SINBAD

Treatment effect of colesvelam for bile acid diarrhoea
– a randomised placebo-controlled trial.

Danish Region Zealand scientific ethics committee: SJ-641
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Abbreviations
Aim
1. To determine the efficacy and safety of colesevelam for treating bile acid diarrhoea (BAD).
2. To correlate both the current scintigraphic 75-Selenium conjugated Tauro-homocholic acid retention test (SeHCAT) and the biochemical markers of BAD fibroblast growth factor 19 (FGF19) and 7α-hydroxy-4-cholesten-3-one (C4), with colesevelam treatment response.

Summary of expected improvements from the study
The present diagnosis of BAD rests on the radionucleotide based SeHCAT test that has limited accessibility. Patients with BAD therefore often endure an extensive diagnostic workup including endoscopies before referral for SeHCAT. Consequently, the patients are often misdiagnosed and suffer impaired quality of life although treatment for BAD exists. A biochemical test will allow timely screening of patients with chronic diarrhoea in analogy with tests for celiac disease, increase awareness of BAD, reduce diagnostic delay, and the exposure to unnecessary invasive examinations. Demonstrating the treatment effect of colesevelam in a randomised placebo-controlled trial and correlating each biochemical test result with the subsequent treatment effect will improve both the diagnostic strategy and the treatment of BAD considerably. This crucial link between test and expected treatment effect is currently missing and providing this will help doctors treat and advice their patients better.

Background
Bile acid diarrhoea
Bile acid diarrhoea (BAD) is a common cause of chronic watery diarrhoea affecting an estimated 1% of the general population (1). BAD is detected in 20–30% of patients with diarrhoea predominant irritable bowel syndrome (IBS-D) (2), up to 40% of patients with microscopic colitis (3), and in patients with Crohn’s disease without inflammatory activity (2, 4-8).

Bile acids are synthesised in the liver, excreted in the bile and facilitate the lipid absorption in the small intestine as micelles. Bile acids that are not thus absorbed are normally reabsorbed in the terminal ileum by the ileal bile acid transporter and returned to the liver via the portal vein. The bile acid pool recirculates approximately 20 times per day and 95–97% is reabsorbed at each passage of the small intestine (4, 6). Non-absorbed bile acids enter the large bowel and give active secretion when present in higher concentrations causing watery diarrhoea.

Bile acid diarrhoea is historically classified as:
- **Type 1**: secondary to disease in the terminal ileum and resection of the terminal ileum.
- **Type 2**: idiopathic or primary BAD
Type 3 secondary to other diseases such as microscopic colitis and cholecystectomy.

New insight into the regulation of bile acid synthesis and its enterohepatic circulation has demonstrated that patients with primary BAD have normal reuptake of bile acids, and overproduction of new bile acids that surpass the ileal re-uptake capacity (4, 6, 7, 9). This has provided new possibilities for both diagnosis and treatment of BAD.

Present diagnosis of bile acid diarrhoea

The present diagnostic test for BAD is the SeHCAT retention test that originally was introduced by Thaysen (10). The $^{75}$Selenium decays by γ-emission with a half-life of 120 days and when bound to Taurine-conjugated homocholic acid (HCAT) it recirculates in the enterohepatic circulation. The ratio between γ-emission measured seven days apart is the test result and reflects the loss to the large intestine. Retention values representing severe (SeHCAT retention 0–5%), moderate (5–10%) and mild BAD (10–15%) have never been validated in placebo-controlled trials (11), but usually moderate and severely reduced retention is associated with diarrhoea and with treatment effect (11). The SeHCAT test is not available in the US and many other countries and $^{75}$Selenium is manufactured in one facility only. Thus, diagnosis of BAD is often delayed and misdiagnosis is common as reflected by the high proportion of BAD among IBS-D patients (2). In lack of better options, clinicians may rely on the subjective treatment effect of a bile acid sequestrant reported by the patient. This approach has not been validated (12) and has several diagnostic pitfalls making the interpretation difficult (12, 13). Bile acids can be measured in the stools but it is cumbersome and used only for research purposes (14).

Biochemical markers of bile acid diarrhoea

New knowledge has emerged on the physiology and regulation of enterohepatic bile acid circulation (15) and of bile acid synthesis (15, 16). 7α-hydroxy-4-cholesten-3-one (C4) is the key intermediary molecule in bile acid synthesis and a marker for the de novo synthesis of bile acids. High levels of C4 in serum are associated with BAD (17-20) and compared with SeHCAT, C4 has a sensitivity of 90% and specificity of 79% for detecting BAD (18, 20). However, C4 requires analysis by high-performance liquid chromatography (HPLC) with tandem mass spectrometry and has thus primarily found clinical use at centres with a special interest in BAD and no access to SeHCAT (14). Several factors may influence C4 of which diurnal variation and food intake are best described (21). Plasma concentration of C4 increases acutely 4–6 hours after intake of alcohol (22). C4 is decreased in cholestasis and may be marginally decreased in hepatic cirrhosis (23). Statins and fibrates decrease the amount of cholesterol available for bile acid synthesis. Thus, atorvastatin lowers a medically or surgically induced elevated C4 (24).
The hormone FGF19 is released from enterocytes in the terminal ileum into the portal circulation in response to bile acid absorption through stimulation of the farnesoid X receptor (16). FGF19 inhibits hepatic bile acid synthesis and relaxes the gallbladder. It also has an insulin-like effect increasing liver gluconeogenesis, decreasing gluconeogenesis, but unlike insulin decreases lipogenesis and increases protein synthesis in the liver (25) – all physiological anti-diabetic effects.

Walters et al. showed that the pathogenesis of primary BAD is an impaired negative hepatic feedback by FGF19 leading to overproduction of bile acids (26, 27). FGF19 correlates inversely with C4 in healthy volunteers (17) and fasting values of FGF19 correlate with SeHCAT (9).

