



LUND UNIVERSITY

The Expression of Serum Antibodies Against Gonadotropin-releasing Hormone (GnRH1), Progonadoliberin-2, Luteinizing Hormone (LH), and Related Receptors in Patients with Gastrointestinal Dysfunction or Diabetes Mellitus.

Roth, Bodil; Berntorp, Kerstin; Ohlsson, Bodil

Published in:
Drug Target Insights

DOI:
[10.4137/DTI.S19352](https://doi.org/10.4137/DTI.S19352)

2014

[Link to publication](#)

Citation for published version (APA):

Roth, B., Berntorp, K., & Ohlsson, B. (2014). The Expression of Serum Antibodies Against Gonadotropin-releasing Hormone (GnRH1), Progonadoliberin-2, Luteinizing Hormone (LH), and Related Receptors in Patients with Gastrointestinal Dysfunction or Diabetes Mellitus. *Drug Target Insights*, 8, 45-50.
<https://doi.org/10.4137/DTI.S19352>

Total number of authors:
3

General rights

Unless other specific re-use rights are stated the following general rights apply:
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

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

The Expression of Serum Antibodies Against Gonadotropin-releasing Hormone (GnRH1), Progonadoliberin-2, Luteinizing Hormone (LH), and Related Receptors in Patients with Gastrointestinal Dysfunction or Diabetes Mellitus

Bodil Roth¹, Kerstin Berntorp² and Bodil Ohlsson¹

Department of Clinical Sciences, ¹Section of Internal Medicine and ²Section of Endocrinology, Skåne University Hospital, Malmö, Sweden. Lund University, Lund, Sweden.

ABSTRACT: Gonadotropin-releasing hormone (GnRH) 1 and 2 and luteinizing hormone (LH) receptors have been described in the gastrointestinal tract. We have previously demonstrated antibodies in serum against GnRH1 in patients with gastrointestinal dysfunction and diabetes mellitus, and antibodies against GnRH receptor, LH, and LH receptor in patients with infertility. The aim of this study was to search for the expression of serum antibodies against GnRH1 with an improved enzyme-linked immune sorbent assay (ELISA), and antibodies against progonadoliberin-2, GnRH2, GnRH receptor, LH, and LH receptor with newly developed ELISAs, in patients with gastrointestinal dysfunction or diabetes mellitus. Healthy blood donors served as controls. Medical records were scrutinized. Our conclusion was that IgM antibodies against GnRH1, progonadoliberin-2, and/or GnRH receptors were more prevalent in patients with functional gastrointestinal disorders, gastrointestinal dysmotility, and/or diabetes mellitus, whereas IgG antibodies against these peptides, and LH- and LH receptor antibodies, were expressed in the same magnitude as in controls.

KEYWORDS: Antibodies, Diabetes mellitus, Gastrointestinal dysmotility, gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), progonadoliberin-2

CITATION: Roth et al. The Expression of Serum Antibodies Against Gonadotropin-releasing Hormone (GnRH1), Progonadoliberin-2, Luteinizing Hormone (LH), and Related Receptors in Patients with Gastrointestinal Dysfunction or Diabetes Mellitus. *Drug Target Insights* 2014;8 45–50 doi:10.4137/DTI.S19352.

RECEIVED: August 13, 2014. **RESUBMITTED:** September 19, 2014. **ACCEPTED FOR PUBLICATION:** October 8, 2014.

