SINBAD

Treatment effect of colesevelam for bile acid diarrhoea

– a randomised placebo-controlled trial.

Danish Region Zealand scientific ethics committee: SJ-641
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Abbreviations

BAD: Bile Acid Diarrhoea, BSF: Bristol Stool Form scale, C4: 7α-hydroxy-4-cholesten-3-one, CDCA: chenodeoxycholic acid, DMA: Danish Medicines Agency, ELISA: enzyme-linked immunosorbent assay, FGF19: fibroblast growth factor 19, GSRS: Gastrointestinal Symptoms Rating Scale, HPLC: high performance liquid chromatography, HRQoL: Heath Related Quality of Life, IBS-D: Diarrhoea-predominant irritable bowel syndrome, LC-MS/MS: liquid chromatography-tandem mass spectrometry, ROC: Receiver Operating Characteristics, SeHCAT: ^{75}Selenium conjugated Tauro-homocholic acid retention test, SF36v2: Short Form 26 version 2, SHS: Short health Scale.

Aims

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 marker of BAD 7α-hydroxy-4-cholesten-3-one (C4) with colesevelam treatment response, and to validate the cut-off values determined in the *VABAD* trial (1).

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 advise 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 (2). BAD is detected in 20–30% of patients with diarrhoea predominant irritable bowel syndrome (IBS-D) (3), up to 40% of patients with microscopic colitis (4), and in patients with Crohn's disease without inflammatory activity (3, 5-9).

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 (5, 7). 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 (5, 7, 8, 10). 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 (11). 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 (12), but usually moderate and severely reduced retention is associated with diarrhoea and with treatment effect (12). 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 (3). 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 (13) and has several diagnostic pitfalls making the interpretation difficult (13, 14). Bile acids can be measured in the stools but it is cumbersome and used only for research purposes (15).

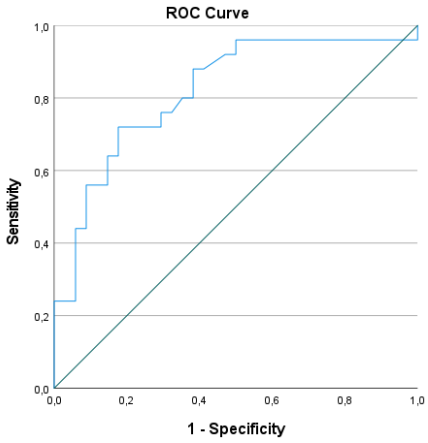
Biochemical markers of bile acid diarrhoea

New knowledge has emerged on the physiology and regulation of enterohepatic bile acid circulation (16) and of bile acid synthesis (16, 17). 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 (18-21) and compared with SeHCAT, C4 has a sensitivity of 90% and specificity of 79% for detecting BAD (19, 21). 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 (15). Upon evaluation of their clinical use of C4, Brydon *et al* chose a 30 ng/mL cut-off. This had correlation to observational treatment response, but unfortunately not to the SeHCAT test (22). Several factors may influence C4 of which diurnal variation and food intake are best described (23). Plasma concentration of C4 increases acutely 4–6 hours after intake of alcohol (24). C4 is decreased in cholestasis and may be marginally decreased in hepatic cirrhosis (25). Statins and fibrates decrease the amount of cholesterols available for bile acid synthesis. Thus, atorvastatin lowers a medically or surgically induced elevated C4 (26).

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 (17). 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 (27) – 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 (28, 29). FGF19 correlates inversely with C4 in healthy volunteers (18) and fasting values of FGF19 correlate with SeHCAT (10). 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%) (10), 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 (30) and then with combinations of the meal plus chenodeoxycholic acid (31) 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 46 ng/mL had optimal combined diagnostic characteristics with a diagnostic accuracy of 74%. 13 (22%) of the 59 patients had C4 > 46 ng/mL. The cut-off > 58 had higher PPV, and 11 of 59 (19%) had C4 > 58ng/mL. We chose the 46 ng/mL cutoff for the primary endpoint to keep false negative test results at a minimum.