Unfortunately, fasting FGF19 alone varies considerably both within and between individuals. A single low fasting value of FGF19 <145pg/mL has 58% sensitivity and 84% specificity for moderate BAD (SeHCAT ≤10%) (9), which in a clinical context is insufficient.

**Biochemical tests in our population – choice of C4 cutoff**

We studied FGF19 after stimulation with a mixed meal (28) and then with combinations of the meal plus chenodeoxycholic acid (29) and pilot results were promising. Based on these preliminary results, we did a prospective validation study (VABAD) of 71 consecutive diarrhoea patients referred for SeHCAT. We found that neither stimulated nor fasting FGF19 were better than C4. The fasting FGF19 receiver operating characteristics (ROC) analysis versus SeHCAT ≤10% showed an area under the curve (AUC) of 0.73 and for C4 it was 0.83. Thus, C4 is the better biochemical test. Of the 71 VABAD patients, 59 fulfilled the Hjortswang criteria for diarrhoea (described below) that is a prerequisite in SINBAD. Table 1 shows ROC results for this subpopulation. The C4 cut-off value of 15.4 ng/mL had optimal diagnostic characteristics. We choose a low cutoff value to keep false negative test results at a minimum.

<table>
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<tr>
<th>TABLE 1</th>
<th>Results from the VABAD study</th>
<th>SeHCAT&lt;=10%</th>
<th>SeHCAT&gt;10%</th>
<th>ROC analysis, AUC (95% CI)</th>
<th>Cutoff for positive test</th>
<th>Sens./spec. (%)</th>
<th>PPV/NPV (%)</th>
</tr>
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<tbody>
<tr>
<td>Fasting C4</td>
<td>N=25 Median (IQR)</td>
<td>30 (16–63)**</td>
<td>7 (4–15)</td>
<td>0.82 (0.71–0.93)**</td>
<td>C4 ≥ 15.4</td>
<td>76 / 80</td>
<td>70 / 81</td>
</tr>
<tr>
<td>Fasting FGF19</td>
<td>N=34 Median (IQR)</td>
<td>75 (57–146)*</td>
<td>115 (8–220)</td>
<td>0.69 (0.54–0.83)*</td>
<td>FGF19 ≤ 85</td>
<td>60 / 77</td>
<td>65 / 72</td>
</tr>
<tr>
<td>Stimulated FGF19</td>
<td>N=34 Median (IQR)</td>
<td>41 (-14–104)</td>
<td>9 (-28–38)</td>
<td>0.39 (0.24–0.54)</td>
<td>ΔFGF19≤22</td>
<td>44 / 41</td>
<td>35 / 50</td>
</tr>
</tbody>
</table>

FGF19 in pg/mL and C4 in ng/mL. The stimulated FGF19Δ0–90min was based on stimulation with a meal and 1250mg chenodeoxycholic acid. The Δ0–90min value is the difference between FGF19 at fasting (0min.) and after 90 min. SeHCAT groups were compared with Mann-Whitney U-test. The statistical significance of the ROC curves was tested nonparametric versus true area=0.50. *p=.02; **p=.00003

Figure 1: Receiver operating characteristics for C4 versus SeHCAT ≤ 10%. The area under the curve is 0.83 (p<0.0001). (Yet
Treatment of Bile Acid Diarrhoea

Based on observational data, sequestrants such as cholestyramine, colesevelam, and colestipol seem effective treatments of BAD (11). They bind to anions like bile acids and these are then excreted with the faeces lowering cholesterol and alleviating symptoms of bile acid diarrhoea. All are licensed for treating hypercholesterolaemia, but cholestyramine has the additional indication for BAD. Cholestyramine is a well-established treatment for BAD, but there are no placebo-controlled trials with cholestyramine for BAD since formulating a placebo for cholestyramine has been impossible. One recent study compared cholestyramine with hydroxypropyl cellulose as an intended placebo in 26 participants with functional diarrhoea (30). Unfortunately, hydroxypropyl cellulose had an active effect on diarrhoea with a per protocol (PP) response rate of 38% compared with 64% for cholestyramine (p=0.22). A 23% dropout rate in the cholestyramine arm reflects that cholestyramine is unpalatable and this dropped the intention to treat (ITT) cholestyramine response rate to 58%.

Colesevelam is used off-label for treating BAD often as second-line therapy due to its higher price, however many BAD patients who do not tolerate cholestyramine benefit from colesevelam, and because of this placebo-controlled studies have been warranted (12, 31). A placebo-controlled trial of colesevelam for suspected BAD in patients with Crohn's disease and diarrhoea despite inflammatory remission (i.e. suspected BAD), found diarrhoea ITT remission rates of 67% (10/15) for colesevelam and 27% (3/11) for placebo (p=0.057). Unfortunately, the extreme inclusion and exclusion criteria caused slow recruitment and the study was terminated prematurely (32).

A recent open, non-controlled trial with obeticholic acid, a potent FXR agonist that stimulates FGF19 synthesis, demonstrated that obeticholic acid reduced bile acid synthesis and had a clinical effect in patients with primary BAD and some cases of type 2 BAD (33). This demonstrates the effect of a new pharmacological class of treatment for BAD.
Design
Randomised, placebo-controlled, parallel groups, double-blinded multicenter, phase IV trial.

Null hypothesis:
Colestevlam and placebo have the same effect on BAD.

Intervention:
Colestevlam (tablets of 625mg) or identical placebo capsules. One to three capsules to be taken twice daily. Start dose is two capsules twice daily. The dose is titrated by a study nurse who is independent of the investigators. Parallel arms of 12 days blinded treatment, of which the first five days is run-in and the last seven days measure endpoints.

