ORIGINAL ARTICLE

Retrospective Cohort Study

Levothyroxine therapy and impaired clearance are the strongest contributors to small intestinal bacterial overgrowth: Results of a retrospective cohort study

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Abstract

AIM

To identify a set of contributors, and weight and rank them on a pathophysiological basis.

METHODS

Patients who have undergone a lactulose or glucose hydrogen breath test to rule out small intestinal bacterial overgrowth (SIBO) for various clinical symptoms, including diarrhoea, weight loss, abdominal pain, cramping or bloating, were seen as eligible for inclusion in a retrospective single-centre study. Clinical data such as co-morbidities, medication, laboratory parameters and other possible risk factors have been identified from the electronic data system. Cases lacking or with substantially incomplete clinical data were excluded from the analysis. Suspected contributors were summarised under four different pathophysiological pathways (impaired gastric acid barrier, impaired intestinal clearance, immunosuppression and miscellaneous factors including thyroid gland variables) and investigated using the χ^2 test, Student's *t*-test and logistic regression models.

RESULTS

A total of 1809 patients who had undergone hydrogen breath testing were analysed. Impairment of the gastric acid barrier (gastrectomy, odds ratio: OR = 3.5, PPI therapy OR = 1.4), impairment of intestinal clearance (any resecting gastric surgery OR = 2.6, any colonic

resection OR = 1.9, stenosis OR = 3.4, gastroparesis OR = 3.4, neuropathy 2.2), immunological factors (any drug-induced immunosuppression OR = 1.8), altered thyroid gland metabolism (hypothyroidism OR = 2.6, levothyroxine therapy OR = 3.0) and diabetes mellitus (OR = 1.9) were associated significantly to SIBO. Any abdominal surgery, ileocecal resection, vagotomy or IgA-deficiency did not have any influence, and a history of appendectomy decreased the risk of SIBO. Multivariate analysis revealed gastric surgery, stenoses, medical immunosuppression and levothyroxine to be the strongest predictors. Levothyroxine therapy was the strongest contributor in a simplified model (OR = 3.0).

CONCLUSION
The most important contributors for the development of SIBO in ascending order are immunosuppression, impairment of intestinal clearance and levothyroxine use, but they do not sufficiently explain its emergence.

Key words: Bacterial overgrowth syndrome; Hydrogen breath tests; Immunosuppression; Intestinal motility; Hypothyroidism

Core tip: Several contributors to small intestinal overgrowth have been described, but the impact of particular risk factors is poorly understood. We aimed to determine the influence of several pathomechanisms, such as impaired gastric acid barrier function, impaired intestinal clearance, impairment of defence mechanisms and miscellaneous factors, as well as to weight and rank a large set of potential contributors by means of a retrospective cohort study of 1809 consecutive patients who had undergone a hydrogen breath test to rule out small intestinal bacterial overgrowth. Overall, levothyroxine therapy, impaired intestinal clearance and immunosuppression are the strongest contributors, while an impaired gastric acid barrier only plays a minor role.

INTRODUCTION

Small intestinal bacterial overgrowth (SIBO) is defined as an increase in the number of bacteria in the upper gastrointestinal tract. The aetiology and pathogenesis of SIBO are incompletely understood. It is believed that endogenous defence mechanisms prevent abun-dant microbial growth in the small intestine[1] under physiological conditions, so that the development of SIBO is usually seen to be associated with disorders of protective antimicrobial mechanisms, anatomical abnormalities or motility disorders. High recurrence rates after successful antibiotic treatment emphasise the need to identify aetiological factors in order to potentially remedy the situation[2].

Impairment of the gastric acid barrier function
Gastric acidity constitutes an effective barrier against the invasion of ingested microorganisms. Although the data is weak and contradictory, reduction of the acid barrier function, as suspected for atrophic gastritis[3,4], use of proton pump inhibitors[5] and gastrectomy[6,7], is thought to lead to higher microbial loads in the small intestine.

Impaired intestinal clearance
Although several reviews highlight anatomic patho-logies associated with small intestinal obstruction and stagnation, for example, strictures, adhesions, tumours of the small bowel, duodenal and jejunal diverticula, previous abdominal surgery such as blind loop syndrome after Billroth-II or Roux-en-Y procedure, or bariatric bypass surgery, to be associated with SIBO, only very few or no data at all support these hypotheses. Overall abdominal surgery was not associated with SIBO in a small retrospective study[8]. However, gastrectomy and bariatric surgery in morbid obese patients often leads to the development of SIBO[6,7,9]; the existence of blind loops might be the common mechanism. Additionally, conditions that predispose stool reflux, such as ileocecal resection or low ileocecal valve pressure, are discussed as SIBO predisposing factors[10].

More evidence exists concerning impaired motility, such as small intestinal pseudo-obstruction and several neurological diseases or diabetes[1,11]. It has been demonstrated for a long time that a subset of patients with SIBO show reduced motility[12] with fewer phase III contractions of the migrating motor complexes and a mutual influence since eradication of bacterial overgrowth improved motility[13]. Gastroparesis, which has been shown to be associated with SIBO[14,15], might indicate gastrointestinal autonomous neuropathy. Otherwise, little is known about the effect of drugs used to deteriorate intestinal motility, even though "narcotics" have been identified as contributing to-wards SIBO[16].