TABLE 1 Results from the VABAD study	**SeHCAT<=10% N=25 Median (IQR)**	**SeHCAT>10% N=34 Median (IQR)**	**ROC analysis, AUC (95% CI)**	**Cutoff for positive test**	**Sens./spec. (%)**	**PPV/NPV (%)**
Fasting C4	47 (21–71)**	11 (9–23)	0.82 (0.71–0.93)**	C4 > 25	72 /82	64 / 80
				C4 > 46	52 / 91	72 / 74
				C4 > 58	44 / 94	77 / 70
Fasting FGF19	75 (57–146)*	115 (8 –220)	0.69 (0.54–0.83)*	FGF19 ≤ 85	60 / 77	65 / 72
FGF19 in pg/mL and C4 in ng/mL. The statistical significance of the ROC curves was tested nonparametric versus true area=0.50. *p=.02; **p=.00003						

Plasma bile acid species

In the VABAD study, patients with bile acid diarrhoea had fewer secondary bile acids in plasma, less lithocolic acid and less sulfate-conjugated bile acid species (1). The diagnostic utility of these possible biomarkers is yet unclear. However, the VABAD study was not powered to make conclusions in this subject. Therefore, we will assess this in further exploratory analyses.

Faecal bile acids in diagnosis of bile acid diarrhoea

Measurements of faecal bile acids are used for diagnosis of BAD in the USA (15). Excretion of total bile acids in 48-hours on a set diet has primarily been used. However, this procedure is cumbersome. Increased faecal primary bile acids > 5% and > 10% possible are alternatives (32, 33). Further, it is expected that patients with bile acid diarrhoea in adjunct to increased primary bile acids in faeces will have less secondary bile acid species. Recently, a combination of primary bile acids >10% in a random single stool sample combined with elevated C4 was shown to be a good diagnostic test (34). Further, primary bile acids correlate well with SeHCAT; levels > 15% had 83% PPV for SeHCAT <10% (35). It is plausible that a combination of C4 with elevated primary fecal bile acids could correlate better to the SeHCAT scintigraphy and to treatment response than C4 alone. However, there only are few reports, and therefore we will assess this in exploratory analyses.

Treatment of Bile Acid Diarrhoea

Based on observational data, sequestrants such as cholestyramine, colesevelam, and colestipol seem effective treatments of BAD (12). 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 (36). Unfortunately, hydroxypropyl cellulose had an active effect with a per protocol (PP) response rate of 38% compared with 64% for cholestyramine (p=0.22). A 23% dropout rate for cholestyramine reflects the side effects of cholestyramine and the intention to treat (ITT) cholestyramine response rate dropped 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 (13, 37). 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 (38).

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 (39). 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:

Colesevelam and placebo have the same effect on BAD.

Intervention:

Colesevelam (tablets of 625mg) or identical placebo capsules. One to three capsules 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.
    - Sexual abstinence from heterosexual intercourse. May be accepted as safe contraception by the investigator in a case by case assessment [a]
    - Vasectomised partner
- Ability to give informed consent after written and oral information in the Danish language

Note a) sexual abstinence from heterosexual intercourse is deemed a highly effective method of anticonception by the Clinical Trials Facilitation Group. It is accepted in this trial only because the duration is short – three weeks.

Exclusion criteria

- Inflammatory bowel disease, including microscopic colitis
- Acute suspected or proven viral gastroenteritis within the recent 4 weeks
- Acute non-viral gastroenteritis within the recent 8 weeks
- Investigator-assessed debilitating chronic disease e.g. WHO performance score 3–5

- Prior treatment with colesevelam
- 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 colesevelam
    - i.e. taken one hour before or 4 hours after colesevelam
- 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 > 46 ng/mL.

Handling of missing data: drop-outs are set as treatment failures in the ITT analysis if less than five of the last seven treatment days that comprise the endpoint assessment period are complete. If five or more of these days are complete, a mean of these days will be used for calculating the Hjortswang response criterium.

Statistical analysis: data will be longitudinally correlated (baseline, run-in, endpoint assessment period for each patient) and nested within study sites. Thus, data will not be independent and cannot correctly be assessed with simple statistical tests. Therefore, we will fit an appropriate generalized linear mixed-effects model with an unstructured covariance pattern of the chance of response with colesevelam versus placebo. In addition, baseline adjustments for the severity of

diarrhoea (mean per day sum of Bristol type 6 and 7 stools) and the severity of bile acid malabsorption (visit 2 C4 value) will be made to avoid skewed randomisation of these prognostic covariates by chance (40). This baseline adjustment is clinically relevant as, in the future, diarrhoea severity and C4 should be available for physicians before starting therapy.
The model fit will be assessed by inspection of scaled residual plots and QQ-plots.

