Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Biochemical Diagnosis of Bile Acid Diarrhea

Prospective Comparison With the ^{75}Seleno-Taurohomocholic Acid Test

Borup, Christian; Wildt, Signe; Rumessen, Jüri; Graff, Jesper; Bouchelouche, Pierre Nordine; Andersen, Trine Borup; Vinter-Jensen, Lars; Zaremba, Anna; German Jørgensen, Søren Peter; Gregersen, Tine; Nøjgaard, Camilla; Timm, Hans Bording; Rainteau, Dominique; Gauliard, Emilie; Munck, Lars Kristian

Published in:
The American Journal of Gastroenterology

DOI (link to publication from Publisher):
10.14309/ajg.0000000000000772

Creative Commons License
CC BY-NC-ND 4.0

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Link to publication from Aalborg University

Citation for published version (APA):
Borup, C., Wildt, S., Rumessen, J., Graff, J., Bouchelouche, P. N., Andersen, T. B., Vinter-Jensen, L., Zaremba, A., German Jørgensen, S. P., Gregersen, T., Nøjgaard, C., Timm, H. B., Rainteau, D., Gauliard, E., & Munck, L. K. (2020). Biochemical Diagnosis of Bile Acid Diarrhea: Prospective Comparison With the ^{75}Seleno-Taurohomocholic Acid Test. *The American Journal of Gastroenterology*, *115*(12), 2086-2094. https://doi.org/10.14309/ajg.0000000000000772

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

COLON

Open

Biochemical Diagnosis of Bile Acid Diarrhea: Prospective Comparison With the ^{75}Seleno-Taurohomocholic Acid Test

Christian Borup, MD[1,2], Signe Wildt, MD, PhD[1], Jüri Rumessen, MD, DMSci[3], Jesper Graff, MD, PhD, DMSci[4], Pierre Nordine Bouchelouche, MD[5], Trine Borup Andersen, MD, PhD[6], Lars Vinter-Jensen, MD, DMSci[7], Anna Zaremba, MD, PhD[7], Søren Peter German Jørgensen, MD, PhD[8], Tine Gregersen, MD, PhD[9], Camilla Nøjgaard, MD, PhD[2], Hans Bording Timm, MD[2], Dominique Rainteau, MD, PhD[10], Emilie Gauliard, PhD[10] and Lars Kristian Munck, MD, DMSci[1]

INTRODUCTION: **The diagnosis of bile acid diarrhea is often missed because the availability of the ^{75}seleno-taurohomocholic acid (SeHCAT) test is limited. We aimed to compare the biomarkers 7α-hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19 (FGF19) with the SeHCAT test.**

METHODS: **Patients with chronic diarrhea without intestinal resection referred for SeHCAT were prospectively recruited for this diagnostic accuracy study. Blood was sampled at fasting and after a stimulation meal with chenodeoxycholic acid. SeHCAT retention ≤10% defined bile acid diarrhea and >10% defined miscellaneous diarrhea. Receiver operating characteristics (ROC) were analyzed with SeHCAT as the gold standard. www.clinicaltrials.gov (NCT03059537).**

RESULTS: **Patients with bile acid diarrhea (n = 26) had mean C4 of 30 ng/mL (95% confidence interval: 19–46) vs 8 (7–11; $P < 0.001$) in the miscellaneous diarrhea group (n = 45). Area under the ROC curve (ROC_{AUC}) for C4 was 0.83 (0.72–0.93). C4 < 15 ng/mL had 85% (74%–96%) negative predictive value; C4 > 48 ng/mL had 82% (59%–100%) positive predictive value. Twenty patients had C4 values 15–48 ng/mL, of whom 11/20 had SeHCAT ≤10%. Median fasting FGF19 was 72 pg/mL (interquartile range: 53–146) vs 119 (84–240) ($P = 0.004$); ROC_{AUC} was 0.71 (0.58–0.83). Stimulated FGF19 responses did not differ ($P = 0.54$).**

DISCUSSION: **We identified C4 thresholds with clinically useful predictive values for the diagnosis of and screening for bile acid diarrhea in patients with chronic watery diarrhea. Further validation of the cutoff values with the placebo-controlled effect of sequestrant therapy is warranted (see Visual Abstract, Supplementary Digital Content 2, http://links.lww.com/AJG/B603).**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/B603 and http://links.lww.com/AJG/B588

Am J Gastroenterol 2020;115:2086–2094. https://doi.org/10.14309/ajg.0000000000000772

INTRODUCTION

Bile acid diarrhea is an often missed cause of chronic watery diarrhea, with an estimated prevalence in the general population of 1% (1). It is categorized into types by pathophysiology. Type 1 bile acid diarrhea is because of impaired bile acid reabsorption from ileal resection or inflammation. Type 2 is caused by an increased bile acid synthesis (2), which mostly is idiopathic (3). Type 3 bile acid diarrhea is because of altered bile acid homeostasis associated with cholecystectomy, coeliac disease, microscopic colitis, abdominal radiotherapy, and chronic pancreatitis (4).

The diagnosis of bile acid diarrhea due to bowel resection is straight forward (5,6). Unfortunately, patients with type 3 and particularly type 2 bile acid diarrhea often remain undiagnosed or even misdiagnosed (7), e.g., 32% of patients with diarrhea-predominant irritable bowel syndrome (IBS-D) have bile acid diarrhea (8). Therefore, increased awareness and accessible diagnostic options are needed.

Scintigraphic measurements of the 1-week retention in the enterohepatic circulation of the synthetic bile acid ^{75}seleno-taurohomocholic acid (SeHCAT) is currently the preferred diagnostic test (9,10). SeHCAT retention ≤5% (severe bile acid diarrhea) and

[1]Department of Internal Medicine, Zealand University Hospital, Koege, Denmark; [2]The Gastro Unit, Section of Medicine, Hvidovre University Hospital, Hvidovre, Denmark; [3]Gastroenterology and Research Unit, Herlev and Gentofte University Hospital, Hellerup, Denmark; [4]Department of Clinical Physiology and Nuclear Medicine, Hvidovre University Hospital, Hvidovre, Denmark; [5]Department of Clinical Biochemistry, Zealand University Hospital, Koege, Denmark; [6]Department of Nuclear Medicine, Aalborg University Hospital, Aalborg, Denmark; [7]Department of Gastroenterology and Hepatology, Aalborg University Hospital, Aalborg, Denmark; [8]Department of Gastroenterology and Hepatology, Aarhus University Hospital, Aarhus, Denmark; [9]Department of Nuclear Imaging and PET, Aarhus University Hospital, Aarhus, Denmark; [10]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Hôpital Saint Antoine, Metomics, Paris, France. **Correspondence**: Christian Borup. E-mail: chrbo@regionsjaelland.dk.
Received February 26, 2020; accepted June 29, 2020; published online July 29, 2020