Study subjects
Inclusion criteria
- Patients referred to Clinical Physiological/Nuclear Medicine departments for SeHCAT at Holbæk, Hvidovre, Aarhus, and Aalborg University Hospitals
- Suspected BAD
- ≥ 18 and < 80 years of age.
- Women of fertile age must use safe contraception during the treatment part of the study
  - spiral or hormonal contraception, ie. birth control pill, hormonal implant, transdermal patch, vaginal ring, or contraceptive depot injection.
- Ability to give informed consent after written and oral information in the Danish language

Exclusion criteria
- Inflammatory bowel disease, including microscopic colitis
- Investigator-assessed debilitating chronic disease e.g. WHO performance score 3–5
- Prior treatment with colestevlam
- Treatment with laxatives or anti-diarrhoeal drugs during the study
  - Except for stable dose the last four weeks of psyllium husk and opioids for pain
- Pregnancy
- Breastfeeding women
- Crucial medication that cannot be separated appropriately from colestevlam
  - i.e. taken one hour before or 4 hours after colestevlam
- Oral anticoagulation, both warfarin, and new oral anticoagulation
- Treatment with cyclosporine within two months
• Bowel obstruction (subileus or ileus)
• Biliary obstruction
• Short bowel syndrome
• Bowel ostomy
• Allergy to colesevelam or its constituents
• Allergy to placebo constituents (excluding lactose)
• Investigator-assessed high risk of non-compliance
• If on statin/fibrate medication, unwilling to pause medication between study visits 1 and 2

Withdrawal criteria
Upon inclusion (i.e. Visit 1) we screen for:
1) Biliary obstruction – plasma total bilirubin must be < 2 x upper normal limit
2) Pregnancy – plasma/urine HCG must be negative in all women of childbearing potential
The screening result must be available before randomisation (i.e. at Visit 2).

Endpoints
Primary endpoint
Placebo-controlled ITT diarrhoea remission rate defined by the Hjortswang criteria for colesevelam in patients with BAD defined by C4 ≥ 15.4 ng/mL.

Secondary endpoints
1. PP analysis for the primary endpoint
2. Placebo-controlled diarrhoea ITT remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by SeHCAT ≤ 10%
3. Placebo-controlled diarrhoea PP remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by SeHCAT ≤ 10%
4. Placebo-controlled effect of colesevelam in patients with bile acid diarrhoea defined with C4
   a. on the absolute number of stools (mean per day over 6 or 7 days)
   b. on the total number of Bristol 6 and 7 stools (mean per day over 6 or 7 days)
5. Placebo-controlled diarrhoea ITT remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by fasting FGF19 ≤ 85 pg/mL
6. The difference in change in Health-related quality of life (HRQoL) in the primary endpoint population by the Short Form 36 version 2 (SF36v2) items (colesevelam vs. placebo)
   a. Physical component score
   b. Mental component score
7. Placebo-controlled subject assessed “good response” ITT rate in the primary endpoint population
8. Placebo-controlled ITT diarrhoea remission rate defined by the Hjortswang criteria for colesevelam in patients with non-BAD, ie. defined by C4 < 15.4 ng/mL

The secondary endpoints are tested with correction for multiple statistical testing and significant results are reported as such.

The secondary endpoints will also be tested at the 0.05 significance level and reported as hypothesis-generating results acknowledging the risk of type 1 error.

Descriptive endpoints and ancillary analytic endpoints

- Placebo-controlled remission rates (ITT+ PP) for colesevelam defined by the Hjortswang criteria in patients with BAD
  - Defined by SeHCAT ≤ 5%
  - Defined by SeHCAT ≤ 15%

- Analysis as the primary endpoint and secondary endpoints 1–4, but in non-BAD patients:
  - Defined by C4 < 15.4 ng/mL
  - Defined by SeHCAT > 10%

- Distribution of C4, FGF19, and SeHCAT results

- ROC analyses comparing different cut-offs for C4, SeHCAT, FGF19, subject assessed “good response”, with the response by Hjortswang criteria
  - Validation of C4 cut-off ≥ 15.4ng/mL with SeHCAT ≤ 10% for diagnosing BAD

- Repeatability of baseline fasting C4 and FGF19 assessed with a Bland-Altman plot
  - Exploratory assessment of the effect of sampling time, statin use, and alcohol intake on C4

Ancillary endpoints for Patient Reported Outcomes

These will be reported in a separate paper. Health-related quality of life (HRQoL) (Questionnaires) correlated to diarrhoea defined by the Hjortswang criteria

- Correlation between HRQoL and Bristol stool scale diarrhoea.
- Correlation between HRQoL and stool number per week.
- Correlation between changes in HRQoL and primary endpoint
- Six-month follow up on HRQoL
- Six-month follow up on the aetiology of diarrhoea
- Six-month follow up on medication
- The response in Short Health Scale (SHS) comparing the answers before treatment with answers during treatment
- Description of sexual dysfunction with HRQoL in the population
Power calculation

Based on the primary endpoint we assume

- a remission rate of 63% for colesevelam and 27% for placebo (30, 32). As we define BAD by C4 in our primary endpoint as opposed to SeHCAT, we have chosen a more conservative remission rate than the 67% reported by Beigel et al. (32).

- two-sided $\alpha = 0.05$, and $\beta = 0.20$ (ie. 80% power); and 1:1 allocation.

Thus, we need 29 subjects with BAD in each arm (G.Power 3.1: z-test of two independent proportions).

As we include all patients with diarrhoea regardless of aetiology, the fraction or prevalence of BAD amongst these eligible patients is crucial. Of 71 subjects in our VABAD study, 59 patients had diarrhoea by Hjortswang criteria and deemed by C4 $\geq$ 15.4ng/mL, 27 of the 59 had BAD (45.7%). In comparison with SeHCAT, Borghede et al. found that 41% of patients referred for SeHCAT had primary BAD defined with SeHCAT (34).