Impairment of immunological mechanisms
Data about the role of the immune system are also scarce and contradictory. A higher bacterial load of jejunal aspirates have been shown in ten paediatric patients with IgA deficiency and seven with other immune syndromes[17], while data concerning adult patients are lacking. In fact, medical therapy is the

most common reason for immunosuppression in adults, though SIBO was not shown to be associated with immunosuppressive medication in patients with Crohn's disease (CD)[18,19], while steroids predisposed to SIBO in a more unselected cohort[20].

Miscellaneous factors

Various other diseases and disorders have been described as being associated with or complicated by SIBO, such as alcohol consumption[21], liver cirrhosis[22-25], non-alcoholic steatohepatitis[26], hypothyroidism[27] or chronic pancreatitis[28].

In summary, several contributors to the development of SIBO have been proposed, but only a few have been proven in clinical studies, which often refer to small and selected cohorts. Additionally, it is uncertain which pathomechanisms are more and which are less important contributors. We aimed, therefore, to (1) evaluate a larger set of potential risk factors; (2) arrange them in a pathogenetic model; and (3) rank the contributors referring to their particular weight in a largely unselected cohort of SIBO and non-SIBO patients in an extensive retrospective cohort study.

MATERIALS AND METHODS

Study population

We conducted a retrospective single-centre study of patients undergoing lactulose or glucose hydrogen breath testing between 1995 and 2010, who were referred to the Department of Gastroenterology at the University Hospital Bergmannsheil Bochum, Germany. Patients underwent breath tests to rule out SIBO for various clinical symptoms, including diarrhoea, weight loss, abdominal pain, cramping or bloating. All cases with an original examination report available were considered eligible for the study. Patients with both missing or incomplete clinical data, or incomplete or aborted examination were excluded. Patients with multiple hydrogen breath tests within the study period were considered only once.

Clinical work-up and reference standard

All patients underwent a hydrogen breath test with lactulose, glucose or both in combination. Additionally, patients underwent a routine diagnostic work-up following a clinical algorithm by a symptom-based diagnostic approach including clinical evaluation (history, symptomatology, clinical examination, complaints, clinical course), laboratory including stool testing, and endoscopy (ileocolonoscopy, esophago-gastroduodenoscopy) including histopathology and transabdominal ultrasound. Further diagnostic tools may have been used, for example, small bowel magnetic resonance imaging, endoscopic ultrasound, small bowel endoscopy, manometry or extended function testing.

Hydrogen breath test

Breath tests were performed according to a stan-dardised protocol with either 25 g lactulose or 75 g glucose in 300 mL water, respectively. Only one test was performed per day. Breath samples were collected using an AlveoSampler™ (Campro Scientific GmbH, Germany) every 10 min over a period of 2 h. In cases of failed hydrogen exhalation during a lactulose breath test, collecting periods were extended up to 180 min.

Analysis of breath test examinations

An increase in hydrogen of at least 20 ppm was considered positive; for a lactulose breath test, this increase had to have occurred at least 15 min before a sustained rise in hydrogen exhalation indicating colonic lactulose metabolism.

Clinical data acquisition

Additional clinical data was collected from the electronic database. Cases lacking or with substantially incomplete clinical data were excluded from the analysis.

Statistical analysis

Statistical analysis was performed with SPSS 23 (IBM, Armonk, United states). The χ^2 test was used to determine statistical significance with categorial variables, and the Student's t-test for metric variables. Regarding the multivariate analysis, binary logistic regression was performed in each pathophysiological pathway suspected separately (hypo-/achlorhydria, impaired clearance, immunosuppression, thyroid gland variables and miscellaneous factors) and with statistically significant variables in a summarising model. Finally, the highest ranked parameter of each particular section was chosen to calculate a simplified ranking model. The odds ratios (OR) and 95%CI were estimated for specific clinical factors using logistic regression models. Analysis was considered significant with a P value $\leqslant$ 0.05.

Ethical considerations

The study was approved by the institutional review board (registration number 4864-13). Informed consent was obtained from the patients before particular examinations.