>5% to ≤10% (moderate) is usually considered diagnostic (8), whereas SeHCAT retention of >10% to ≤15% may be an indeterminate result. Cutoffs ranging from <8% to <11% have found most use (11). The SeHCAT test is highly sensitive and specific (12), but it has limited capacity and availability. This delays the diagnosis of bile acid diarrhea and consequently, the patients may undergo unnecessary radiological and endoscopic examinations (7). A readily available test is therefore needed (13). The bile acid precursor 7α-hydroxy-4-cholesten-3-one (C4) reflects the bile acid synthesis rate (14), and patients with bile acid diarrhea of any subtype have elevated C4 blood levels (15–17). C4 and SeHCAT correlate well, particularly in patients with an ileal resection or a right-sided hemicolectomy (15,18), and previous studies have included many of these patients (15–17). However, recent research recommends that given an *a priori* risk of disease >95%, no testing is needed in this subgroup (19–21). Fibroblast growth factor 19 (FGF19) is released by enterocytes in the terminal ileum on reabsorption of bile acids that activate the farnesoid X receptor (22). Fasting FGF19 is inversely correlated with C4 (23) and directly with SeHCAT retention, but overlap in fasting FGF19 values between patients with bile acid diarrhea and diarrhea controls impairs its diagnostic usefulness (24). Our pilot studies indicated that stimulation of FGF19 with a meal (25) and particularly taken together with the farnesoid X receptor agonist chenodeoxycholic acid (26) could improve its diagnostic capability. Therefore, this study aimed to examine the diagnostic utility of fasting FGF19, stimulated FGF19, and of C4, in a prospective cohort of patients referred for SeHCAT.

MATERIALS AND METHODS

Design and patients

This prospective multicenter diagnostic accuracy study recruited from March 22 to November 3, 2017. Patients aged 18–80 years referred for SeHCAT were eligible. Exclusion criteria were small bowel or right hemicolonic resection, inflammatory bowel disease with the need of systemic steroids within 4 weeks of SeHCAT day 1, sequestrant treatment within 1 week, use of laxatives or antidiarrheal medication except for a stable dose of psyllium husk and opioids for pain, cirrhosis, biliary obstruction, frequent biliary cholics, cholecystitis, pregnancy, breastfeeding, and allergy to chenodeoxycholic acid or eggs in the test meal.

Our pilot study cutoff values for C4 and stimulated FGF19 (the 90-minute increment and the total area under the curve) did not support extrapolation to the current study. Therefore, our cutoff values will be exploratory.

The study was approved by the Ethics Committee for the Zealand Region (SJ-546), and data registries were approved by the Zealand Regional Data Protection Agency (Datatilsynet) (REG-089-2016). All patients voluntarily gave written informed consent. The Danish Medical Agency approved chenodeoxycholic acid for study use (EudraCT: 2016-002217-22). The study was registered on ClinicalTrials.gov (NCT03059537).

Procedures

The SeHCAT test was performed according to the local guidelines. We defined bile acid diarrhea as SeHCAT retention ≤10% in the primary analyses. Retention >10% defined miscellaneous diarrhea.

The SeHCAT test involves 2 visits 1 week apart at the department of nuclear medicine. At SeHCAT visit 1, demographic characteristics, medical history, and concomitant medication were recorded and the questionnaires (i) Short form 36 version 2 (27) and (ii) Short health scale (28) were administered. Stool frequency and Bristol Stool Form were registered by a standardized diary for 6 days between SeHCAT visit 1 and 2. The diary results were assessed according to the Hjortswang diarrhea activity criteria that define diarrhea as a mean of ≥3 stools per day and/or a mean of ≥1 liquid stool per day (29). These criteria have been validated in microscopic colitis (29) and were used in the lack of disease-specific criteria. Fasting blood samples were collected at SeHCAT visit 2, and the patients then ingested 1,250 mg chenodeoxycholic acid, which defined t = 0 minutes, followed by the test meal consisting of 2 boiled eggs, 2 slices of toast, and 500 mL tap water, as previously described (26). EDTA plasma samples for FGF19 and C4 were taken at fasting immediately before t = 0, at t = 90, 120, and 150 minutes.

Patient files were subsequently reviewed for final diagnoses and records of treatment response.

Biochemistry

Fasting blood samples from visit 2 were analyzed locally for total cholesterol, low- and high-density lipoprotein-cholesterol, triglycerides, and glucose. FGF19 was analyzed with ELISA as previously reported (R&D Systems, MN) (25,26). C4 and bile acid species were analyzed with high-performance liquid chromatography tandem mass spectrometry as previously described (30,31). The biochemical technicians were blinded from other data.

Power calculation

We defined a receiver operating characteristics (ROC) area under the curve (ROC_{AUC}) of 0.80 as the least acceptable test performance and expected 33% of patients to have SeHCAT ≤10% (8). With $\alpha = 0.01$ and $\beta = 0.10$, we needed 60 patients. Accounting for uncertainties, we aimed to include 70 patients.

Statistical analyses

Continuous data are presented as medians with interquartile ranges (IQRs) or as means with 95% confidence intervals (CIs) and compared accordingly with the Mann-Whitney *U* test or the Student *t* test. Correlations (r_s) were analyzed with Spearman rank test. The cumulated areas under FGF19 curves were calculated with the trapezoidal rule. Diagnostic values and likelihood ratios were calculated (32,33). The above analyses were performed with IBM SPSS Statistics version 26. Multivariate logistic regression models were fitted with R software (version 3.5.2) to predict bile acid diarrhea (SeHCAT ≤ 10%). Among tentative covariates, C4, FGF19, age, and mean stool number were significant in univariate testing. A stepwise backward elimination resulted in the same covariates. Exploring the plasma bile acid profile, the sum of sulfate conjugated bile acid species had a high predictive value that was used in a subsequent model. Two-sided *P* values < 0.05 were considered significant.

RESULTS

We invited 209 patients and 78 were included (see Figure S1: STARD flow diagram, Supplementary Digital Content 1, http://links.lww.com/AJG/B588). Seventy-one patients completed the protocol, of whom 25 were recruited at Zealand University Hospital, 22 at Hvidovre University Hospital, 19 at Aalborg University Hospital, and 5 at Aarhus University Hospital. Bile acid diarrhea was diagnosed in 36%, 32%, 37%, and 60%, respectively.