ACADEMIC EDITOR: Anuj Chauhan, Editor in Chief

TYPE: Original Research

FUNDING: Grants from the Bengt Ihre Foundation, the King Gustaf V:s and Queen Victoria Free Mason's Foundation, Albert Pålsson Foundation, and the Development Foundation of Region Skåne sponsored this study. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

COPYRIGHT: © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

CORRESPONDENCE: bodil.ohlsson@med.lu.se

Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder, occurring in 10–15% of the population. The etiology is unknown, and no organic, pathognomonic changes have been established so far.¹ Relatively severe gastrointestinal dysmotility is often found as a complication in diabetes mellitus and rheumatologic and neurologic diseases.^{2,3} As women are affected by these disorders more often than men, hormonal influences on the gastrointestinal tract have been assumed.⁴

We have recently described the expression of gonadotropin-releasing hormone (GnRH, 1 and 2) and receptors for luteinizing

hormone (LH) in the human enteric nervous system (ENS).^{5–8} The effects of these peptides are not known, but a few studies have described an influence by GnRH and LH on the migrating motor complex (MMC).^{9–11} We have developed an in-house enzyme-linked immune sorbent assay (ELISA) to describe the presence of serum antibodies against GnRH1 in patients with IBS, dysmotility, and diabetes mellitus, but not in inflammatory bowel disease (IBD) or celiac disease,^{12,13} as well as in patients with functional gastrointestinal complaints in association with primary Sjögren's syndrome.^{3,14} The pathophysiological role of these antibodies is unknown. The original



ELISA was associated with a high degree of background signaling.^{3,5,6,12,13} The method has now been further improved and we have also developed ELISAs for the GnRH receptor, LH, and LH receptor.^{14–16} Furthermore, we have developed an ELISA for measurement of antibodies against GnRH2 and its precursor, progonadoliberin-2, as these peptides are abundantly expressed in the ENS,^{8,17} and could hypothetically be more important for gastrointestinal function than GnRH1.

The aim of the present study was to search for the expression of antibodies against GnRH1, progonadoliberin-2, GnRH2, GnRH receptor, LH, and LH receptor with new, sensitive ELISAs, in a population of patients with symptoms and signs of functional gastrointestinal disorders or gastrointestinal dysmotility, and/or diabetes mellitus, and compare these to healthy blood donors, to confirm the presence of autoantibodies in these patients.

Materials and Methods

The study was performed in accordance with the Helsinki declaration and after approval by the ethics review board of Lund University. Consecutive patients visiting the out-patient clinic of the Departments of Gastroenterology or Endocrinology, Skåne University Hospital, Malmö, were included. All patients and blood donors had given their written, informed consent to participate in the study.

Patients suffering from IBS or gastrointestinal dysmotility. Consecutive patients suffering from gastrointestinal symptoms suggesting IBS or dysmotility over a 2-year period (2003–2004) were investigated depending on the severity of the symptoms.¹² They underwent extensive examinations to exclude organic gastrointestinal disorders, eg endoscopy, radiologic examination, and abdominal ultrasound. This had been performed repeatedly for several years in some cases. Routine blood and urine samples were analyzed. The gastrointestinal examination was then further complemented in order to evaluate gastrointestinal motility by manometry, gastric transit time, and full-thickness biopsies of the bowel wall, depending on the clinical picture.^{5,18–22} Patients with functional sub-occlusion without mechanical obstruction, but abnormality in the antroduodenal manometry, were diagnosed as having chronic intestinal pseudo-obstruction (CIPO).²³ The criterion for enteric dysmotility (ED) was abnormality in the small bowel manometry, without sub-occlusion episodes.²⁴ The diagnosis idiopathic gastroparesis was set when delayed gastric emptying rate was the only finding, without engagement of other segments. Patients with gastrointestinal symptoms fulfilling the Rome-III criteria, with no abnormal pattern on antroduodenal manometry, were diagnosed as having IBS.¹ Patients were invited to participate in the study when organic, gastrointestinal diseases were excluded, and one of the above-mentioned diagnoses was set.

Patients suffering from diabetes mellitus. Consecutive patients suffering from gastrointestinal complaints in addition to diabetes mellitus were examined during 2003–2004.^{2,12}

The patients were examined by gastroduodenoscopy, esophageal manometry, and/or gastric emptying scintigraphy to evaluate the gastrointestinal motility.^{18–20,22} Patients diagnosed as suffering from diabetes-related dysmotility, or who fulfilled the criteria for IBS, were invited to participate in the study.