RESULTS

Basic characterics

A total of 3715 hydrogen breath test examinations was considered eligible, 1586 of these were excluded due to missing or insufficient clinical information, and 320 records were excluded due to repetitive examinations of the same subject (Figure 1), therefore, our study population in summary contained a total of 1809 patients, with a slight, but not significant excess of women (Table 1). The age did not differ significantly between the gender groups. The overall basic characteristics of the patients were equally distributed as displayed, but patients with SIBO took slightly more
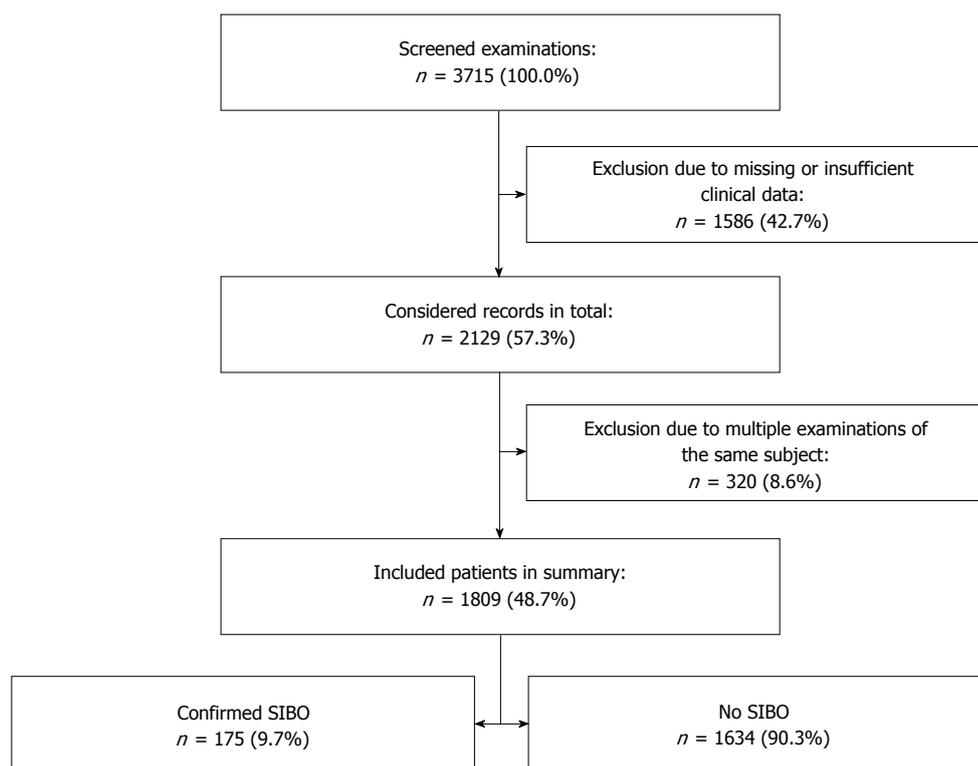
Figure 1 Study flow chart. A total of 3715 hydrogen breath examinations was eligible; 1586 were excluded due to missing or insufficient clinical data. From the remaining 2129 records, 320 were excluded because of multiple breath tests in the same subject, so that a summary total of 1809 patients were included in our study. 175 (9.7%) showed SIBO in terms, while 1634 did not (90.3%). SIBO: Small intestinal bacterial overgrowth.

Table 1 Basic characteristics

	SIBO		Non-SIBO		χ^2 or Student's *t*-test
	n = 175	%	*n* = 1634	%	*P* value
Age (yr)	48.7 ± 17.9	-	49.3 ± 18.0	-	0.70
Sex (female)	107	61.1	924	56.5	0.139
Pathological LHBT	102	58.3	212	13.0	< 0.001[1]
Pathological GHBT	146	83.4	9	0.6	< 0.001[1]
Diarrhoea	72	41.1	648	39.7	0.380
Constipation	4	2.3	38	2.3	0.616
Weight Loss	39	22.3	313	19.2	0.185
Malabsorption	9	5.1	53	3.2	0.138
Iron Deficiency Anaemia	2	1.1	38	2.3	0.240
Vitamin B12 Anaemia	1	0.6	4	0.2	0.399
Number of drugs used	1.61	-	1.32	-	0.005[1]
Supplementation of folic acid	3	1.7	22	1.3	0.442
Supplementation of iron	17	9.7	18	1.1	0.022[1]
Supplementation of vitamin B12	6	3.4	18	1.1	0.023[1]
Abdominal ultrasound	152	86.9	1508	92.3	0.022[1]
EGD	120	68.6	1187	72.6	0.146
Colonoscopy	100	57.1	1069	65.4	0.019[1]
Enteroclysis	45	25.7	357	21.8	0.143
Video capsule endoscopy	7	4.0	19	1.2	0.009[1]
Enteroscopy	1	0.6	4	0.2	0.399

[1]Statistically significant with *P* value < 0.05 in χ^2 or Student's *t*-test. As expected, the SIBO group showed a pathological hydrogen breath test. Symptoms did not differ significantly. Patients with SIBO took more drugs and were more likely to supplement vitamin B12 or iron. During the hospital stay, patients with SIBO underwent small bowel diagnostic procedures more often, while patients without SIBO were more likely to undergo other diagnostic procedures. SIBO: Small intestinal bacterial overgrowth; LHBT: Lactulose Hydrogen Breath Test; GHBT: Glucose Hydrogen Breath Test; EGD: Esophagoga stroduodenoscopy.

drugs (1.32% *vs* 1.61%), especially spasmolytics and antiemetics (4.1% *vs* 14.9% and 8.8% *vs* 11.6%, respectively). Supplementation of iron and cobalamin was more common in SIBO patients.