Twenty-six patients (37%) had bile acid diarrhea with SeHCAT ≤ 10%. Four patients had type 1 bile acid diarrhea because of nonresected Crohn's disease, 15 patients had type 2, and 7 patients had type 3 because of cholecystectomy (n = 4),

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

Table 1. Patient characteristics

	SeHCAT > 10% Miscellaneous diarrhea	SeHCAT ≤ 10% Bile acid diarrhea			
	n = 45	All types n = 26	Type 1 n = 4	Type 2 n = 15	Type 3 n = 7
Demographic characteristics					
Sex, female	27 (60%)	17 (65%)	2 (50%)	10 (67%)	5 (71%)
Age, yr	55 (45–64)[a]	45 (34–49)[a]	39 (37–44)	46 (27–51)	46 (41–54)
Body mass index, (kg/m^2)	26 (23–31)	30 (25–36)	30 (28–34)	30 (22–39)	30 (29–46)
Current smoking	13 (29%)	5 (19%)	2 (50%)	2 (13%)	1 (14%)
Diary results					
No. of stools per day	3 (2–4)[a]	4 (3–5)[a]	5 (4–5)	3 (3–4)	5 (4–6)
Bristol Stool Form Scale per stool	6 (5–6)	6 (6–6)	6 (6–7)	6 (5–6)	6 (6–6)
Mean number of stools ≥3.0 per day	23 (51%)	20 (77%)	4 (100%)	9 (60%)	7 (100%)
Mean Bristol type 6 and 7 per day ≥ 1.0	32 (71%)	24 (92%)	4 (100%)	14 (93%)	6 (86%)
Hjortswang diarrhea criteria, positive	34 (76%)	25 (96%)	4 (100%)	14 (93%)	7 (100%)
Reported symptoms					
Urgency	43 (98%)[b]	26 (100%)	3 (75%)	15 (100%)	6 (100%)
Fecal incontinence	32 (72%)[b]	21 (81%)	4 (100%)	12 (80%)	4 (67%)
Nocturnal diarrhea	22 (50%)[b]	15 (58%)	3 (75%)	8 (53%)	4 (67%)
Abdominal pain	31 (72%)[c]	15 (60%)[d]	3 (75%)	8 (57%)[e]	4 (67%)
Bloating	40 (89%)	21 (84%)[d]	3 (75%)	12 (80%)	4 (80%)[f]
Diarrhea duration					
1–2 yr	9 (20%)[b]	4 (16%)[d]	1 (25%)	3 (21%)[e]	0 (0%)
>2 yr	19 (43%)[b]	17 (68%)[d]	2 (50%)	10 (71%)[e]	5 (71%)
Patient chart data					
Response to sequestrant treatment	N/A				
Good		18 (69%)	3 (75%)	9 (60%)	6 (86%)
Insufficient		4 (15%)	1 (25%)	3 (20%)	1 (14%)
Intolerant		2 (8%)	0 (0%)	2 (13%)	0 (0%)
Not recorded in the patient file		2 (8%)	0 (0%)	1 (7%)	0 (0%)
Ultimate sequestrant treatment	N/A				
Colestyramine		17 (65%)	3 (75%)	9 (60%)	5 (71%)
Colesevelam		3 (12%)	0 (0%)	2 (13%)	1 (14%)
None		6 (23%)	1 (25%)	4 (27%)	1 (14%)

Demographic characteristics, diary stool registration, and reported subjective symptoms registered at study visit 1. Treatment response and ultimate sequestrant treatment were obtained in a subsequent review of patient files. SeHCAT retention ≤ 10% defined bile acid diarrhea; data from these patients are also shown divided by the 3 subtypes of bile acid diarrhea. Categorical data are shown as n (%) and continuous data as median with interquartile range.
^{75}SeHCAT, 75-seleno tauro-homocholic acid retention test; N/A, not applicable.
[a]Mann-Whitney *U* test, $P < 0.05$. Unless given in the top row, the denominator was, b: 44, c: 43, d: 25, e: 14, f: 5.

microscopic colitis (n = 1), coeliac disease (n = 1), or pancreatic insufficiency (n = 1). The final diagnoses in the miscellaneous diarrhea group were IBS (n = 16), functional diarrhea (n = 11), indeterminate bile acid diarrhea (SeHCAT 10.1–15%) (n = 11), ulcerative colitis (n = 3), Crohns' disease (n = 2), constipation diarrhea (n = 1), and pancreatic insufficiency (n = 1). The patients with bile acid diarrhea were younger and had more frequent bowel movements, and all but one fulfilled the Hjortswang diarrhea activity criteria. The symptoms reported by the 2 groups were similar (Table 1).

7α-hydroxy-4-cholesten-3-one

Mean C4 was higher in patients with bile acid diarrhea (30 ng/mL, 95% CI: 19–46) than in patients with miscellaneous diarrhea (8 ng/mL, 95% CI: 7–11; $P < 0.0001$) (Table 2). C4 correlated inversely with SeHCAT ($r_s = -0.64$; n = 71, $P < 0.0001$) (see Figure S2, Supplementary Digital

Table 2. Fasting C4, fasting, and stimulated FGF19 specified by SeHCAT subgroups

SeHCAT Retention	C4 Mean (95% CI) 0 min	FGF19 Median (IQR) 0 min	90 min	120 min	150 min	Δ90–0	Total AUC
≤10% n = 26	30[b] (19–46)	72[a] (53–146)	140 (81–191)	233 (142–294)	239 (122–356)	36 (−14 to 104)	23,740 (16,339–35,781)
>10% n = 45	8[b] (7–11)	119[a] (84–240)	144 (85–230)	198 (139–371)	261 (116–440)	10 (−28 to 51)	28,770 (17,052–35,834)
≤5 % n = 16	40 (26–61)	67 (52–172)	116 (93–185)	233 (186–294)	244 (178–356)	22 (−22 to 63)	23,991 (16,339–29,383)
>5 to ≤10% n = 10	18 (7–49)	79 (53–134)	152 (53–318)	217 (74–552)	162 (99–649)	92 (19 to 184)	22,117 (9,045–44,667)
>10 to ≤15% n = 12	13 (8–19)	150 (96–223)	159 (107–452)	249 (161–455)	291 (139–464)	26 (−44 to 213)	29,552 (20,332–56,614)
>15% n = 33	7 (5–10)	112 (82–240)	140 (84–204)	175 (124–371)	261 (96–440)	8 (−28 to 38)	28,770 (14,308–34,232)

Mean 7α-hydroxy-4-cholesten-3-one (C4) in ng/mL with 95% CI; median FGF19 in pg/mL with IQRs. Both C4 and FGF19 were measured at fasting before the stimulation: ingestion of the study meal plus 1,250 chenodeoxycholic acid (t = 0 min). Subsequent measurements for FGF19 were performed after 90, 120, and 150 minutes. The calculated increment in FGF19 from fasting to t = 90 minutes is shown (Δ90–0). The total area under the FGF19 curve (AUC) was calculated with the trapezoidal rule (pg/ml • min).
AUC, area under ROC curve; CI, confidence interval; FGF19, fibroblast growth factor 19; IQR, interquartile range; ^{75}SeHCAT, 75-seleno tauro-homocholic acid retention test.
[a]Mann-Whitney *U* test; $P = 0.004$.
[b]Student *t* test; $P < 0.00001$.