Allowing for dropout (10%) and to include 2 x 29 subjects with BAD we thus need:

$$\frac{2 \times 29 \text{ BAD subjects}}{45.7\% \text{BAD subjects among subjects with diarrhoea}} \times 110\% = 140 \text{ subjects with diarrhoea.}$$

Methods

Bristol stool form scale

Bristol stool scale classifies stool from hard lumps (1 on the scale) to watery (7 on the scale). A one-week stool diary is usually collected in clinical trials (35, 36). Time and form of stools are noted and further information on pain, urge and incontinence can be obtained. In patients undergoing diagnostic workup for diarrhoea, those with an organic cause of diarrhoea more often have $\geq$3 stools per day and watery stool consistency (37) compared with functional diarrhoea.

Response criteria in chronic watery diarrhoea – Hjortswangs criteria

Hjortswang et al. have validated that disease activity defined by stool consistency and frequency correlates with reduced quality of life determined by health-related quality of life (HRQoL) questionnaires in patients with collagenous colitis (35, 38) and lymphocytic colitis (39).

- Activity is defined as $\geq$ 3 stools per day or $\geq$ 1 watery stool (BSF 6-7) per day as the mean of seven (six) days.
- Remission is defined as <3 stools per day and < 1 watery stool per day as the mean of seven (six) days.
In conjunction with these definitions, it was recently documented that both stool frequency and consistency correlated with an organic cause of chronic diarrhoea, including BAD, with consistency being the most powerful predictor (37). As there are no validated activity criteria for BAD and chronic diarrhoea is the major symptom, we will apply Hjortswang’s criteria for activity and remission.

Diarrhoea remission rates are calculated from comparison of the baseline Study Diary 1 (i.e. Study Day 1 – 7) with the endpoint part of the Study Diary 2 (i.e. Study Day 14 – 20).

**Patient-reported outcomes**

**Short Health Scale and Short Form 36 version 2**

The Short Health Scale (SHS) is a simple index using four 0 – 100 VAS scales to estimate symptom burden, social function, disease anxiety, and well-being (40). The SHS correlates to the more elaborate HRQoL questionnaires both with the severity of symptoms and global assessment and has been validated in English and in Swedish in patients with both inflammatory and functional bowel disorders (35, 38, 40-43).

The SHS has not been validated in BAD nor in Danish. Therefore, we will compare the result of an SHS questionnaire with the more elaborate SF36v2 and disease activity defined by the Hjortswang criteria.

**Gastrointestinal Symptom Rating Scale**

The Gastrointestinal Symptom Rating Scale (GSRS) quantifies gastrointestinal symptoms on 15 Likert scales grading symptoms from 1 (mild) to 7 (severe). Added questions specify symptom from the upper and the lower gastrointestinal tract. The GSRS questionnaire is validated in Danish and widely recognised (44).

**Health-related quality of life and sexual dysfunction**

Sexual dysfunction from chronic diarrhoea due to inflammatory bowel syndrome is common but often the problem goes unnoticed. The questionnaires Female Sexual Distress Scale (FSDS), Female Sexual Function Index (FSFI), and International Index of Erectile Function (IIEF) address the issue in general and the novel IBD-SEX questionnaire address sexual dysfunction from inflammatory bowel disease (IBD) and chronic diarrhoea (45, 46). The IBS-SEX questionnaire is validated in Danish language and validation in Danish patients with IBD is ongoing.

**Patient-reported “good treatment response”**

A diagnostic therapeutic trial is a common practice instead of testing for BAD. Patients with suspected BAD are treated with a sequestrant and assess the subjective effect. We ask the
subjects in the intervention group if they had a good response. In order to compare this with the dichotomous Hjortswang response criteria, answers are:

Question: “Do you think that the treatment relieved your diarrhoea?”
   a) “No, it was not sufficiently effective”; b) “Yes, it was sufficiently effective”.

In Danish
“Synes du behandlingen fjerne din diarré?”
   a) ”Nej, den var ikke tilstrækkelig effektiv”; b) ”Ja, min diarré forsvandt under behandlingen”.

**Blinding**

**Initial blinding:** Over-encapsulation makes colesevelam and placebo tablets identical. These are produced, packed, and uniquely numbered at the Hospital Pharmacy of the Capital Region, from where packages are distributed to each study centre.

**Maintaining blinding:** Due to the expected treatment effect and possible side effects, maintaining subject blinding could be difficult. This potential bias cannot be avoided. To avoid investigators to be unblinded, dose titration and AE registration during treatment are performed by an independent study nurse.

We assess subject blinding early in the treatment period and again at treatment end. We assess investigator blinding at treatment end. Each assessment the question is: “Do you think you/the subject is given a) Colesevelam, b) Don’t know, c) Placebo. With this information, we quantify both initial and maintained blinding to estimate the size of potential bias (47-50).

The blinding continues until the last subject has completed the clinical study phase and all data entries have been checked and locked. At this time point, the blinding is unveiled, all subject are informed, and the treatment is documented in the medical chart.

**Emergency unblinding**

Sealed envelopes for emergency unblinding are enclosed with each delivery from the Pharmacy to a study centre for the local primary investigator with a copy to the Sponsor. The local investigator or Sponsor may use such an envelope to unblind the treatment at any time and without restrictions if it is deemed necessary. The study subjects are also instructed to keep the contact information for the primary investigator who is contacted in case of any emergency that could warrant unblinding of the study intervention.

In case of unblinding, two dated signatures are needed on the envelope; in an emergency, these need not be from investigator or subinvestigator. Signing the envelope states that it will be opened, and beforehand is intact and untampered.
Randomisation
A randomisation list is made by the Hospital Pharmacy of the Capital Region by computer randomisation distributing consecutive unique numbers in variable block sizes of two, four, or six to one of the two treatment arms (www.randomization.com). The different block sizes hinder deduction of the allocation of the ultimate slots in a block and secure a 1:1 allocation. The Hospital Pharmacy of the Capital Region keeps the randomization lists separately and exclusively until study end.