Sensitivity analyses: The robustness of the statistical modelling of the primary endpoint will be addressed in sensitivity analyses of different approaches.
Different handling of missing values: 1) any missing diary day of the last seven treatment days and the patient treatment is set to failure; 2) excluding patients who drop out due to large pill size (the DB caps used for trial blinding are significantly larger than the colesevelam tablet), 3) using multiple imputations for missing values.
Strict Hjortswang's response criteria: no watery stools (Bristol stool type 6 or 7) during the last seven treatment days.
Different C4 cut-off used to define bile acid diarrhoea: C4 > 25 ng/mL; C4 > 58 ng/mL.
If possible, given that the study is not powered for stratification, we will assess remission rates in stratification intervals defined by C4 0–=25 ng/mL, >25–46 ng/mL.
Different statistical modelling and assessment: 1) added adjustment for patient sex and age, 2) model without baseline adjustment, 3) model without random effect of study site, 4) a simple Fisher's exact test of a 2x2 table (response versus treatment allocation) reporting remission rates.

Secondary endpoints

1. PP analysis for the primary endpoint

Statistical analysis: this is an efficacy (*de jure*) estimand for colesevelam. Modelling as for the primary endpoint but with imputation of missing data as if all patients had adhered (41, 42). This includes all patients with bile acid diarrhoea and complies with a *per protocol* principle.

2. Placebo-controlled diarrhoea ITT remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by SeHCAT ≤ 10%

Statistical analysis: as the primary endpoint but with baseline adjustment for SeHCAT, not C4.
Sensitivity analyses: as for the primary endpoint. Instead of differing C4 cutoffs, the SeHCAT cutoffs ≤ 5%, and ≤ 15% will be assessed.

3. Placebo-controlled diarrhoea PP remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by SeHCAT ≤ 10%

Statistical analysis: this is an efficacy (*de jure*) estimand for colesevelam. Modelling as for the primary endpoint with baseline adjustment for SeHCAT and with imputation of missing data as if all patients had adhered.

4. Placebo-controlled effect of colesevelam in all treated patients

Statistical analysis: as for the primary endpoint but instead of the binary Hjortswang response criteria as outcome, continuous variables will be modelled with suiting baseline adjustment:

   a. the absolute number of stools (mean per day over 6 or 7 days)
   b. the total number of Bristol 6 and 7 stools (mean per day over 6 or 7 days)

5. The *de facto* effectiveness of colesevelam on health related quality of life assessed as the sum of Short Health Scale scores.

Statistical analysis: modelling with adjustment for baseline SHS score

The secondary endpoints are tested with correction for multiple statistical testingand 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.

Expected missing data and drop-outs

We anticipate droput/missingness due to
1) side effects to colesevelam. We presume this to be 'missing at random'.
Drop-out from this cause could dilute the observed effect of colesevelam. This reflects real-world effect and is accounted for by analysing both an effectiveness (ITT) and an efficacy (PP) estimand.
2) lack of effect from placebo. Presumably somewhat dependent on baseline diarrhoea severity, which is controlled for in the modelling.
Drop-out from this cause probably has no effect on the primary endpoint.
3) due to the (large) pill size. We presume this will be 'missing at random'.
Drop-out from this risks diluting the observed effect of colesevelam. This is only specific to the trial context and not future clinical practise. We take this into account in a sensitivity analysis excluding these cases.

Statistical analysis plan for ancillary endpoints

Diagnostic validation endpoints

- Placebo-controlled diarrhoea ITT remission rate for colesevelam defined by the Hjortswang criteria in patients with BAD defined by fasting FGF19 ≤ 60 pg/mL
- ROC analyses to assess the negative predictive values of C4 < 15 ng/mL, FGF19 > 204 pg/mL, SeHCAT >10%, respectively with diarrhoea remission defined by Hjortswang criteria as gold standard test.
- Repeatability of baseline fasting C4 and FGF19 assessed with marginal limits of agreements (Bland-Altman plot) comparing visit 2 samples with 'optimal' visit 1 samples (ie. fulfilling

visit 2 sampling criteria: fasting, not using statins within a week, no alcohol consumption within 24h)

- Exploratory assessment of the effect of sampling time, statin use, and alcohol intake on C4. This is a regression model of C4 at visit 2 explained by: C4 at visit 1, sampling time, alcohol consumption, statin use, respectively.
- ROC-AUC for all visit 1 C4 samples versus SeHCAT ≤ 10% as gold standard
- Placebo-controlled subjectively assessed "good response" ITT rate in the primary endpoint population
- Diagnostic values for subjectively assessed "good response" in the colesevelam-arm
    - C4 > 46 ng/mL
    - SeHCAT ≤10%

Descriptive statistical analyses

- correlations between each of FGF19, C4, and SeHCAT and baseline mean stools and number of watery stools
- correlations between each of FGF19, C4, and SeHCAT and change in mean stool number and number of watery stools in the colesevelam population

Exploratory analyses of bile acid species in plasma and feces

We will explore the distribution of bile acid species in feces and plasma regarding bile acid diarrhoea both defined with C4 and SeHCAT. Explorative statistical modeling will be done to analyse the diagnostic and prognostic performance of bile acid parameters. Bile acid species will primarily be assessed as sums of primary and secondary species given as a percentage of the total amount of bile acids. Percentages of the total amount of bile acids have less variability than absolute amounts (43). Secondary analyses of single bile acid species and conjugation forms either on absolute values or percentages may be performed given a relevant signal in the primary exploratory analysis.