Content 1, http://links.lww.com/AJG/B588) and with FGF19 ($r_s = -0.33$; n = 71, $P = 0.005$). ROC analysis of fasting C4 and SeHCAT retention ≤ 10% gave a ROC_{AUC} of 0.83 (0.72–0.93; $P < 0.0001$). C4 <15 ng/mL had an 85% (74%–96%) negative predictive value (NPV) to exclude, and C4 > 48 ng/mL had an 82% (59%–100%) positive predictive value (PPV) to diagnose bile acid diarrhea. Twenty patients had C4 of 15–48 ng/mL, 11 (55%) of these had SeHCAT ≤ 10% (Figure 1A). The cutoff C4 > 30 ng/mL had a PPV of 78% (59%–97%) and NPV of 77% (66%–89%) (Table 3). See Table S2, Supplementary Digital Content 1, http://links.lww.com/AJG/B588 for more cutoff values.

Four patients with SeHCAT retention of 13, 16, 18, and 23% had high values of C4 (range 40–58 ng/mL) (Figure 1A and Figure S1, Supplementary Digital Content 1, http://links.lww.com/AJG/B588). One of these had microscopic colitis and one had ulcerative colitis. Six patients with SeHCAT ≤ 10% had low C4 values (<15 ng/mL). One of these used statin for hypercholesterolaemia. C4 was unaffected by controlling for cholesterol.

FGF19

Median fasting FGF19 was lower in patients with bile acid diarrhea (72, IQR 53–146 pg/mL) than in patients with miscellaneous diarrhea (119, IQR 84–240 pg/mL; $P = 0.004$). There was no difference in FGF19 stimulated with a meal plus chenodeoxycholic acid for the 90-minute increment in FGF19, the total area under the FGF19 curves ($FGF19_{AUC}$), or FGF19 at 120 or 150 minutes (Table 2). Similarly, low fasting FGF19 correlated with low SeHCAT retention ($r_s = 0.31$; $P = 0.01$). whereas the stimulated total $FGF19_{AUC}$ and SeHCAT did not ($r_s = 0.07$; $P = 0.55$).

The ROC analysis of fasting FGF19 with SeHCAT ≤ 10% gave ROC_{AUC} of 0.71 (95% CI: 0.58–0.83; $P = 0.004$). FGF19 <60 pg/mL had a 75% (51%–100%) PPV for diagnosis and had 78% overall diagnostic accuracy; FGF19 ≥204 pg/mL had 88% (73%–100%) NPV to exclude moderate to severe bile acid diarrhea (Table 3). Forty-two patients had FGF19 of 60–204 pg/mL, of whom 15 (36%) had SeHCAT ≤ 10%. See Table S3, Supplementary Digital Content 1, http://links.lww.com/AJG/B588 for more cutoff values.

Plasma bile acids

The patients with bile acid diarrhea had less secondary bile acids (0.58 μM [IQR: 0.20–1.22] vs 1.04 μM [0.62–1.53]; $P = 0.008$, Bonferroni corrected not significant), significantly less sulfate conjugated bile acid species (0.16 μM [0.05–0.36] vs 0.36 μM [0.23–0.65]; $P = 0.001$), and less lithocholic acid species (0.16 μM [0.03–0.36] vs 0.36 μM [0.21–0.63]; $P = 0.001$) (see Table S1, Supplementary Digital Content 1, http://links.lww.com/AJG/B588).

Exploratory predictive modeling

An exploratory logistic regression model to predict SeHCAT ≤ 10% improved the ROC_{AUC} to 0.88 (0.79–0.96) by controlling the logarithm of C4 for age and mean stools per day. Including the logarithm of FGF19 increased the ROC_{AUC} to 0.91 (0.84–0.98) (Table 4). The covariates sex, body mass index, Bristol Stool Form, any cholesterol, or plasma triglycerides did not improve the model. Further exploratory analysis showed that a model with C4, age, and the sum of sulfate conjugated bile acid species in plasma achieved ROC_{AUC} 0.91 (0.83–0.98). See supplementary material (Supplementary Digital Content 1, http://links.lww.com/AJG/B588) for model equations.

Patient chart treatment response

Subsequent chart review of observational treatment response was available for most patients with SeHCAT <15% according to local treatment practice. Treatment response was reported by 18 of 26 patients with SeHCAT ≤ 10%, by 8 of 11 patients with C4 > 48 ng/mL, and of 5 of 12 patients with FGF19 <60 pg/mL (Table 5).

