared to 3 patients (60.0%) in the placebo group (P = 0.13). Regarding the secondary outcome parameter “change in quality-of-life”, no statistically significant changes were found between the colesevelam and the placebo group.

In the PP population (colesevelam n = 7, placebo n = 4) there was no significant difference regarding the primary endpoint (5 patients (71.4%) in the colesevelam group reached the primary endpoint vs. 1 patients (25.0%) in the placebo group; P = 0.24), but significant differences in liquid stools/day for patients treated with colesevelam (median 5.0/day (mean 4.8/day) at baseline to median 2.0/day (mean 2.1/day) at termination visit; P = 0.002), but not in the placebo group (median 4.2/day (mean 4.2/day) at baseline to median 3.9/day (mean 3.9/day) at termination visit; P = 0.78). In the colesevelam group, 7 patients (100%) had improvement of at least one level in the Bristol stool chart compared to 2 patients (50.0%) in the placebo group (P = 0.11). Regarding the secondary outcome parameter “change in quality-of-life”, no statistically significant changes were found between the colesevelam and the placebo group.

3.5. Subgroup analysis of patients regarding baseline cholestenone values

In further analyses patients were divided in two groups regarding their cholestenone levels at baseline (high cholestenone levels group and low cholestenone levels group). An arbitrary cut-off for “high cholestenone levels” was set at ≥ 200 mg/dL. In the colesevelam group, patients of the high cholestenone levels group had a reduction in median liquid stools from 4.5/day (mean value 4.5/day) at baseline to median 2.3/day (mean value 2.4/day) at followup, which was statistically significant (P = 0.02). Patients of the low cholestenone levels group (<200 mg/dL) had a reduction in median liquid stools from 5.0/day (mean value 4.9/day) at baseline to 2.0/day (mean value 2.6/day) at follow up, which did not reach statistical significance (P = 0.16).

In the placebo group, patients of the high cholestenone level group had a reduction in median liquid stools from 3.3/day (mean value 3.8/day) at baseline to 3.0/day (mean value 3.6/day) at followup (P = 0.85), while of the low cholestenone level group had a reduction in median liquid stools from 4.5/day (mean value 4.2/day) at baseline to 3.2/day (mean value 3.0/day) at followup (P = 0.42).
3.6. Adverse events and serious adverse event

During the study period, no suspected unexpected serious adverse reaction (SUSAR) occurred. Overall, we observed 8 mild adverse events (AEs) in 6 patients treated with colesevelam and 6 mild AEs in 4 patients receiving placebo. In the colesevelam group, two AEs were assigned to be related to the study drug (two patients with constipation) and three AEs possibly related (exanthema of the lower legs, bloating, and acne at the temporal forehead). In the placebo group, two AEs were assigned to be related (one patient with nausea and one with constipation), and one AE possibly related (lid edema, pruritus). There were two severe adverse events (SAEs) in two patients of the colesevelam group, both not related to the study medication: hospitalization after car accident and hospitalization for anal bougienage of a preexisting anal CD-related stenosis. All AEs resolved after discontinuation of the study drugs. Three AEs (constipation in the colesevelam group and constipation and nausea in the placebo group) led to withdrawal of the study drug and pretermination of the study. All SAEs and AEs are shown in Table 2.

4. Discussion

In this study, we conducted an investigator-initiated, randomized, placebo-controlled multicentre trial to evaluate the efficacy and safety of colesevelam in CD patients with BAM-associated diarrhea, which is a common feature of CD and is also associated with irritable bowel syndrome with diarrhoea (IBS-D).22–24 Recent open label studies, which were published in abstract form only,14,15 demonstrated positive effects of colesevelam on BAM-associated diarrhea. In our study, we confirm these results; however, the required number of patients to be recruited was not achieved which may limit the interpretation of the data. In the ITT analysis (primary analysis), we found that for the primary endpoint of this study, which was >30% reduction of liquid stools/day from baseline to termination visit after 4 weeks of treatment with 3 × 2 tablets colesevelam 625 mg or placebo, there was a trend towards a significant difference between the colesevelam and placebo group (ITT: P = 0.057). In the PP analysis, this risk difference was statistically significant (PP: P = 0.036). In patients treated with colesevelam, we found a reduction of the number of liquid stools per day from week 0 to week 4 (PP: P = 0.02; ITT: P = 0.01) as secondary outcome parameter. In contrast, patients receiving placebo had no statistically significant reduction of liquid stools per day (PP: P = 0.61; ITT: P = 0.42). The effect of colesevelam on the reduction of liquid stools might be dependent on the primary cholestenone level, since a subgroup analysis revealed significant reductions of liquid stools/day in colesevelam-treated patients with cholestenone values ≥200 mg/dL compared to patients <200 mg/dL, which had no statistical reduction.