Biochemistry
FGF19 is analysed by commercially available ELISA assay (R&D Systems, MN, USA). C4 is analysed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (51). These samples are prepared and frozen for bulk analysis once inclusion is finished. Routine blood samples are analysed in the hospital laboratories of participating centres. These include ALT, ALP, amylase, HDL, LDL and total cholesterol, triglycerides, bilirubin, glucose with sampling time specified elsewhere. In total, these samples comprise 24 mL blood from each subject. We accept differences in laboratory reference values and procedures in between study centres as these analyses do not comprise endpoints.

Biobank
Study Biobank: Frozen plasma samples for FGF19 and C4 are stored in a biobank for analysis once the last participant has finished the study. After analysis, surplus plasma is re-frozen and stored for a period of maximum 15 years, but only if the subject has given consent to the biobank for future scientific work described below. Analysis of bile acid species including C4 is done in Paris (see cooperating departments, page 3). Biobank for future scientific work: This is created for future, yet unspecified scientific work. 22mL blood is required to be stored as 4mL plasma and 10mL EDTA-blood. Furthermore, a stool sample is collected for this biobank. Separate informed consent is obtained for the biobank. Consent may be withdrawn and the samples are then destroyed. This biobank is stored for a period of 15 years and then destroyed. The biobank will not be given to the third party. Data security is monitored by the Danish Data Security Agency.

Study medication
Colesevelam
Colesevelam is registered for treating hypercholesterolemia; however, it is extensively used off-label as a sequestrant treating bile acid diarrhoea (12, 13, 31, 32). The treatment effect is comparable to that of colestyramine and it is considerably better tolerated (12, 32). Colesevelam
tablets are bought from Sanofi-Aventis by the Hospital Pharmacy of the Capital Region of Denmark. The Pharmacy encapsulates colesevelam and placebo tablets (Capsugel® DBcaps®, size AAA) in accordance with Good Distribution Practise and Good Manufacturing Practise and documents this.

**Purpose:** the active arm of the double-blinded randomised intervention.

**Dose:** We strive for a standard dose of three capsules each of 625mg twice daily taken before breakfast, lunch, or dinner. To avoid drop-out due to dose-related adverse reactions such as nausea, constipation, and vomiting we use dose escalation and titration.

Start dosage: 2 tablets twice daily for two days. If the subject has no or limited side effects, the dose is increased to 3 tablets twice daily. In case of severe constipation, nausea, or vomiting the dose may be decreased (to one capsule twice daily). Minimum dose is one capsule twice daily. Dosing once daily is not allowed. All subjects are contacted by telephone on day 2 to ensure dose escalation if possible. If the dose is changed the subjects is contacted again 2–3 days afterwards to follow up.

Communication regarding dose and registration of AEs is kept between the subject and a study nurse does not partake in endpoint registration nor in reporting of the study. If the nurse needs advice from a doctor, this is first sought from a doctor who is not affiliated with the study; ie. not from an investigator. In matters regarding possible serious adverse events, the decision of emergency unblinding, study participation etc. the investigator must be involved.

**Side effects to colesevelam:** See the exert below from Summary of Product Characteristics (updated 01.02.2017).
4.8 Undesirable effects

Summary of the safety profile
The most frequently occurring adverse reactions are flatulence and constipation, found within the gastrointestinal disorders system organ class.

Tabulated list of adverse reactions
In controlled clinical studies involving approximately 1400 patients and during post-approval use, the following adverse reactions were reported in patients given Cholestagel.

The reporting rate is classified as very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100), rare (≥1/10,000 to <1/1,000), very rare (<1/10,000) and not known (cannot be estimated from the available data).

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<thead>
<tr>
<th>Nervous system disorders</th>
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<tbody>
<tr>
<td>Common: Headache</td>
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<table>
<thead>
<tr>
<th>Gastrointestinal disorders</th>
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</thead>
<tbody>
<tr>
<td>Very common: Flatulence*, constipation*</td>
</tr>
<tr>
<td>Common: Vomiting, diarrhoea*, dyspepsia*, abdominal pain, abnormal stools, nausea, abdominal distension</td>
</tr>
<tr>
<td>Uncommon: Dysphagia</td>
</tr>
<tr>
<td>Very rare: Pancreatitis</td>
</tr>
<tr>
<td>Not known: Intestinal obstruction*,**</td>
</tr>
</tbody>
</table>

Musculoskeletal and connective tissue disorders

<table>
<thead>
<tr>
<th>Common: Myalgia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigations</td>
</tr>
<tr>
<td>Common: Serum triglycerides increased</td>
</tr>
<tr>
<td>Uncommon: Serum transaminases increased</td>
</tr>
</tbody>
</table>

* see section below for further information
** adverse reactions from post-marketing experience

Description of selected adverse events (continued from exert)
The background incidence of flatulence and diarrhoea were higher in patients receiving placebo in the same controlled clinical studies. Only constipation and dyspepsia were reported by a higher percentage among those receiving Cholestagel (colesevelam), compared with placebo.

The incidence of intestinal obstruction is likely to be increased among patients with a history of bowel obstruction or removal. Cholestagel in combination with statins and in combination with ezetimibe was well tolerated and the adverse reactions observed were consistent with the known safety profile of statins or ezetimibe alone.

Placebo
Matrix placebo tablets (17mm size) with same capsule (Capsugel® DBcaps®, size AAA) making these identical to the colesevelam capsules.

The tablet matrix does not affect bowel function. Constituents are as follows per tablet:

- Lactose monohydrate 330mg
- Potato starch 335mg
- Gelatine 12mg
- Magnesium stearate 3.5mg
- Talc 31.5 mg
The placebo capsules (Capsugel® DBcaps®, size AAA) dissolve in the stomach giving a negligible delay in availability of colesevelam of 2–3 minutes.

No side effects are expected from the placebo tablets.