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

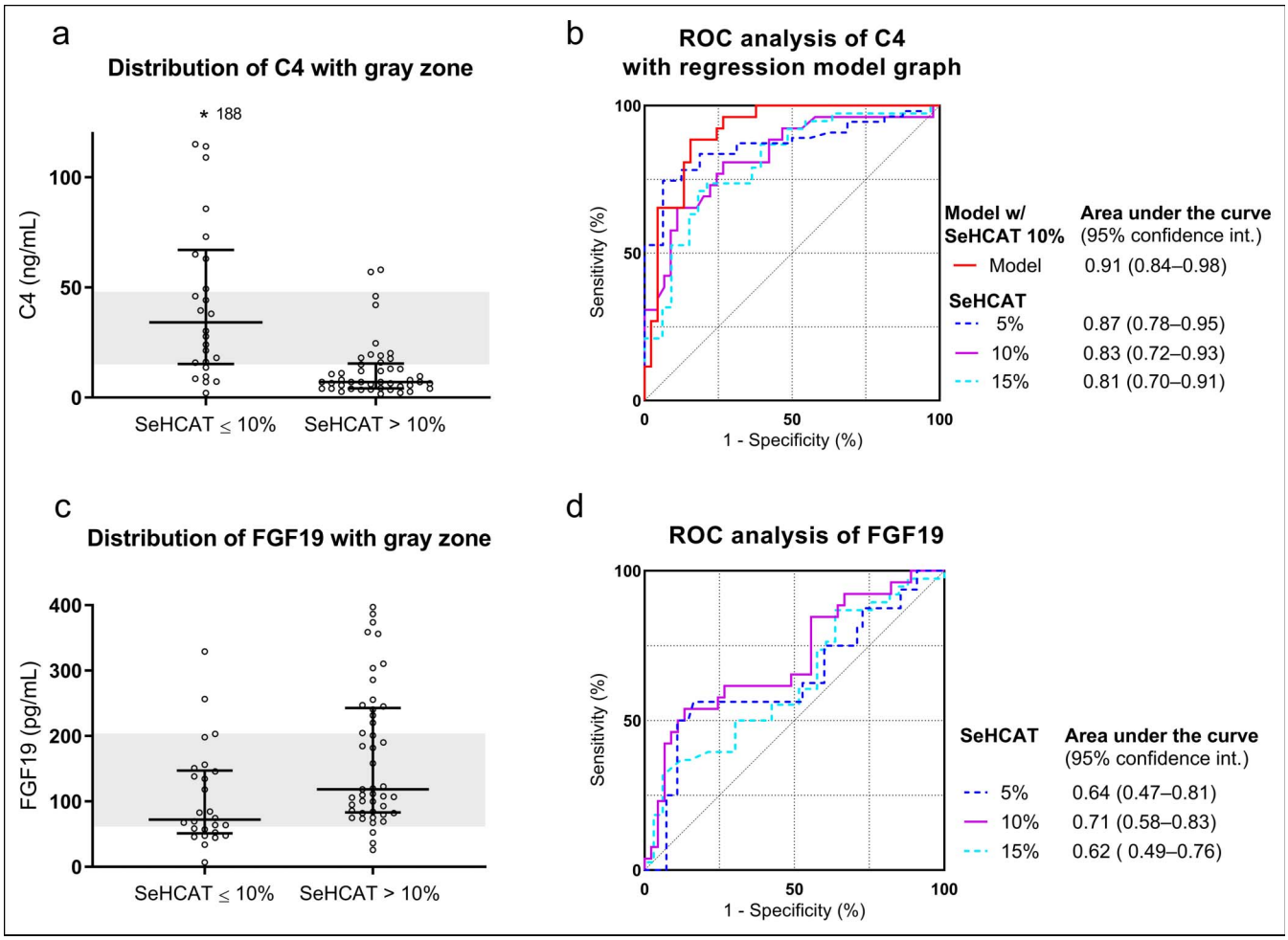

Figure 1. (**a**) The distribution of 7α-hydroxy-4-cholesten-3-one (C4) divided by 75-seleno-taurohomocholic acid (SeHCAT) retention in 71 patients. Retention ≤ 10% defined bile acid diarrhea (n = 26). A gray zone of C4 values from 15 to 48 ng/mL is shown. (**b**) Receiver operator characteristics (ROC) curves for C4. The red line shows the ROC analysis of an exploratory logistic regression model controlling for C4, FGF19, age, and the mean number of stools to predict SeHCAT ≤ 10%. (**c**) The distribution of fibroblast growth factor 19 (FGF19) divided by SeHCAT retention; FGF19 from 60 to 204 pg/mL is shown as a gray zone. (**d**) ROC curves for FGF19.

Adverse events

There were 2 serious adverse events deemed nonrelated to chenodeoxycholic acid: one patient with pneumonia and one patient with acute norovirus gastroenteritis. Common side effects were transient diarrhea (28% of the participants) and abdominal pain (24%).

DISCUSSION

This prospective diagnostic accuracy study compared FGF19 and C4 for diagnosing bile acid diarrhea with SeHCAT ≤ 10% as the gold standard test. We verify that C4 has potential clinical value both for screening and diagnosis of bile acid diarrhea, and we confirm that the diagnostic performance is high also in patients without intestinal resection. C4 <15 ng/mL had 85% NPV and C4 > 48 ng/mL had 82% PPV. Exploratory logistic regression showed that the accuracy of C4 might be improved by controlling for mean stool number, age, and FGF19.

Our diagnostic performance of fasting C4 was slightly lower than that in a previous comparable study (15) that reported an 87% sensitivity and 86% sensitivity for C4 > 30 ng/mL compared with SeHCAT. However, 12 of these 46 patients had bile acid diarrhea because of ileal resection. Recent recommendations are not to test these patients with >95% *a priori* risk of disease (19–21), so we excluded such patients to focus on a population with an intermediate prevalence of the disease, such as the 32% prevalence of bile acid diarrhea in IBS-D (8). This likely explains the difference in test performance. Higher diagnostic cutoffs at approximately 48 ng/mL, which is the upper normal limit (16), have been used in patients with chronic diarrhea of various aetiologies (16) and patients with Crohn's disease (34,35). We substantiate the use of this cutoff with an 82% PPV in our population. Values <48 ng/mL have also been suggested for screening (36) but only had 72% NPV in our population (Table 3), where C4 <15 ng/mL was a better cutoff, with 85% NPV. Combining C4 > 48 ng/mL for diagnosis and C4 <15 ng/mL for screening correctly categorized 43 of 51 patients. Twenty patients had C4 values in the range of 15–48 ng/mL, of whom 11 (55%) had SeHCAT ≤ 10% (Figure 1). To avoid missing the diagnosis, patients with intermediate C4 values could be given an empirical treatment. However, the interpretation of this is difficult and recent guidelines do not recommend empirical treatment for primary diagnosis (37,38). Our present data on treatment outcome is retrospective and lack and lack a control group; however, the data suggest that most patients with C4 > 48 ng/mL have a beneficial response. C4 is of potential clinical value,