The SIBO patients were more likely to undergo small bowel visualisation (video capsule endoscopy

Table 2 Hypo-/achlorhydria

	SIBO		Non-SIBO		χ^2 test	OR	RR
	n = 175	%	*n* = 1634	%	*P* value		
Gastrectomy	4	2.3	11	0.7	0.049[1]	3.451	0.295
Atrophic gastritis	2	1.1	5	0.3	0.141	-	-
PPI therapy	70	40.0	519	31.8	0.034[1]	1.432	0.794
Ulcer indicated PPI therapy	5	2.9	47	2.9	0.611	-	-
Gastritis indicated PPI therapy	20	11.4	195	11.9	0.481	-	-
GERD indicated PPI therapy	20	11.4	179	11.0	0.464	-	-
Indicated PPI therapy	42	24.0	395	24.2	0.522	-	-
Not indicated PPI therapy	58	33.1	370	22.6	0.002[1]	1.728	0.693

[1]Statistically significant with $P < 0.05$ in χ^2 test. Patients with SIBO had a history of gastrectomy more often and were more likely to take PPI. In subgroup analysis, those patients who received PPI due to peptic ulcer disease, gastritis or GERD had the same risk of developing SIBO, while those patients who did not exhibit such indications had a 1.7-fold increased risk of showing SIBO. SIBO: Small intestinal bacterial overgrowth; PPI: Proton pump inhibitor; GERD: Gastroesophageal reflux disease.

Table 3 Impaired clearance

	SIBO		Non-SIBO		χ^2 test	OR	RR
	n = 175	%	*n* = 1634	%	*P* value		
Any abdominal surgery	51	29.1	487	29.8	0.466	-	-
Obstetric surgery	2	1.1	45	2.8	0.135	-	-
Abdominal w/o obstetric surgery	49	28.0	444	27.2	0.438	-	-
Gastrectomy	4	2.3	11	0.7	0.049[1]	3.451	0.295
BII-resection	5	2.9	31	1.9	0.265	-	-
Existence of blind loops	8	4.6	41	2.5	0.094[2]	1.861	0.549
Any resecting gastric surgery	12	6.9	45	2.8	0.007[1]	2.600	0.402
Resection of Ileocecum	7	4.0	41	2.5	0.176	-	-
Appendectomy	7	4.0	134	8.2	0.027[1]	0.466	2.050
Functional appendectomy	14	8.0	175	10.7	0.163	-	-
Cholecystectomy	8	4.6	127	7.8	0.078[2]	0.568	1.700
Resection of small intestine	11	6.3	80	4.9	0.260	-	-
Any colonic resection	19	10.9	97	5.9	0.013[1]	1.930	0.547
Sigmoid resection	3	1.7	13	0.8	0.196	-	-
Vagotomy	2	1.1	6	0.4	0.177	-	-
Fistula	2	1.1	16	1.0	0.532	-	-
Stenosis	10	5.7	29	1.8	0.003[1]	3.354	0.311
Impaired motility	17	9.7	9	0.6	< 0.001[1]	5.157	0.202
Gastroparesis	5	2.9	24	1.5	0.030[1]	3.403	0.300
Neuropathy	9	5.1	39	2.4	0.037[1]	2.217	0.464
Opioid use	10	5.7	55	3.4	0.090[2]	1.740	0.589

[1]Statistical significant with P value $P < 0.05$; [2]Statistical tendency with P value $0.05 < P < 0.1$ χ^2 test each. Overall abdominal surgery was not associated with an increased risk of developing SIBO. Resecting gastric surgery in general and gastrectomy was associated with the development of SIBO. Patients with SIBO were less likely to have a history of appendectomy and - as a tendency - cholecystectomy. Ileocecal resection had no impact on the development of SIBO. As a further anatomical alteration, stenoses were more frequent in SIBO patients. Impaired motility, as shown by gastroparesis, led to SIBO; patients with neuropathy or opioid medication are patients at risk. SIBO: Small intestinal bacterial overgrowth.

4.0% *vs* 1.2%) while colonoscopy was performed more often in the non-SIBO group (57.1% *vs* 65.4%).

Suspected risk factor analysis
Hypo-/achlorhydria: Patients with a history of total gastrectomy were more likely to develop SIBO (2.3% *vs* 0.7%). The OR was 3.45 (Table 2). Current PPI therapy led to a higher SIBO rate (40.0% *vs* 31.8%), although the effect was small (OR = 1.43).

Impaired bowel clearance - anatomical alterations and surgery: Overall abdominal surgery was not associated with a higher risk of SIBO (Table 3). Patients after gastrectomy and patients with a history of any resecting gastric surgery had a higher prevalence of SIBO (6.9% *vs* 2.8%, OR = 2.60). By contrast, neither Bilroth-II resection nor the existence of blind intestinal loops exhibited a higher prevalence of SIBO, although the latter group showed a tendency (4.6% *vs* 2.5%). Loss of the ileocecal valve did not increase risk, while a history of appendectomy occurred more often in non-SIBO patients (8.2% *vs* 4.6%), indicating a protective factor with an OR of 0.46. On the other hand, functional appendectomy (including those patients with ileocecal resection) did not show such an effect. Obstetric surgery, sigmoid and small intestinal resection did not affect development of SIBO, while any colonic resection was associated with