**Study plan**

**Pre-screening – Written invitation**

This has two steps:

1. Eligible participants as deemed by inclusion and exclusion criteria are either given or send the written invitation by the Gastroenterological department when referred for SeHCAT or send from the Clinical Physiological Nuclear Medicine department to referred patients. All pre-screened participants are anonymously registered on a pre-screening list.

2. At the first SeHCAT visit a sub-investigator asks the potential subject, if the written invitation is received, read, and understood. **Further, we ask if they consider participation and/or would like further information from the investigator (doctor).**

The investigator assesses eligibility, informs and may include the patient. If an eligible subject opts to participate in this visit (first SeHCAT visit) is called **Study visit 1.**

**Study visit 1 – Inclusion and start of baseline registration**

**Study Day 1.**

After inclusion, a blood sample for the study biobank is drawn. This sample is used to assess the concordance between repeated tests, and we register the given conditions for this sample to explore how sampling time, fasting, medication etc. affect the C4 sample.

**Case Record Form**

At inclusion the (sub)investigator creates an electronic Case Record Form for the study subject to document:

- Medical history
  - Physical status
  - Medication of interest: antidiabetic, analgetic, anti-hypercholesteremia, anti-diarrhoea, laxatives, systemic glucocorticoids, recent (3 mo.) systemic antibiotics.
  - Prior medical and surgical history
  - Results within 12 months of any diagnostic workup regarding diarrhoea
- Microbiological stool samples
- Fecal-calprotectin
- Fecal-elastase
- Biochemical analyses for lactose intolerance, coeliac disease, thyrotoxicosis
- Endoscopies, regarding coeliac disease and inflammatory bowel disease including histology
- Breath tests for malabsorption or bacterial overgrowth
  - Use of tobacco and alcohol
    - Baseline blood analyses (ALT, ALP, bilirubin, amylase)
    - Weight (measured) and height

The (sub)investigator needs access to the subject’s medical file and chart. This is specified in participant information and is given in the written informed consent.

All subjects complete the baseline questionnaires (Q-base) consisting of
1) Short Form 36 version 2 (SF36v2 two week recall) on HRQoL
2) Gastrointestinal Symptom Rating Scale (GSRS)
3) Short Health Scale focused on the gastrointestinal effect on HRQoL.

We ask all subjects if they as a voluntary supplement would opt to answer the questionnaires addressing sexual dysfunction due to chronic diarrhoea. If yes; we send the questionnaire to the subject to answer at home before Visit 2 and again at the six-month follow-up.

All subjects are given:
1. the baseline diary
2. a kit for stool sampling at home

**Optional stool sample for biobank for future scientific work**

The subject may opt to take a collection kit for stool sample at home on study day 6 or 7 to make 75-Se radiation negligible. The sample is immediately placed in the subjects’ -18 – -23°C freezer. The subject brings the sample on study visit 2 for placement in a - 80°C freezer. If the subject continues to treatment, he/she may opt for a second sample taken on treatment day 18–20 and deliver it on Visit 3.

Stool sampling is not mandatory for study participation and is only planned if it is feasible according to the individual circumstances.

**Notification to the institution that referred for SeHCAT**

The (sub)investigator e-mails the institution that referred for SeHCAT with instructions to contact the investigator if they find participation inappropriate. This notification is only optional on the condition that the subject consents to the notification.
Study visit 2 – Assessment of eligibility by stool diary results

Study Day 8. Concurrent with the second SeHCAT visit, all subjects meet in the fasting state, the only exception is drinking still water. Subjects must not ingest alcohol on the day before visit 2.

Fasting blood samples are drawn from all subjects, no later than 10:00 AM.

The blood samples include: 4mL plasma for C4 and for FGF19 analysis (total 8mL). Routine samples for: p-triglycerides, p-cholesterols (total, High and Low-Density Lipoproteins), fasting p-glucose.

Any violation of alcohol abstinence the day before study visit 2 and of pausing statin/fibrate use leads to exclusion as a protocol violation. Minor violations of fasting (drinking thin fluids like coffee or tea, also with milk added) are noted in the CRF and the patient can participate in the study. All other violations lead to exclusion or rescheduling for fasting blood sampling another day.

The (sub)investigator collects the baseline study diary and tallies this.

Study Diary 1 – baseline

Subjects are screened for baseline diarrhoea as defined by Hjortswang’s criteria:

- ≥3 stools per day or ≥ 1 watery stool per day (Bristol Stool form 6 or 7) as a mean of seven days (minimum six days).

The baseline week is the week between the first and second SeHCAT retention test.

The result of the baseline stool diary determines whether the subject objectively has diarrhoea as specified above.

Subjects without diarrhoea:

These subjects do not proceed to the intervention, thus, we do not register adverse events for these subjects.

SeHCAT results are registered once available and the subjects are reminded of the six-month follow-up on the subject’s medical chart and the aetiology of the subject’s health complaints. The follow-up is done by telephone and the questionnaires are distributed electronically, or in special circumstances by mail. After the six-month follow-up, study participation ends for the subjects without diarrhoea.

Subjects with diarrhoea:

This is the ITT population. Adverse Event registration starts now (study day 8) and includes all subjects with diarrhoea.
The (sub)investigator dispatches the relevant double blinded treatment packet according to the randomisation sequence.

**Start of intervention and possibility for delay of intervention**

If the time/hour of the day for visit 2 enables the subject to take two doses of the medication this day, the intervention may start on visit 2, which subtracts one day from the time schedule below. For logistic reasons in the collaboration between the Department of Nuclear Medicine and the Department of Gastroenterology at the local study centre, the treatment start may be delayed for a maximum of seven days. In case the start is postponed, the postponed days are not counted as study days.

**Study Diary 2 – treatment period**

This diary registers the number of daily stools and consistency according to the Bristol stool form. The participants also note any AEs. The Study Diary 2 is commenced at Study Visit 2 and entails three periods

1. Study Visit 2 (Study day 8)
2. Run-in period of five days (Study day 9 – 13)
3. Registration of Primary endpoint (Study day 14 – 20)

These periods are not separated in the diary. The diary also forms the subject’s study participation card, with contact information to the relevant investigator or study nurse.