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

Table 3. Receiver operating characteristics for C4 and FGF19 in SeHCAT subgroups

SeHCAT retention	ROC_{AUC}	Pos. cutoff	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Positive likelihood ratio	Negative likelihood ratio	Diagnostic accuracy (%)
				Fasting C4					
≤5% (n = 16)	0.87 (0.78–0.95)	>15	94 (82–100)	71 (59–83)	48 (31–66)	98 (93–100)	3.2 (2.1–5.0)	0.1 (0.01–0.6)	76
		>30	69 (46–91)	87 (78–96)	61 (39–84)	91 (83–98)	5.4 (2.5–11.6)	0.4 (0.2–0.7)	83
		>48	38 (14–61)	91 (83–99)	55 (25–84)	83 (74–93)	4.1 (1.4–11.8)	0.7 (0.5–1.0)	79
≤10% (n = 26)	0.83 (0.72–0.93)	>15	77 (61–93)	76 (63–88)	65 (48–81)	85 (74–96)	3.1 (1.8–5.5)	0.3 (0.1–0.6)	76
		>30	54 (35–73)	91 (82–99)	78 (59–97)	77 (66–89)	6.1 (2.2–16.4)	0.5 (0.3–0.8)	78
		>48	35 (16–53)	96 (90–100)	82 (59–100)	72 (60–83)	7.8 (1.8–33)	0.7 (0.5–0.9)	73
≤15% (n = 38)	0.81 (0.70–0.91)	>15	66 (51–81)	82 (69–95)	81 (67–95)	68 (53–82)	3.6 (1.7–7.7)	0.4 (0.3–0.7)	73
		>30	39 (24–55)	91 (81–100)	83 (66–100)	57 (43–70)	4.3 (1.4–13.7)	0.7 (0.5–0.9)	63
		>48	24 (10–37)	94 (86–100)	82 (59–100)	52 (39–64)	3.9 (0.9–16.8)	0.8 (0.7–1.0)	56
				Fasting FGF19					
≤5% (n = 16)	0.64 (0.47–0.81)	<60 <204	38 (14–61) 88 (71–100)	89 (81–97) 27 (15–39)	50 (22–78) 26 (14–38)	83 (73–93) 88 (73–100)	3.4 (1.3–9.2) 1.2 (0.9–1.5)	0.7 (0.5–1.0) 0.5 (0.1–1.8)	78 41
≤10% (n = 26)	0.71 (0.58–0.83)	<60	35 (16–53)	93 (86–100)	75 (51–100)	71 (60–83)	5.2 (1.5–17.5)	0.7 (0.5–0.9)	72
		<204	92 (82–100)	33 (20–47)	44 (31–58)	88 (73–100)	1.4 (1.1–1.8)	0.2 (0.1–0.9)	41
≤15% (n = 38)	0.62 (0.49–0.76)	<60 <204	26 (12–40) 87 (76–98)	93 (86–100) 36 (20–53)	83 (62–100) 61 (48–74)	53 (40–65) 71 (49–92)	4.3 (1.0–18) 1.3 (1.0–1.8)	0.8 (0.6–0.97) 0.4 (0.1–0.9)	58 63

ROC for 7α-hydroxy-4-cholesten-3-one (C4) with cutoffs in ng/mL and FGF19 with cutoffs in pg/mL, both with SeHCAT retention test as gold standard. Bile acid diarrhea is defined by SeHCAT retention values: ≤5% severe; 5 to ≤10% moderate, and 10 to ≤15% indeterminate. Brackets show 95% confidence intervals.

FGF19, fibroblast growth factor 19; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristics; ROC_{AUC}, area under the ROC curve; SeHCAT, 75-seleno-taurohomocholic acid.

Table 4. An exploratory predictive logistic regression model

Covariate	OR	95% CI	*P*	Estimate	SE
C4, 50% increase	1.7	1.3–2.3	<0.001	3.2	0.04
Age, 5 year increase	0.7	0.5–0.9	0.01	−0.36	0.14
Stools/d, mean increase of 1	1.5	1.1–2.3	0.03	0.43	0.19
FGF19, 50% increase	0.6	0.4–0.9	0.04	−0.47	0.23
ROC 0.91 (95% CI: 0.84–0.98)					

Exploratory multivariate logistic regression model to predict bile acid diarrhea defined as SeHCAT retention ≤10%. Univariate testing was significant for each covariate (>10% vs ≤ 10%). Both C4 and FGF19 have undergone a logarithmic transformation. The model has an intercept of 25.4, with estimate 3.2 and SE of 3.2. A model index cutoff > 0.32 has 88% sensitivity, 84% specificity, and a likelihood ratio of 5.7. An index cutoff > 0.7 has 65% sensitivity, 96% specificity, and a positive likelihood ratio of 16.
OR, odds ratio; CI, confidence interval; FGF19, fibroblast growth factor 19; ROC, receiver operating characteristics; SE, standard error; SeHCAT, 75-seleno-taurohomocholic acid.

especially where SeHCAT is unavailable. Because C4 is measured at a single time point, it is less robust than the 1-week SeHCAT examination that quantifies the bile acids loss over numerous enterohepatic cycles. However, the symptoms of bile acid diarrhea are generally persistent (39,40) and replicate C4 measurements are consistent (36). More extensive testing for bile acid diarrhea is needed to avoid missing the diagnosis (7,8). Early testing of patients with chronic watery diarrhea could be considered, and because C4 is much cheaper than SeHCAT, this is feasible. Testing would also be relevant in patients with functional diarrhea or IBS-D, as has been recommended in the 2019 AGA clinical practice guidelines (41).

Table 5. Observational treatment response from chart review

	Patient chart treatment response			
	Yes	No	Intolerant	Missing data
SeHCAT retention (%)				
≤5	12	3	1	1
>5 to ≤10	6	0	1	2
>10 to ≤15	7	3	1	1
>15	1	3	1	28
C4 (ng/mL)				
<15	9	5	1	25
15 to 30	7	1	1	4
>30 to 48	2	2	1	2
>48	8	1	1	1
FGF19 (pg/mL)				
<60	5	3	0	4
60 to 204	18	5	4	15
>204	3	1	0	13

Treatment response from chart review. Treatment was initiated and effect evaluated by the patient's tending physician.
FGF19, fibroblast growth factor 19; SeHCAT, 75-seleno-taurohomocholic acid.

Different purposes and cutoffs have been suggested for fasting FGF19. It is a marker of ileal resection and inflammation (42), and a study found fasting FGF19 to have a ROC_{AUC} of 0.74 and suggested a 145 pg/mL cutoff for bile acid diarrhea (24). Later studies have used FGF19 for diagnosis with a cutoff ≤ 61.7 pg/mL in IBS-D and functional diarrhea (36), and in patients with Crohn's disease, a similar cutoff <60 pg/mL has been used (34,35). Our ROC_{AUC} of 0.71 replicates the previous findings, and we substantiate using the diagnostic cutoff of FGF19 <60 pg/mL that in our population had 75% PPV. Similarly, FGF19 > 204 pg/mL had a high NPV of 88%. As reported in previous studies, we found a large overlap in FGF19 between diarrhea controls and bile acid diarrhea. In fact, 59% of our patients had FGF19 values between 60 and 204 pg/mL, so the performance of FGF19 is low.

In contrast to our pilot comparison of patients with severe type 2 bile acid diarrhea and healthy volunteers (26), stimulation with chenodeoxycholic acid and a meal did not improve the diagnostic characteristics of FGF19 in this study. Others have shown that incubation of ileal biopsies with chenodeoxycholic acid caused a 16-fold FGF19 increase in biopsies from patients with bile acid diarrhea and a 182-fold increase in biopsies from patients with idiopathic diarrhea (3). However, our 26 patients with bile acid diarrhea had a normal magnitude and temporal increase in FGF19 (Table 2), resembling the response in healthy volunteers (26,43,44).