In addition, consecutive patients with diabetes mellitus, independent of the presence or absence of gastrointestinal symptoms, were considered for inclusion for a further 2-year period. Inclusion criteria for the study were age >18 years and diabetes mellitus. The first patient who fulfilled the criteria at each consultation at the Section of Endocrinology, Skåne University Hospital, Malmö, January 2008–February 2010, was asked to take part in the study when visiting the clinic for routine follow-up. Exclusion criteria were renal failure requiring dialysis and severe cardiac morbidity. Examinations of esophageal motility and gastric emptying were performed.^{18–20,22} For further description of the patients, see Berntorp et al.¹³

At the time of inclusion, all patients with diabetes mellitus completed a questionnaire concerning symptoms related to disturbances of the gastrointestinal tract (“loss of appetite, swallowing disturbances, meal-related cough, early satiety, nausea, vomiting, weight loss, abdominal fullness, bloating, regurgitation, constipation, diarrhea, evacuation incontinence, symptomatic postprandial hypoglycemia, and postprandial perspiration”), which had previously been used for these patients.^{2,25,26}

Controls. Blood samples from 200 consecutive, healthy blood donors (100 women), mean age of 42 ± 13 years, were collected at Skåne University Hospital, Malmö, and provided a control group for the antibody analyses.

Study design. The patients and controls gave a blood sample, which consisted of 5.0 mL blood drawn into SST-tubes (366566, BD Vacutainer, Plymouth, UK) containing gel and a coagulation activator. After the tubes had been turned 10 times, they were centrifuged for 15 minutes at 1,500 G. Serum was separated, measured into aliquots, and frozen at -20°C and analyzed for the expression of antibodies. Medical records were reviewed concerning duration of gastrointestinal symptoms, co-existing diseases, therapy treatments, hereditary factors, and routine laboratory analyses.

Measurement of antibodies. Analyses of antibodies (IgM and IgG) against GnRH1, LH, and their receptors were carried out by ELISAs developed in-house as described previously.^{15,16} Serum was chosen because heparin and citrate can affect the analyses and because serum had been taken from the control group. The method has been improved from the original ELISA for GnRH antibodies,^{3,5,6,12,13} and we now use GnRH conjugated to ovalbumin (OVA) (90215.02, Innovagen, Lund, Sweden) with 16 mol GnRH/mol OVA instead of a small peptide, ie 10 amino acids, as antigen. This provides a more stable and efficient analysis. Another improvement is the calculation of relative units (RU) from a



constructed standard curve generated from 200 blood donors, while in earlier studies^{3,5,6,12,13} any value above 0 was considered positive. The absorbance was measured at 405 nm after 30 minutes (GnRH1, GnRH receptor, LH receptor, and LH IgM) or 60 minutes (LH IgG) of incubation at room temperature (RT). The grades of anti-LH-, anti-GnRH1-, and anti-GnRH receptor antibodies were calculated as RU based on each standard curve. To construct standard curves, rabbit anti-human LH antibodies (MBS535386, MyBiosource, San Diego, CA, USA) were diluted from 1:3,000 to 1:192,000, mouse anti-human GnRH antibodies (ab62432, Abcam, Cambridge, USA) were diluted from 1:2,000 to 1:32,000, and rabbit anti-human GnRH receptor antibodies (90217.09, Innovagen) were diluted from 1:8,000 to 1:128,000. Anti-LH receptor antibodies were calculated as absorbance values multiplied by 1,000. In both cases, the background values of each sample were subtracted before calculation. The cut-off value for the presence of antibodies in healthy blood donors was set as RU >97.5th percentile. The intra-assay correlation coefficient of variation (CV) of GnRH and GnRH receptor IgM antibodies was 10% and 8%, respectively ($n = 6$), and the inter-assay CV was 11% and 6%, respectively ($n = 12$). Because of the lack of positive serum, no intra-assay or inter-assay CV of IgG antibodies was calculated. The intra-assay CV of LH IgG and LH IgM was 5.6% and 9.2%, respectively ($n = 8$), and the inter-assay CV was 7.7% and 6.1%, respectively ($n = 17$). Because of the lack of an appropriate commercial antibody, no intra-assay or inter-assay CV of LH receptor was calculated. Since antibodies against LH and LH receptors were not expressed in patients with diabetes mellitus, these analyses were omitted in our IBS and dysmotility patients.