Table 4 Immunological factors							
	SIBO		Non-SIBO		χ^2 test	OR	RR
	n = 175	%	*n* = 1634	%	*P* value		
IgA-deficiency	0 of 6	0.0	13 of 75	17.3	0.337	-	-
IgG-deficiency	3 of 5	60.0	15 of 52	28.8	0.175	-	-
IgM-deficiency	0 of 5	0.0	15 of 54	21.7	0.311	-	-
5-Aminosalicylates	24	13.7	183	11.2	0.191	-	-
Steroid use	36	20.6	222	13.6	0.010[1]	1.647	0.660
Immunosuppressant use	8	4.6	31	1.9	0.029[1]	2.477	0.415
Azathioprin use	7	4.0	31	1.9	0.067[2]	2.155	0.474
Metotrexate use	3	1.7	0	0.0	0.001[1]	n/a	n/a
Any drug-induced immunosuppression	39	22.3	230	14.1	0.004[1]	1.751	0.632
Steroid plus immunosuppressant	7	4.0	23	1.4	0.021[1]	2.918	0.352

[1]Statistical significant with *P* value *P* < 0.05; [2]Statistical tendency with *P* value 0.05 < *P* < 0.1 χ^2 test each. Deficiencies of immunoglobulins were not associated with a higher prevalence of SIBO. On the other hand, pharmacologically induced immunosuppression with steroids alone, with an immunosuppressant alone or with both in combination lead to a higher risk of developing of SIBO. SIBO: Small intestinal bacterial overgrowth.

an OR of 1.93 (10.9% *vs* 5.9%). Cholecystectomy tended to be protective (4.6% *vs* 7.8%). Stenoses of the intestinal tract were associated with SIBO (5.7% *vs* 1.8%) with an OR of 3.36.

Impaired bowel clearance - functionally impaired clearance: Impaired motility (9.7% *vs* 0.6%), gastroparesis (2.9% *vs* 1.5%) and neuropathy (5.1% *vs* 2.4%), but not vagotomy were associated with higher risks of SIBO; referring to Table 3, the ORs were 5.16, 3.40 and 2.22, respectively. Prevalence of SIBO tended to be higher under opioid medication, but did not achieve statistical significance (5.7% *vs* 3.4%).

Immunological factors: Immunoglobulin deficiency did not change the risk of developing SIBO (Table 4), while the use of steroids (20.6% *vs* 13.6%) or classical immunosuppressants (4.6% *vs* 1.9%), any immunosuppressive therapy (22.3% *vs* 14.1%) and the combination of steroids and immunosuppressants led to a higher risk of SIBO (4.0% *vs* 1.4%). The OR was highest in the combination group (steroid plus immunosuppressant: OR = 2.92).

Other factors
Thyroid gland metabolism: As referred to in Table 5, patients with hypothyroidism and patients with levothyroxine therapy showed a higher prevalence of SIBO (9.7% *vs* 4.0% and 17.1% *vs* 6.5%, respectively) while a history of thyroidectomy slightly failed to.

Miscellaneous: Diabetes mellitus was associated with a 1.90-fold increased risk of developing SIBO (14.3% *vs* 8.1%; Table 5). Sigmoid, but not colonic diverticulosis was associated with a lower prevalence of SIBO (4.6% *vs* 9.2%).

Multivariate analysis: The different pathomechanistic pathways were tested in a binary logistic regression analysis. The strongest particular independent vari-

ables were PPI therapy and gastrectomy for hypo- or achlorhydria. Both showed statistical significance with an OR of 1.45 and 3.64, respectively.

All variables which potentially impair intestinal clearance were studied in a further model. Any resecting gastric surgery, stenoses, gastroparesis and any colonic resection were significantly associated with the presence of SIBO (*P* < 0.05): the ORs were 6.49, 3.19, 3.25 and 1.85, respectively, while gastrectomy, neuropathy, existence of blind loops, appendectomy and cholecystectomy were not.

The model for immunosuppression did not show any significant parameters. Binary logistic regression for thyroid gland variables proved statistically significant for levothyroxine use with an OR of 2.8, while thyroidectomy and hypothyroidism did not. The only significant parameter in the model of miscellaneous variables was sigmoid diverticulosis with an OR of 0.453; the other factors were opioid use, smoking, diabetes and ulcerative colitis.

Finally, the variables PPI therapy, history of gastrectomy, history of any resecting gastric surgery, presence of stenoses, use of levothyroxine, presence of diabetes, neuropathy or gastroparesis, medical immunosuppression and therapy with opioids have been included in a summarising model (Table 6). The Omnibus test results are highly significant (*P* < 0.001). The Hosmer-Lemeshow test (*P* = 0.500) indicates that the independent variables form a good model to predict SIBO (54 cases observed, 56 cases expected); Nagelkerke's R^2 was 0.070. Variables with significant influence were any resecting gastric surgery (*P* = 0.037, OR = 2.40), stenoses (*P* = 0.008, OR = 2.81), any medical immunosuppression (*P* = 0.036, OR = 1.53), levothyroxine therapy (*P* < 0.001, OR = 2.92) and presence of sigmoid diverticulosis (*P* = 0.028, OR = 2.30).