Stool consistency, which was quantified by the visual Bristol stool chart, improved significantly in the colesevelam group but not in the placebo group. However, we found no differences between the two study groups regarding quality of life. There were significant more male patients in the colesevelam group which could be a potential bias of the study results; however, currently there are no data that male patients have better response rates to bile acid sequestrants than female patients.

As mentioned above, the required number of 46 patients could have not been recruited into this study. A main problem of enrolling patients in this study was the very stringent inclusion criteria, which did not allow patients with inflammatory activity to be included in the study, generating several screening and pre-screening failures due to elevated CRP and/or CDAI values. We choose these inclusion criteria together with the measurement of serum cholestenone to confirm the presence of BAM and rule out inflammatory activity of CD which could have had impacts on stool frequency or consistency. Since this study was an investigator-initiated trial (IIT) with limited financial support, we had only 5 national study sites active and despite several study calls in several publications of the national IBD organizations, there was a prolonged recruitment of patients. An additional problem of our study was the small sample size in several analyses of

<table>
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<th>PatID</th>
<th>Age</th>
<th>Sex</th>
<th>Group</th>
<th>Type</th>
<th>Description</th>
<th>Relation to study drug</th>
<th>Action taken</th>
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<td>F</td>
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<td>SAE</td>
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<td>AE</td>
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<td>No</td>
<td>None</td>
<td>Resolved</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>F</td>
<td>Placebo</td>
<td>AE</td>
<td>Lid edema, pruritus</td>
<td>Possible</td>
<td>None</td>
<td>Resolved</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>F</td>
<td>Placebo</td>
<td>AE</td>
<td>Menstrual pain</td>
<td>No</td>
<td>None</td>
<td>Resolved</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>F</td>
<td>Placebo</td>
<td>AE</td>
<td>Constipation</td>
<td>Yes</td>
<td>Treatment stop</td>
<td>Resolved</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>F</td>
<td>Placebo</td>
<td>AE</td>
<td>Upper respiratory tract infection</td>
<td>No</td>
<td>None</td>
<td>Resolved</td>
</tr>
</tbody>
</table>
patient subgroups. Therefore, the results of statistical analyses have to be interpreted with caution, since for non-categorical variables the Student's t-test was used. However, a very recent analysis demonstrated reliable results using Student's t-test even in extremely small sample sizes.25

There was no assessment of fecal calprotectin as a good inflammatory marker as inclusion criteria, which is a further limitation of this study. However, there are data that in addition to calprotectin also CRP values correlate with intestinal inflammation in IBD patients.26 Therefore, a CRP cut-off \( \leq 1 \) mg/dL, which we used as inclusion criterion, can exclude relevant inflammatory activity of CD. Since an elevated number of stools increase the CDAI value and many CD patients have chronically elevated CRP values, these were further major obstacles to enroll patients. Moreover, since inclusion criteria had to fit at screening as well at baseline visit, some patients did only meet the inclusion criteria at the screening visit but not at baseline visit and should not have been randomized, which resulted in a relative high rate of patients who could not be analyzed per-protocol (24%).