On study day 9, subjects set an “X” in the diary according to what treatment they think they are receiving: a) Colesevelam, b) Don’t know, c) Placebo. This is used to assess initial blinding.

**Telephone consultation: Start of intervention**

**Study day 10**

The intervention has started on study day 9. The study nurse calls the subject to note possible early AEs, aid compliance, and to titre the dose. If the nurse suspects an SAE, she contacts the investigator.

**Titration of intervention dose**

If the subject experiences lack of effect or the adverse symptoms constipation, vomiting, or nausea that could be caused by too high a dose of colesevelam the study nurse may taper or increase the daily dosage to three or one capsule(s) twice daily.

Whenever the dose is changed, a follow-up telephone consult is planned within 2–3 days.
**Telephone consultation: Start of endpoint period – compliance and AEs**

**Study day 14 (± 1 day)**

The study nurse calls the subject to note possible early AEs, aid compliance, and to titrate the dose if indicated. This consult is mandatory regardless of follow-up on any prior dose titration.

**Study visit 3 – Treatment end and repeated questionnaires (Q-2)**

**Study day 21 (+1–3 days).** The treatment ends at the end of day 20.

Before (same day or 1-2 days ahead of) the clinical contact on visit 3, the investigator answers if he/she has had any contact with the subject, perhaps through the study nurse: yes/no. Further if yes, what he/she thinks the allocated treatment was: “a) Colesevelam, b) Don’t know, c) Placebo.

The (sub)investigator then in the order below:

- distributes repeated questionnaires (Q-int)
  - SF36v2, SHS, GSRS
    - If the subject participates in the voluntary questionnaire on HRQoL and sexual dysfunction, this questionnaire is distributed again by e-mail
  - After the last question in the questionnaire, the subject is asked to mark which treatment he/she thinks to have received. This will assess the maintenance of blinding.
- collects the Study Diary 2 and checks this for deficiencies
- obtains a history of AEs and deem if any AE the nurse has noted is related to the intervention, ie. AR. This can only be done by a doctor
- collects surplus medication, counts the number of return capsules and assesses compliance
- orders follow up blood analysis to assess biochemical side effects (sampled in 3 – 4 days)
  - ALT, ALP, bilirubin, amylase,
- schedules study end telephone consultation
- confirms agreement for the six-month follow-up

The six-month follow-up is included in the study consent. This follow-up is identical to the one described above for subjects without diarrhoea.

**End of the clinical study phase**

**Study day 26 (± 3 days).** AE registration continues for minimum 72 hours after the end of treatment. Colesevelam is not absorbed from the gastrointestinal tract. Normal gastrointestinal transit is 36 – 48 hours leaving minimal if any traces of colesevelam at this time.

The investigator telephones the subject (this also applies to subjects in open-label treatment):

- Assess biochemical AEs
  - Informs the subject of these results
- Takes a history of AEs
• Takes action if needed (blood sampling, extended AE registration etc.)

The blinding is not unveiled at this time.

The central study nurse notes what he/she thinks the treatment was (questions as above).

**Six-month follow-up**

The local (sub)investigator notes the result of the diagnostic work-up of diarrhoea.

The questionnaires (SF36v2, SHS, GSRS) are distributed electronically. This questionnaire also asks if, what, and how any medicine against diarrhoea is taken.

If the subject has consented to answer the questionnaires regarding sexual dysfunction these are also distributed via e-mail.

Analysis of primary and secondary endpoints does not wait until the follow-up is finished.

**Adverse events**

All adverse events (AEs) and adverse reactions (ARs) are registered by an AE/AR table in the study Case Record Form and documented at study visits as specified above.

Serious AEs (SAE) and serious ARs (SAR) are defined as an AE or AR that is life-threatening or leading to death, that leads to hospitalization or prolonging of hospitalization, that causes seriously or sustained disability or incapacity to work, or causes a congenital anomaly.

All SAEs and SARs will be reported to sponsor as soon as possible and within 24 hours. A SAR that is not expected is deemed Suspected Unexpected SAR (SUSAR). If the event is life-threatening or deadly sponsor reports to the DMA and to the Ethical Committee immediately and within 7 days with follow up within further 8 days.

All other SUSARs are reported to DMA within 15 days of sponsors knowing.

A SAR is deemed SUSAR if not listed in the AE table for colesevelam.

All SAR’s are reported to the DMA and the Ethical Committee in yearly reports and after study completion (sooner than 3 months from the last visit, last patient).

**Schedule**

Inclusion starts in the second half of 2018/start of 2019 and is estimated to complete within 18 months. Primary data collection ends two weeks after the last participant is included. The questionnaire follow-up ends six months after this time point.

**Rights**

Sponsor is senior author on the planned publications, and the coordinating investigator is the first author. All protocol authors and the site investigators as listed on page 1 and page 2 have had the opportunity to comment and influence the protocol. These persons are authors on the planned main publication. All rights are specified in agreements between each study centre and Sponsor.
Planned publications
The main publication reports the primary and secondary endpoints described above, and authorship is described in detail clinical trial agreements. Secondary publications based on tertiary or ancillary endpoints and from the biobank for future scientific work will have at least one author from each study centre. Authorship is based on the Vancouver criteria.

Ethics
The study is conducted in accordance with the Helsinki declaration with guidelines from the International Conference on Harmonization of Good Clinical Practice (ICH-GCP). The Danish Medicines Agency, DMA (Lægemiddelstyrelsen) is applied for permission to using colesevelam and placebo. The GCP units as specified on page 3 monitor the study. Registers with personal data are subject to the Danish Data Protection Agency, and will be handled in accordance with ”Lov om behandling af personoplysninger”. Data registers are subject to the “Region Sjællands paraplygodkendelse” and are applied as such.