Of the variables "impairment of acid barrier" (PPI therapy and gastrectomy), "impairment of intestinal clearance" (history of any resecting gastric surgery, presence of stenoses, gastroparesis), "impairment of immune response" (any medical suppression) or

Table 5 Miscellaneous variables

	SIBO		Non-SIBO		χ^2 test	OR	RR
	n = 175	%	*n* = 1634	%	*P* value		
Thyroid gland surgery	7	4.0	33	2.0	0.085[2]	2.021	0.505
Hypothyroidism	17	9.7	66	4.0	0.002[1]	2.556	0.416
Hyperthyroidism	1	0.6	24	1.5	0.287	-	-
Levothyroxine use	30	17.1	106	6.5	< 0.001[1]	2.982	0.378
Adipositas	31	17.7	307	18.8	0.410	-	-
Diabetes mellitus	25	14.3	132	8.1	0.006[1]	1.896	0.565
Steatosis hepatis	22	12.6	158	9.7	0.139	-	-
Hepatitis	7	4.0	61	3.7	0.492	-	-
Liver cirrhosis	2	1.1	35	2.1	0.289	-	-
Renal insufficiency	2	1.1	16	1.0	0.532	-	-
Colonic diverticulosis	6	3.4	37	2.3	0.229	-	-
Sigmoid diverticulosis	8	4.6	151	9.2	0.020[1]	0.470	2.021
Crohn's disease	19	10.9	134	8.2	0.146	1.363	0.755
Ulcerative colitis	2	1.1	52	3.2	0.092[2]	0.352	2.785
Alcoholism	3	1.7	36	2.2	0.468	-	-
Smokers	10	5.7	52	3.2	0.070[2]	1.844	0.557
NSAID use	29	16.6	284	17.4	0.442	-	-
Laxative use	3	1.7	58	3.5	0.142	-	-
Antidiarrhoics use	9	5.1	118	7.2	0.195	-	-
Spasmolytics use	9	5.1	257	15.7	< 0.001[1]	0.290	3.058
Antiemetics use	22	12.6	144	8.8	0.071[2]	1.488	0.701
Irritable bowel syndrome	8	4.6	322	19.7	< 0.001[1]	0.195	4.311

[1]Statistical significant with *P*-value $P < 0.05$; [2]Statistical tendency with *P*-value $0.05 < P < 0.1$ χ^2 test each. Patients with hypothyroidism and substitution of levothyroxine show a higher risk of SIBO. In the case of thyroidectomy, statistical significance was not achieved. The presence of diverticulosis of the sigmoid, but not of the entire colon was associated with a decreased risk of SIBO. Patients with Crohn's disease did not exhibit a higher prevalence of SIBO, and ulcerative colitis tended to be protective, but did not reach statistical significance. Patents without SIBO were more likely to use spasmolytics and to suffer from irritable bowel syndrome. SIBO: Small intestinal bacterial overgrowth; NSAID: Non-steroidal anti-inflammatory drugs.

Table 6 Multivariate analysis

Equation variables	Regression coefficient B	Wald	Significance	Exp (B)	95%CI for Exp (B)	
					Lower limit	Upper limit
PPI therapy	0.241	2.015	0.156	1.273	0.912	1.776
Gastrectomy	0.600	0.676	0.411	1.821	0.436	7.604
Any resecting gastric surgery	0.875	4.369	0.037	2.399	1.056	5.450
Stenoses	1.033	7.011	0.008	2.809	1.308	6.033
Gastroparesis	1.016	3.244	0.072	2.762	0.914	8.345
Any colon resection	0.479	3.012	0.083	1.614	0.940	2.772
Any medical immunosuppression	0.428	4.380	0.036	1.534	1.028	2.291
Levothyroxine therapy	1.070	20.980	0.000	2.916	1.845	4.609
Diabetes mellitus	-0.453	3.223	0.073	0.636	0.388	1.042
Sigmoid diverticulosis	0.832	4.835	0.028	2.298	1.095	4.823

Binary logistic regression analysis for risk factors for SIBO. All parameters found to be significant in previous analyses were used for analysis with binary logistic regression. A history of gastric surgery, stenoses, medical immunosuppression, levothyroxine therapy and sigmoid diverticulosis showed statistical significance as contributors to SIBO. SIBO: Small intestinal bacterial overgrowth; Exp: Exponent.

hypothyroidism (levothyroxine supplementation), the latter three were significantly associated with SIBO with odds ratios of 2.2, 1.6 and 3.0, respectively, in a simplified model in which every factor that was significant in the particular logistic regression was summarised (Table 7).

DISCUSSION

The pathogenesis of SIBO and underlying predisposing conditions are insufficiently understood. Several risk factors have been proposed, but most studies refer to one or very few variables in selected populations. Furthermore, no study investigated and ranked the underlying main pathomechanistic pathways. In this retrospective study, we sought to identify, categorise and, finally, rank the influence of potential contributors to SIBO in a large and widely unselected population. Three main pathogenetic pathways have been hypothesised: hypo- or achlorhydria, impaired intestinal clearance and immunosuppression. Further factors of unknown action, such as hypothyroidism, inflammatory bowel disease or sigmoid diverticulosis, were also considered.