Our explorative logistic regression models indicate how the diagnostic precision of C4 might be improved. Controlling for age and number of stools increase the ROC_{AUC} of C4 from 0.81 to 0.88; adding FGF19 gave ROC_{AUC} of 0.91 (Table 4). Our patients with bile acid diarrhea had less sulfate conjugated bile acids in plasma (Suppl. Table S1). The intestinal loss of bile acids in patients with bile acid diarrhea may result in fewer sulfate-conjugated species for urinary excretion (31,45), and this together with age and C4 achieved ROC_{AUC} of 0.91. The exploratory models need validation and should include other factors such as cholecystectomy, abdominal radiotherapy, inflammatory bowel disease, and ileal resection (21,37).

We recruited patients at 4 sites which could have contributed to heterogeneity, but the prevalence of bile acid diarrhea at the centers was similar. Our sample size was sufficient to confirm the diagnostic characteristics of C4 and fasting FGF19. Nevertheless, the diagnostic options need validation with the placebo-controlled effect of sequestrant treatment.

In conclusion, we have identified C4 values with clinically useful predictive values for the diagnosis of and screening for bile acid diarrhea in a prospective population of patients without previous intestinal resection investigated with SeHCAT. Implementation of C4 testing in patients with chronic watery diarrhea could facilitate easier and earlier identification of patients with bile acid diarrhea. We have suggested ways to optimize the accuracy and clinical utilization of a C4-based test. Validation of the diagnostic modalities and cutoff values with the placebo-controlled effect of sequestrant therapy is warranted to predict beneficial treatment response.

CONFLICTS OF INTEREST

Guarantor of the article: Lars Kristian Munck, MD, DMSci.

Specific author contributions: C.B., S.W., J.R., P.N.B., and L.K.M. did study concept and design. C.B., J.G., T.B.A., L.V.J., A.Z., S.P.G.J., T.G., C.N., and H.B.T. collected data. P.B., D.R,. and E.G. did the biochemical analyses. C.B. did the statistical analysis and primary interpretation with L.K.M., S.W., J.R., and J.G. C.B. drafted the

manuscript. All authors critically revised and approved the final manuscript.
Financial support: The study was supported by the Region Zealand Scientific Fund (Grant number: 15-000342). Sigma-tau Rare Disease Ltd. provided chenodeoxycholic acid for study use without restrictions as an independent scientific grant.
Potential competing interests: S.W. has been an advisory board member for Tillotts and Takeda. All other authors: no disclosures.

Study Highlights

WHAT IS KNOWN

- ✓ One-third of patients with IBS-D have bile acid diarrhea.
- ✓ Access to the ^{75}SeHCAT acid test is limited.
- ✓ Bile acid diarrhea is treatable with sequestrant therapy.

WHAT IS NEW HERE

- ✓ Biochemical screening for and diagnosis of bile acid diarrhea is feasible.
- ✓ 7α-hydroxy-4-cholesten-3-one (C4) test was the superior biochemical test.
- ✓ Validity was confirmed in patients without intestinal resection.