The study is registered in the ClinicalTrials.gov register.
Pregnant women are excluded. This study entices no significant risks or strain for the study subjects. The study gives crucial information on the correlation between both the SeHCAT scintigraphy and the biochemical diagnostics to the effect of colesevelam treatment. If our thesis is confirmed, bile acid diarrhoea may be diagnosed exclusively on basis of a blood test thus eliminating the need for radiation exposure and providing the possibility for easy and early diagnosis at local facilities thereby making it possible for many more patients to be identified and treated. Academically, it will be possible to re-examine individuals with BAD to describe changes over time. This improves the diagnostic algorithm and provides great benefit to both future patients and potentially also to study subjects.

We offer subjects reimbursement for documented extra expenses for transportation.

Recruiting
Patients referred for SeHCAT at study centres are eligible. Referring doctors/investigators may hand out the written information directly or (sub)investigators at SeHCAT centres may send the written invitation alongside the SeHCAT appointment. The potential participants are given the folder: ”Før du beslutter dig”. (Before you decide). They are informed that this enquiry is regarding a medical scientific study, that participation is voluntary, and that it will have no influence on current and future examinations and treatment, whether the patient decides to participate in the study or not.

Information and consent
The potential participant is invited to bring a bystander to the first SeHCAT day. A sub-investigator asks for interest in participation or in an information meeting, which then is arranged on the same day. The meeting is held in an office without time constraints. The written material is explained and questions are answered. It is the responsibility of the investigator (doctor) that the potential
participant understands the information and is qualified for giving informed consent. If the potential participant after the information meeting need further time for reflection a maximum of 24 hours may be given. Informed consent is given on the criteria above. The doctor signs to guarantee this.

Participation is voluntary and consent may be withdrawn at any time with no reason given. Consent for biological material for the biobank is given separately on the same occasion. Likewise regarding consent for the investigator to contact the referring institution. If the study reveals important information on the health of the subjects, he or she is informed unless the subject in the informed consent specifically wished not to be informed.

Results
Upon conclusion of the study participants are offered a short synopsis of its results in plain Danish. Participants may beforehand decline to this. All data and information are kept in accordance to:”Lov om behandling af personoplysninger” and ”Lov om patienters retsstillinger”. The results - positive, negative or inconclusive - will be published in international peer-reviewed journals.

Risks and nuisances
In total 50 mL full-blood is drawn with routine sterile technique and this is not considered a risk. Colesevelam is well known as an off-label treatment for BAD. Common side effects are headache and constipation (10%) but it is generally well tolerated. See full description of side effects above. Longtime treatment with colesevelam may give malabsorption of fatty nutrients and vitamins, but this is irrelevant in the short context of this study.

Data security and access
Anonymized data are kept in Microsoft Access with password protection and audit trail and in IBM SPSS. Register with social security number (CPR) and all other personal data is kept in a file on SharePoint Teamsite under Region Zealand with logged access only for (sub)investigators with responsibility for data collection or data analysis. Backup discs are kept in a secure and locked facility within the department. Study data and metadata is anonymized after study end and saved in a repository for public access. The biological material is saved anonymized and registered in the above-mentioned files and is destroyed 10 years after study end. Registers are under the supervision of the Danish Data Protection Agency.
Data from the participants’ medical chart
The participants’ medical chart data are reviewed for information on diarrhoea work-out and to screen for inclusion and exclusion criteria. This includes biochemical tests and the shared medicines chart (FMK).
For monitoring of study safety and conduct the (sub)investigators, Danish Medicinal Agency, and the GCP units need access to the subjects’ medical chart.

Economy
The study is investigator-initiated. The “Overlæge Johan Boserup and Lise Boserup Foundation” has donated DKK 120.000 for biochemical analysis and assistance. The Civilengineer H.C. Bechgaard & wife Ella Mary Bechgaard’s Fund has donated DKK 50.000. Production of colesvelam and placebo is funded by the Axel Muusfeldt Foundation (DKK 198.505) and the Aase and Ejnar Danielsen Foundation (DKK 100.000). The Region Zealand Scientific Fund has granted DKK 352.600. The “Fabrikant Vilhelm Pedersen og hustrus mindelegat” donated DKK 2.000.000 after recommendation by The Novo Nordisk Foundation.
Funds are administrated by the Zealand University Hospital.
Both central and local investigators have no personal economic gain by the study.

Compensation and insurance in case of injury caused by the study
Sponsor and local sites are public institutions and are as such covered by the general patient compensation *patienterstatningen*.

References
33. Walters JR, Johnston IM, Nolan JD, Vassie C, Pruzanski ME, Shapiro DA. The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. Aliment Pharmacol Ther. 2015;41(1):54-64.
Appendix 1: Study overview chart

<table>
<thead>
<tr>
<th>Day: - X</th>
<th>Study Visit 1 (day 1)</th>
<th>Study Visit 2 (day 8)</th>
<th>Telephone Consultations</th>
<th>Study Visit 3 (day 21 (+1-3 days))</th>
<th>Telephone (day 26 (+/- 2 days))</th>
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<td>14, 15, 16 ......, 20, 21</td>
<td>22, 23, 24, 25, 26</td>
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</tbody>
</table>

Prescreening:
- Inclusion
- Blood sample
- Questionnaires 1

SeHCAT 1
- Screening Diary
- opt. stool sample
- Yes

Diarrhoea by Hjortswang criteria?
- No

SeHCAT 2
- Study blood sample
- Treatment start, run-in, titration
- Endpoint Diary
- opt. stool sample
- Questionnaires 2

Clinical Study End
- Biochemistry End

Randomization
- First dose day 9
- Last dose day 20

Dispatch
- Colesevelam
- Placebo

Collect
- AEs?
- AEs?

AE registration: Start
- END

Study overview chart: SeHCAT: \(^{75}\)Selenium tauro-homocholic acid retention test, AE: adverse event

EudraCT number: 2016-001452-22