Table 7 Binary logistic regression for categorised variables						
Equation variables	Regression coefficient B	Wald	Significance	Exp (B)	95%CI for Exp (B)	
					Lower limit	Upper limit
Impaired acid barrier	0.277	2.780	0.095	1.319	0.953	1.827
Impaired clearance	0.772	14.463	0.000[1]	2.164	1.454	3.221
Impaired immune response	0.453	5.161	0.023[1]	1.572	1.064	2.324
Hypothyroidism	1.083	22.514	0.000[1]	2.953	1.888	4.620

[1]Statistically significant with *P* value < 0.05 in binary logistic regression. Regression analysis for categorised variables: CI: Confidence Interval. Exp (B): Exponent B. A simplified model, which included a leading factor of each pathophysiological pathway, revealed that, in ascending order, impaired immune response, impaired intestinal clearance and hypothyroidism are the key pathways for the development of small intestinal bacterial overgrowth.

Hypo-/achlorhydria

Both PPI therapy and, even more predominantly, gastrectomy were significantly associated with SIBO (Table 2). These findings coincide with results in the literature, although glucose hydrogen breath tests usually failed to find an association between SIBO and PPI therapy[5-7]. Consequently, gastrectomy was a stronger predictor of SIBO in binary regression analysis than PPI therapy (OR 3.6 *vs* 1.5). Moreover, the altered anatomical situation with the establishment of blind loops might play a role in the development of SIBO after gastrectomy, although our data does not confirm blind loops as a pathophysiological factor (Table 3). However, both variables are of minor relevance and lost their significance in multivariate models.

Impaired intestinal clearance

Anatomic modifications which might lead to impairment of intestinal clearance derive mostly from surgery, but, similar to a smaller retrospective study by Petrone *et al*[8], overall abdominal surgery was not associated with an increased risk of SIBO, neither were sigmoid nor ileocecal resection nor obstetric surgery (Table 3). Our data, therefore, does not confirm the previously assumed protective function of the ileocecal valve[10]. By contrast, total gastrectomy, any resecting gastric surgery and stenoses which lead to stasis of the chymus contributed to the development of SIBO with odds ratios between 1.9 and 3.6. Since blind loops of any reason were astonishingly not associated to SIBO, the combination of intestinal diversion and loss of acid barrier is of greater relevance.

Since small intestinal motility is difficult to quantify as a promoter of clearance, this effect might be estimated by measuring gastroparesis as a surrogate parameter. This association has already been described in other studies[14,15]. Our data shows that conditions believed to be associated with intestinal paralysis, such as polyneuropathy, are indeed associated with gastroparesis ($P < 0.001$, χ^2 test). Both are linked to SIBO, but multivariate analysis reveals that gastroparesis is the better parameter (Tables 3 and 6), by which it is easier to quantify than intestinal motility itself.

Any resecting gastric surgery, stenoses and gastroparesis were associated with SIBO in binary regression analysis, and the particular OR varied between 1.8 and 6.5, with the highest value for overall gastric surgery.

Immunosuppression

Data about the role of the immune system is scarce, biased and contradictory. Higher bacterial loads of jejunal aspirates have been shown in ten paediatric patients with IgA deficiency and seven with other immune syndromes[17]. Therapy with immunosuppressives in CD did not increase the occurrence of SIBO[18,19], while steroids did so in a more unselected cohort by lowered levels of IgA, as hypothesised by the authors[20]. Since neither IgA nor IgG deficiency were associated to SIBO in our analysis, we conclude that IgA deficiency does not - either directly or indirectly triggered by steroids - contribute to SIBO in adult patients. On the other hand, therapy with classical immunosuppressants, such as azathioprine or methotrexate, with steroids alone or in combination with immunosuppressants leads to a higher risk of SIBO (Table 4), while other anti-inflammatory drugs, such as 5-aminosalicylates, that usually serve as reference did not change the risk, suggesting that immunosuppression is the underlying mechanism. The association was quite loose, with odds ratios between 1.6 and 2.5, but was still found to be a minor contributor to SIBO in the multivariate analysis therapy with any immunosuppressant drug, *i.e.*, azathioprine, methotrexate or steroids.

Thyroid gland metabolism

Hypothyroidism and levothyroxine therapy are the most strongly associated to SIBO in our cohort. A case control study by Lauritano *et al*[27] has already revealed a high prevalence of SIBO in patients with autoimmune thyroiditis and hypothyroidism, but the influence of the autoimmune process was a questionable biasing factor. Multivariate analysis confirmed that levothyroxine therapy is a stronger predictor of SIBO than hypothyroidism. The underlying mechanism is unclear. One might speculate that hypothyroidism leads to hypomotility, but, surprisingly, levothyroxine therapy was even more associated to SIBO and not able to reverse the effect of hypothyroidism.

Miscellaneous variables

Although SIBO was more frequent in patients with

cholecystectomy in one study[29], our data propose that cholecystectomy is a protective factor. This finding is supported by another retrospective study[21]. We assume that the prolonged contact time and the optimised environment due to constant excretion of the bile allow its antimicrobial effects to better develop as a potential underlying mechanism.

Though reported as a slight risk factor for the development[20] and recurrence[2] of SIBO, in our cohort, appendectomy was prevalent more often in non-SIBO patients (8.2% *vs* 4.6%, $P = 0.027$, χ^2 test), indicating that it is a protective factor with an OR of 0.455. The potential underlying mechanism is unclear, but an effect on other bowel diseases, especially ulcerative colitis, has already been shown[30].