Elucidation of further aspects of treatment effect

- Placebo-controlled ITT diarrhoea remission rate defined by the Hjortswang criteria for colesevelam in patients with BAD by C4 ≥ 46 ng/mL AND
    - fecal primary bile acids >10%, and >15%
- Logistic regression model predicting remission in the colesevelam group for
    - C4 controlled for baseline mean stools/day
        - Sensitivity, specificity, PPV and NPV for C4 > 46 ng/mL
            - Model characteristics reported with mean stools/day
    - SeHCAT controlled for baseline mean stools/day
        - Sensitivity, specificity, PPV and NPV for SeHCAT ≤10%

- Six-month follow up on the aetiology of diarrhoea
- Six -month follow up on medication
- Exploratory regression analyses to determine if fecal or plasma bile acid profile may be a predictor for diagnosis and for treatment response.
- Placebo-controlled change in fecal bile acids during treatment with colesevelam.

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

- The 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)
        - Physical component score and Mental component score
    - Each of the 4 items in the SHS
- Correlation between HRQoL and Bristol stool scale diarrhoea.
- Correlation between HRQoL and stool number per week.
- Correlation between changes in HRQoL and primary endpoint
- Description of sexual dysfunction with HRQoL in the population
- Six-month follow up on HRQoL

Power calculation

Based on the primary endpoint we assume

- a remission rate of 67% for colesevelam and 27% for placebo (36, 38).

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

Thus, we need 23 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 ≥ 46 ng/mL, 16 of the 59 had BAD (27%). In comparison with SeHCAT, Borghede et al. found that 41% of patients referred for SeHCAT had primary BAD defined with SeHCAT (44).

Thus to include 2 x 23 patients with BAD defined with C4 > 46 ng/mL, we need

$$\frac{\text{2 x 23 BAD subjects}}{\text{27\% BAD subjects among patients with diarrhoea}} = 170\ patients$$ 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 (45, 46). 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 ≥3 stools per day and watery stool consistency (47) 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 (45, 48) and lymphocytic colitis (49).

- Activity is defined as ≥ 3 stools per day **or** ≥ 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 (47). 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 (50). 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 (45, 48, 50-53).
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 (54).

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 (55, 56). 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 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 fjernede 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 (57-60).

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

All samples are prepared and immediately frozen for later bulk analysis once inclusion is finished. 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) in the Department de Chimie, Sorbonne Universités, Paris (Dominique Rainteau) (61). C4 will also be analysed in the department of Clinical Biochemistry, Rigshospitalet, Copenhagen (Svend H. Hansen). This founds future clinical availability of the C4 analysis in Denmark. Therefore, we will use the Copenhagen C4 measurements for the endpoint analyses. Ancillary analyses comparing C4 from the two laboratories will be done.
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 (13, 14, 37, 38). The treatment effect is comparable to that of colestyramine and it is considerably better tolerated (13, 38). 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 2–3 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 28.11.2018).

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).

Nervous system disorders
Common: Headache
Gastrointestinal disorders
Very common: Flatulence*, constipation*
Common: Vomiting, diarrhoea*, dyspepsia*, abdominal pain, abnormal stools, nausea, abdominal distension
Uncommon: Dysphagia
Very rare: Pancreatitis
Not known: Intestinal obstruction*,**
Musculoskeletal and connective tissue disorders
Uncommon: Myalgia
Investigations
Common: Serum triglycerides increased
Uncommon :Serum transaminases increased

* 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 assess eligibility, informs and may include the patient as specified under "Ethics". If an eligible subject opts to participate, 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
    - 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 are related to the intervention, ie. AR. This can only be done by a doctor
- collects surplus medication, counts the number of return capsules and assess 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. If visit 3 (above) is scheduled after these 72 hours, the AE registration at visit 3 is final. The investigator may opt to only telephone the patient is an end-of-trial blood samples is abnormal. Otherwise 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 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 "R*egion 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: "*Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt*". (Your rights as a participant in a research project). 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 with:”Lov om behandling af personoplysninger” and ”Lov om patienters retsstilling”.
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.