To perform competitive ELISA, sera from patients with antibodies above the cut-off level were incubated with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)-T with various amounts of GnRH1, GnRH receptor, or LH receptor, all three both conjugated and unconjugated with an OVA peptide (Innovagen) or LH (MBS537383, MyBiosource), ie 50, 100, or 200 ng/100 μ L, 30 minutes prior to the application to the microtiter plates.

A new in-house ELISA was set up for the analysis of IgM antibodies against progonadoliberin-2, the precursor of GnRH2.¹⁷ The microtiter plates (456537, Nunc, Roskilde, Denmark) were provided with a layer of recombinant progonadoliberin-2 (MBS1014236, MyBiosource) in PBS or PBS only (an internal blank). After an overnight incubation at 4°C, the plates were washed three times with PBS-T and thereafter blocked with 0.5% BSA (A7030, Sigma, St Louis, USA) in PBS-T. Sera from patients and healthy blood donors diluted to 1:400, or IgG antibodies against human GnRH2 raised in rabbits (MBS6004097, MyBiosource) in serial dilution (to provide a standard curve) with BSA in PBS-T, were added to the plates in triplicate (two wells coated with progonadoliberin-2 and one well coated with PBS) and incubated at RT for 2 hours. The washing procedure was repeated, and the

deposition of autoantibodies directed to progonadoliberin-2 was detected using biotinylated, rabbit anti-human IgM (673211, MP Biomedicals, Santa Ana, California, USA), or goat anti-rabbit IgG (B7389, Sigma), diluted in PBS-T. A phosphatase substrate kit (37620, Pierce, Rockford, IL, USA) was used to develop a color reaction. The absorbance was measured at 405 nm after 30 minutes of incubation at RT. Antibody levels are presented as RU (values after subtracted background), and the concentration in each doublet is interpolated from the standard curve. The cut-off value to define the expression of antibodies in the healthy blood donors was set to RU >97.5th percentile. The intra-assay CV was 5.7% ($n = 8$) and inter-assay CV was 8.3% ($n = 8$). As IgG antibodies against GnRH1 or its receptor have not been shown to be of importance in any previous study, those were not analyzed for progonadoliberin-2.

Statistical methods. Data are described as mean \pm standard deviation (SD) or median (interquartile range [IQR]). Fischer's exact test was used for dichotomous variables and the Mann-Whitney *U*-test for continuous variables. $P < 0.05$ was considered as statistically significant.

Results

Patients suffering from IBS or dysmotility. Forty-five patients (37 women [82%]), mean age 43 ± 17 years, were included. The mean duration of the gastrointestinal symptoms was 16 ± 15 years. Twenty-five patients were diagnosed as having IBS, 11 of these patients had the alternating type of IBS (IBS-A), eight had constipation-predominant IBS (IBS-C), and six had diarrhea-predominant IBS (IBS-D). Twelve patients suffered from ED, five from CIPO, and three from idiopathic gastroparesis (Table 1).

Apart from the gastrointestinal diagnosis, five patients had current or recent treatment for depression, and two cases of each of the following diseases were noted: asthma bronchialis,

Table 1. The expression of IgM antibodies in patients with IBS or dysmotility.

	GnRH1 <i>n</i> (%)	PROGONADOLIBERIN-2 <i>n</i> (%)	GnRH RECEPTOR <i>n</i> (%)
IBS-A (<i>n</i> = 11)	4 (36)	3 (27)	2 (18)
IBS-C (<i>n</i> = 8)		1 (12)	
IBS-D (<i>n</i> = 6)			
ED (<i>n</i> = 12)	2 (17)		1 (8)
CIPO (<i>n</i> = 5)			1 (2)
Gastroparesis (<i>n</i> = 3)			

Note: The cut-off value to define the expression of antibodies in the healthy blood donors was set to relative unit (RU) >97.5th percentile.