REFERENCES

1. Walters JR. Defining primary bile acid diarrhea: Making the diagnosis and recognizing the disorder. Expert Rev Gastroenterol Hepatol 2010;4(5):561–7.
2. Walters JR, Tasleem AM, Omer OS, et al. A new mechanism for bile acid diarrhea: Defective feedback inhibition of bile acid biosynthesis. Clin Gastroenterol Hepatol 2009;7(11):1189–94.
3. Johnston IM, Nolan JD, Pattni SS, et al. Characterizing factors associated with differences in FGF19 blood levels and synthesis in patients with primary bile acid diarrhea. Am J Gastroenterol 2016;111(3):423–32.
4. Mottacki N, Simren M, Bajor A. Review article: Bile acid diarrhoea - pathogenesis, diagnosis and management. Aliment Pharmacol Ther 2016; 43(8):884–98.
5. Wildt S, Norby Rasmussen S, Lysgard Madsen J, et al. Bile acid malabsorption in patients with chronic diarrhoea: Clinical value of SeHCAT test. Scand J Gastroenterol 2003;38(8):826–30.
6. Skouras T, Dodd S, Prasad Y, et al. Brief report: Length of ileal resection correlates with severity of bile acid malabsorption in crohn's disease. Int J Colorectal Dis 2019;34(1):185–8.
7. Fernandes DCR, Poon D, White LL, et al. What is the cost of delayed diagnosis of bile acid malabsorption and bile acid diarrhoea?. Frontline Gastroenterol 2019;10(1):72–6.
8. Wedlake L, A'Hern R, Russell D, et al. Systematic review: The prevalence of idiopathic bile acid malabsorption as diagnosed by SeHCAT scanning in patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2009;30(7):707–17.
9. Notghi A, O'Brien J, Low CS, et al. Measuring SeHCAT retention: A technical note. Nucl Med Commun 2011;32(10):960–6.
10. Valentin N, Camilleri M, Altayar O, et al. Biomarkers for bile acid diarrhoea in functional bowel disorder with diarrhoea: A systematic review and meta-analysis. Gut 2016;65(12):1951–9.
11. Wilcox C, Turner J, Green J. Systematic review: The management of chronic diarrhoea due to bile acid malabsorption. Aliment Pharmacol Ther 2014;39(9):923–39.
12. Sciarretta G, Vicini G, Fagioli G, et al. Use of 23-selena-25-homocholyltaurine to detect bile acid malabsorption in patients with illeal dysfunction or diarrhea. Gastroenterology 1986;91(1):1–9.
13. Vijayvargiya P, Camilleri M. Current practice in the diagnosis of bile acid diarrhea. Gastroenterology 2019;156(5):1233–8.
14. Axelson M, Aly A, Sjovall J. Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. FEBS Lett 1988; 239(2):324–8.
15. Brydon WG, Nyhlin H, Eastwood MA, et al. Serum 7 alpha-hydroxy-4-cholesten-3-one and selenohomocholyltaurine (SeHCAT) whole body retention in the assessment of bile acid induced diarrhoea. Eur J Gastroenterol Hepatol 1996;8(2):117–23.
16. Sauter GH, Munzing W, von Ritter C, et al. Bile acid malabsorption as a cause of chronic diarrhea: Diagnostic value of 7alpha-hydroxy-4-cholesten-3-one in serum. Dig Dis Sci 1999;44(1):14–9.
17. Eusufzai S, Axelson M, Angelin B, et al. Serum 7 alpha-hydroxy-4-cholesten-3-one concentrations in the evaluation of bile acid malabsorption in patients with diarrhoea: Correlation to SeHCAT test. Gut 1993;34(5):698–701.
18. Brydon WG, Walters JR, Ghosh S, et al. Hydroxypropyl cellulose as therapy for chronic diarrhoea in patients with bile acid malabsorption - possible mechanisms. Aliment Pharmacol Ther 2016;44(3):306–7.
19. Murray IA, Murray LK, Woolson KL, et al. Incidence and predictive factors for positive (75)SeHCAT test: Improving the diagnosis of bile acid diarrhoea. Scand J Gastroenterol 2017;52(6-7):698–703.
20. Gracie DJ, Kane JS, Mumtaz S, et al. Prevalence of, and predictors of, bile acid malabsorption in outpatients with chronic diarrhea. Neurogastroenterol Motil 2012;24(11):983–e538.
21. Lim SJ, Gracie DJ, Kane JS, et al. Prevalence of, and predictors of, bile acid diarrhea in outpatients with chronic diarrhea: A follow-up study. Neurogastroenterol Motil 2019;31(9):e13666.
22. Walters JR. Bile acid diarrhoea and FGF19: New views on diagnosis, pathogenesis and therapy. Nat Rev Gastroenterol Hepatol. 2014;11(7): 426–34.
23. Pattni SS, Brydon WG, Dew T, et al. Fibroblast growth factor 19 and 7alpha-Hydroxy-4-Cholesten-3-one in the diagnosis of patients with possible bile acid diarrhea. Clin Transl Gastroenterol 2012;3:e18.
24. Pattni SS, Brydon WG, Dew T, et al. Fibroblast growth factor 19 in patients with bile acid diarrhoea: A prospective comparison of FGF19 serum assay and SeHCAT retention. Aliment Pharmacol Ther 2013; 38(8):967–76.
25. Borup C, Syversen C, Bouchelouche P, et al. Diagnosis of bile acid diarrhoea by fasting and postprandial measurements of fibroblast growth factor 19. Eur J Gastroenterol Hepatol 2015;27(12):1399–402.
26. Borup C, Wildt S, Rumessen JJ, et al. Chenodeoxycholic acid stimulated fibroblast growth factor 19 response - a potential biochemical test for bile acid diarrhoea. Aliment Pharmacol Ther 2017;45(11):1433–42.
27. Jenkinson C, Coulter A, Wright L. Short form 36 (SF36) health survey questionnaire: Normative data for adults of working age. Bmj 1993; 306(6890):1437–40.
28. Krarup AL, Peterson E, Ringstrom G, et al. The Short health scale: A simple, valid, reliable, and responsive way of measuring subjective health in patients with irritable bowel syndrome. J Clin Gastroenterol 2015; 49(7):565–70.
29. Hjortswang H, Tysk C, Bohr J, et al. Defining clinical criteria for clinical remission and disease activity in collagenous colitis. Inflamm Bowel Dis 2009;15(12):1875–81.
30. Dior M, Delagreverie H, Duboc H, et al. Interplay between bile acid metabolism and microbiota in irritable bowel syndrome. Neurogastroenterol Motil 2016;28(9):1330–40.
31. Humbert L, Maubert MA, Wolf C, et al. Bile acid profiling in human biological samples: Comparison of extraction procedures and application to normal and cholestatic patients. J Chromatogr B Analyt Technol Biomed Life Sci 2012;899:135–45.
32. Deeks JJ, Altman DG. Diagnostic tests 4: Likelihood ratios. BMJ 2004; 329(7458):168–9.
33. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ 1994; 309(6947):102.
34. Battat R, Duijvestein M, Vande Casteele N, et al. Serum concentrations of 7alpha-hydroxy-4-cholesten-3-one are associated with bile acid diarrhea in patients with crohn's disease. Clin Gastroenterol Hepatol 2019;17(13): 2722–30 e4.
35. Lenicek M, Duricova D, Komarek V, et al. Bile acid malabsorption in inflammatory bowel disease: Assessment by serum markers. Inflamm Bowel Dis 2011;17(6):1322–7.
36. Vijayvargiya P, Camilleri M, Carlson P, et al. Performance characteristics of serum C4 and FGF19 measurements to exclude the diagnosis of bile acid diarrhoea in IBS-diarrhoea and functional diarrhoea. Aliment Pharmacol Ther 2017;46(6):581–8.
37. Sadowski DC, Camilleri M, Chey WD, et al. Canadian association of gastroenterology clinical practice guideline on the management of bile acid diarrhea. Clin Gastroenterol Hepatol 2020;18(1):24–41.e1.

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The American College of Gastroenterology

38. Orekoya O, McLaughlin J, Leitao E, et al. Quantifying bile acid malabsorption helps predict response and tailor sequestrant therapy. Clin Med (Lond) 2015;15(3):252–7.
39. Damsgaard B, Dalby HR, Krogh K, et al. Long-term effect of medical treatment of diarrhoea in 377 patients with SeHCAT scan diagnosed bile acid malabsorption from 2003 to 2016; a retrospective study. Aliment Pharmacol Ther 2018;47(7):951–7.
40. Rossel P, Sortsoe Jensen H, Qvist P, et al. Prognosis of adult-onset idiopathic bile acid malabsorption. Scand J Gastroenterol 1999;34(6):587–90.
41. Smalley W, Falck-Ytter C, Carrasco-Labra A, et al. AGA clinical practice guidelines on the laboratory evaluation of functional diarrhea and diarrhea-predominant irritable bowel syndrome in adults (IBS-D). Gastroenterology 2019;157(3):851–4.
42. Nolan JD, Johnston IM, Pattni SS, et al. Diarrhea in crohn's disease: Investigating the role of the ileal hormone fibroblast growth factor 19. J Crohn's colitis 2015;9(2):125–31.
43. Morton GJ, Kaiyala KJ, Foster-Schubert KE, et al. Carbohydrate feeding dissociates the postprandial FGF19 response from circulating bile acid levels in humans. J Clin Endocrinol Metab 2014;99(2):E241–5.
44. Friedrich D, Marschall HU, Lammert F. Response of fibroblast growth factor 19 and bile acid synthesis after a body weight-adjusted oral fat tolerance test in overweight and obese NAFLD patients: A non-randomized controlled pilot trial. BMC Gastroenterol 2018;18(1):76.
45. Alnouti Y. Bile acid sulfation: A pathway of bile acid elimination and detoxification. Toxicol Sci 2009;108(2):225–46.

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.