In our study, we confirmed that colesevelam is well tolerated, which has been demonstrated in previous studies.10 The majority of colesevelam-related adverse events were gastrointestinal (GI) symptoms like constipation, bloating and nausea which are well known side effects of bile acid sequestrating agents. We chose the highest approved dose of colesevelam (6 × 625 mg/day), which may result in a higher rate of GI-related adverse effects than lower doses. In clinical practice, most patients reduce the colesevelam dose when side effects occur which was not possible in our study. Only one patient, who received colesevelam, terminated the study due to constipation. Interestingly, also a patient receiving placebo had constipation and also terminated the study prematurely. Of note, all observed side effects in our study were mild and completely resolved after discontinuation of the study drug. In terms of better patient compliance, the good tolerability of colesevelam might be an advantage compared to cholestyramine which often causes side effects such as constipation, nausea, bloating, flatulence, bloating and abdominal pain. However, since the study period was only 4 weeks, we cannot provide long-term safety data for the use of colesevelam in patients with CD disease and BAM. Long-term side effects should be the same that observed in colesevelam-treated patients with type 2 diabetes, which were mainly constipation and dyspepsia.27 However, other potential long-term side effects of colesevelam like vitamin malabsorption, particularly of fatty soluble vitamins such as vitamin K potentially resulting in coagulopathy, might be more problematic in IBD patients with per se malabsorptive problems. Therefore, monitoring for vitamin deficiency in patients with long-term colesevelam therapy should be advocated in CD patients. In CD patients treated with cyclosporine, trough level measurements are necessary when starting or stopping colesevelam, since it decreases the bioavailability of cyclosporine. All other oral medication should also be taken with at least two hour delay after colesevelam intake, since intestinal absorption of other drugs may be altered by colesevelam.

The results of our study regarding the reduction of liquid stools in patients with BAM by colesevelam are in line with previous experiences for the use of other bile acid sequestrants in BAM. A retrospective study observed treatment responses to cholestyramine among patients suffering from chronic watery diarrhea.28 In this study, BAM due to ileal dysfunction (type I) was found in 77 patients, idiopathic bile acid malabsorption (type II) was found in 68 patients, and 56 patients with other conditions had bile acid malabsorption (type III). Of the 150 patients with BAM, more than half of them reported positive effects on their bowel habits after cholestyramine therapy. Importantly, most severe BAM in CD patients occurs after resection of the terminal ileum, but BAM can occur in up to 50% in unresected CD patients, regardless of the disease localization.29 About half of the patients included in our trial had previous ileoceleal resection (ICR) but we could not find a correlation between high cholestenone values and ICR and there was no significant difference in the ICR rate between both groups.

Since the diagnosis of BAM in CD patients is difficult, laboratory tests for BAM should become a part of the algorithm for diagnosis of CD with diarrhea to identify patients who might respond to therapies such as bile acid sequestrants. Elevated serum levels of cholestenone as indirect marker of increased synthesis of bile acids and the ileal hormone fibroblast growth factor 19 (FGF19), which is a regulator of bile acid synthesis, are valuable tests to detect patients suffering from BAM. In a recent study of patients with BAM-associated diarrhea compared with a control group with not BAM-associated diarrhea, negative and positive predictive values of FGF19 values \( < 145 \) pg/mL for a SeHCAT value <10% were 82% and 61%, respectively.30 Therefore, both are good markers to detect BAM with an indirect, proportional relationship between levels of cholestenone and FGF19 (\( P < 0.001 \)).31

In conclusion, we observed effects of colesevelam in CD patients with BAM-associated diarrhea in terms of reduction of liquid stool frequency and improvement of stool consistency with a low incidence of side effects. Colesevelam may therefore serve as an alternative treatment option for patients who are intolerant or do not respond to cholestyramine or other bile acid sequestrants.

**Authors' contribution to the paper**

FB study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; obtained funding; study supervision

NT acquisition of data; critical revision of the manuscript

SH acquisition of data; critical revision of the manuscript

FL acquisition of data; critical revision of the manuscript

JM acquisition of data; critical revision of the manuscript

SIB statistical analysis

CR technical and material support; critical revision of the manuscript

BG administrative support; study supervision; critical revision of the manuscript

SB acquisition of data; drafting of the manuscript; critical revision of the manuscript

TO study concept and design; obtained funding; drafting of the manuscript; critical revision of the manuscript.

All authors read and approved the final manuscript.
Conflict of competing interests statement

There are no potential conflicts of interests of any author and co-author related to this manuscript. Genzyme/Sanofi was not involved in writing or statistical analysis of this study.

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