As in previous studies[11], diabetes was associated with SIBO, but lost its effect in multivariate analysis (Tables 5 and 6), proposing that diabetes does not contribute directly, but as a consequence of complications such as neuropathy, which is supported by a strong association between diabetes and gastroparesis ($P < 0.001$, χ^2 test).

Weighting of the particular etiological pathways

Binary logistic regression reveals the history of any resecting gastric surgery, stenoses, medical immunosuppression, levothyroxine supplementation or presence of sigmoid diverticulosis to be associated to a higher risk of SIBO (Table 6). In this model, a reduced gastric acid barrier lost its significance as a pathogenetic factor. The highest odds ratios were seen for factors that, similar to stenoses, lead to impaired clearance, on the one hand, and supplementation of levothyroxine, on the other hand (Table 6). In this model, several independent factors of impaired clearance, but only one of immunosuppression and thyroid gland metabolism were confirmed.

In a simplified model in which any impairment of the acid barrier, of intestinal clearance, or of immune response and hypothyroidism were analysed, the impaired acid barrier again lost its significance (Table 7). Hypothyroidism or, respectively, levothyroxine therapy was most the important single factor with an odds ratio of 3.0; the second most important was impaired intestinal clearance (OR = 2.2) and the third most important was impaired immune response (OR = 1.6).

Limitations

Due to selective information on outpatients, our study population consists mostly of inpatients, which might have led to a certain bias. Furthermore, the retrospective study design itself is associated with certain limitations, such as primary lack or secondary loss of information, uncertainty of adherence to protocols or unequal power between groups. We, therefore, included only a subset of possible contributors that were available for retrospective analysis. Diagnosis of SIBO referred to clinical work-up and is mainly based on the hydrogen breath test. That approach covers

advantages such as independence from jejunal cultures, which is neither well standardised nor able to culture all respective flora, but also disadvantages such as unclear reliability. Finally, not every potential contributor who underwent clinical work-up was carefully ruled out in every subject. Therefore, some suspected contributors, such as gastroparesis or polyneuropathy, might be underreported.

To the best of our knowledge, this is the largest study that investigated contributors to SIBO in a widely spread population. The results confirm that impairment of acid barrier, impaired intestinal clearance, immunosuppression and especially levo-thyroxine therapy/hypothyroidism to be important pathomechanistic pathways for the development of SIBO. The strongest effects derive from levothyroxine supplementation/hypothyroidism, although the relevant mechanism of action remains unclear, and intestinal stasis.

COMMENTS

Background
Small intestinal bacterial overgrowth (SIBO) is characterised by an excessive colonisation of the small bowel. Pathogenesis is incompletely understood, but recurrence rates after successful antibiotic treatment are high. Several risk factors that contribute to the development have been proposed, and some potential contributors have been shown in mostly small studies with selected populations.

Research frontiers
SIBO is defined as an increase in the number of bacteria in the upper gastrointestinal tract. Related symptoms are nonspecific and include bloating, abdominal pain or diarrhea, and thus overlap widely with symptoms of irritable bowel syndrome. Diagnosis of SIBO still is a challenging clinical problem. Several methods are available, but up to now, a standard of choice is not yet defined. Furthermore, the pathogenesis of SIBO is incompletely understood and studies are needed to identify etiology and pathogenesis. Therapy of SIBO is unsatisfactory; empiric and often broad-spectrum antibiotic treatment fails frequently and suffers from high recurrence rates.

Innovations and breakthroughs
The aim of the study was to identify a set of risk factors, weight and rank them on a pathophysiological basis in a large and widely unselected population. Potential contributors were summarised under four different pathophysiological pathways. Impairment of the gastric acid barrier (gastrectomy, odds ratio: OR = 3.5, PPI therapy OR = 1.4), impairment of intestinal clearance (any resecting gastric surgery OR = 2.6, any colonic resection OR = 1.9, stenosis OR = 3.4, gastroparesis OR = 3.4, neuropathy 2.2), immunological factors (any drug-induced immunosuppression OR = 1.8), altered thyroid gland

metabolism (hypothyroidism OR = 2.6, levothyroxine therapy OR = 3.0) and diabetes mellitus (OR = 1.9) were associated significantly to SIBO. Any abdominal surgery, ileocecal resection, vagotomy or IgA-deficiency did not have any influence, and a history of appendectomy decreased the risk of SIBO. Multivariate analysis revealed gastric surgery, stenoses, medical immunosuppression and levothyroxine to be the strongest predictors. Levothyroxine therapy was the strongest contributor in a simplified model (OR = 3.0).

Applications

The most important contributors for the development of SIBO in ascending order are immunosuppression, impairment of intestinal clearance and levothyroxine use, but they do not sufficiently explain its emergence. The knowledge of respective contributors potentially enables to intervene in the pathogenesis and therefore minimise the risk of recurrence.

Terminology

SIBO is characterised by an increase of bacterial colonisation of the small bowel.

Peer-review

It is an interesting and well performed study.

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