Primary data responsibility

Lars Kristian Munck and Christian Borup

Investigators with data access for analysis

Signe Wildt, Jüri Johannes Rumessen, Morten Dahl, Jesper Graff

Investigators at participating centres, as fully listed on page 2 and 3: Bente Sonne, Camilla Nøjgaard, Hans B. Timm, Søren Peter German Jørgensen, Tine Nygaard Gregersen, Trine Borup Andersen, Anna Zaremba, Lars Vinter-Jensen, Dominique Rainteau.

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 colesevelam and placebo is funded by the Axel Muusfeldt Foundation (DKK 198.505) and the Aase and Ejnar Danielsen Foundation (DKK 100.000).
Approximately DKK 900.000 is budgeted for PhD salary, of which 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*.

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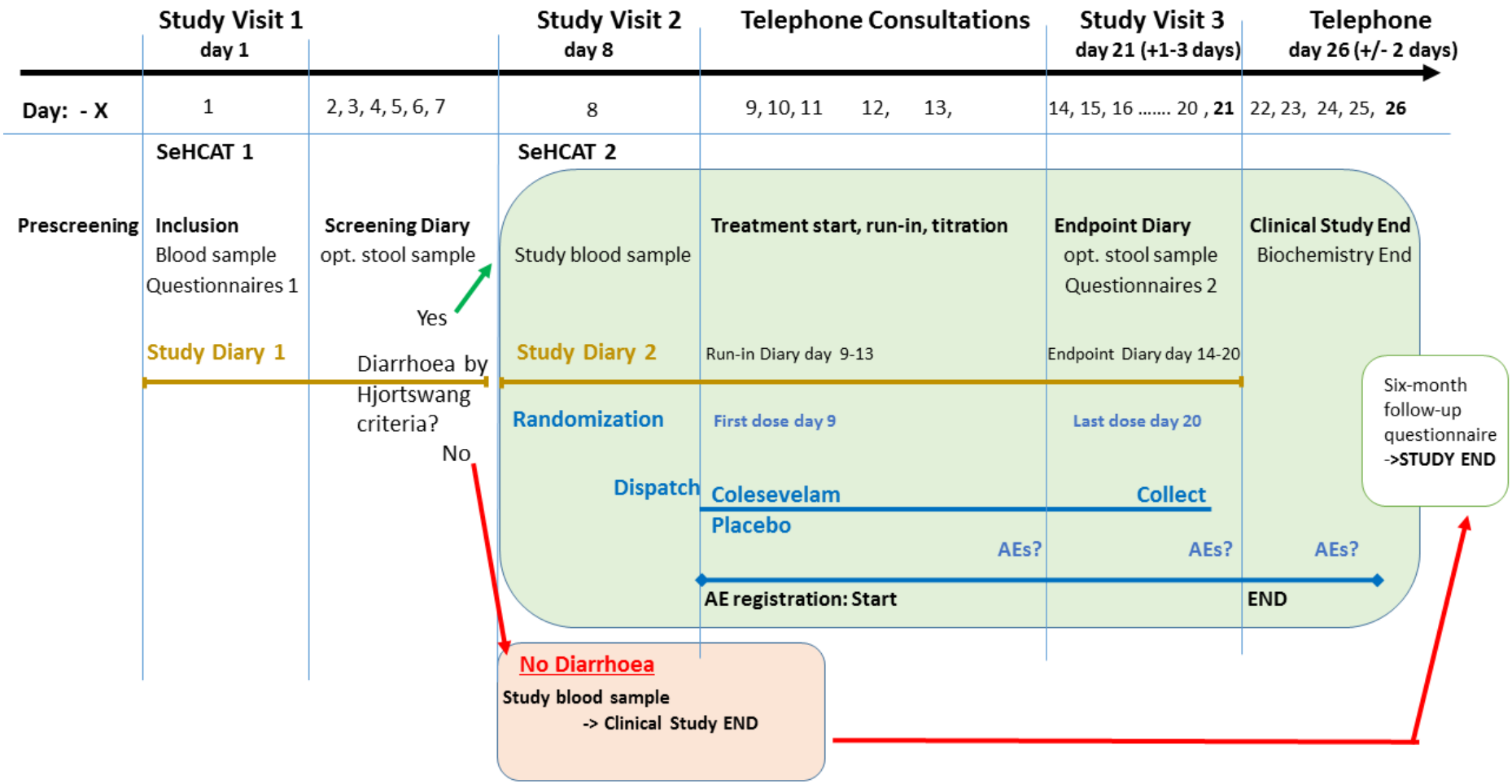
Appendix 1: Study overview chart



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