Abbreviations: CIPO, chronic intestinal pseudo-obstruction; ED, enteric dysmotility; GnRH, gonadotropin-releasing hormone; IBS-A, alternating type of IBS; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; *n*, number.



endometriosis, hypertension, hypothyroidism, and rheumatologic diseases. Other sporadic diseases were migraine, panic attacks, Parkinson's disease, and thyrotoxicosis. All routine blood samples were within the reference values (data not shown).

Patients suffering from diabetes mellitus and gastrointestinal complaints. Nineteen patients with diabetes mellitus and gastrointestinal complaints (10 women [53%]), mean age 50 ± 11 years, were included. The mean duration of diabetes mellitus was 31 ± 12 years, and of gastrointestinal complaints was 5 ± 4 years.

Seventeen patients had type 1 diabetes and two had type 2 diabetes. All were on insulin treatment and were under acceptable metabolic control (HbA1c: 64.9 ± 10.1 mmol/mol). All 19 patients were examined using gastric emptying scintigraphy, and 12 using esophageal manometry. Gastroparesis was found in 10 patients and esophageal dysmotility in eight patients. Six of the patients had abnormal findings in both examinations, with no correlation between the abnormalities in the two organs. Most of the patients also suffered from secondary complications of diabetes mellitus, such as retinopathy (79%), autonomic neuropathy (53%), and peripheral neuropathy (47%). As some of the patients with diabetes mellitus and gastrointestinal complaints had no pathological changes as found in the examinations ($n = 3$), they were diagnosed as IBS patients. The most common symptoms were abdominal fullness (95%), bloating (79%), early satiety (68%), and constipation (68%). Four of the patients had hypertension, two had hypothyroiditis, two had chronic pancreatitis, and one had atrophic gastritis, depression, hypopituitarism, and rheumatoid arthritis, respectively.

Patients suffering from diabetes mellitus. Forty patients with diabetes mellitus (27 women [68%]), mean age 51 ± 13 years, were included in the study. The patients were insulin-treated (type 1 diabetes: 37 patients [92%]) and were under acceptable metabolic control (HbA1c: 65.2 ± 9.5 mmol/mol). The duration of diabetes mellitus was 26 ± 13 years. Esophageal dysmotility was more often found (60%) than gastroparesis (20%). Twelve percent had dysmotility in both esophagus and the stomach. The most common diabetes complication was retinopathy (65%), followed by peripheral neuropathy (48%) and angiopathy (28%). Although the patients were included

consecutively, regardless of gastrointestinal symptoms, the majority reported symptoms related to food intake and the gastrointestinal tract. The most common symptoms were bloating (48%), abdominal fullness (40%), and early satiety (35%). Apart from diabetes mellitus, three patients suffered from hypothyroidism and one from Addison's disease, atoxic adenoma, celiac disease, pernicious anemia, Sjögren's syndrome, thyrotoxicosis, and vitiligo, respectively.

Serum antibodies. The distribution of antibodies among subgroups of IBS or dysmotility patients is shown in Table 1. When calculated as a whole group, they had a higher prevalence of IgM antibodies against GnRH1 and progonadoliberin-2, and a tendency to a higher prevalence of IgM antibodies against GnRH receptor, than healthy controls (Table 2). The expression of IgG antibodies against GnRH1 and GnRH receptor did not differ from that of the controls ($p = 1.000$ and $p = 0.625$, respectively). The expression of any of the antibodies was not associated with age ($p = 0.311$), duration of symptoms ($p = 0.743$), or subgroup of patients ($p = 0.353$). When comparing patients with IBS and dysmotility, the prevalence of antibodies was equal ($p = 1.000$).

Patients with gastrointestinal complaints related to diabetes mellitus only expressed IgM antibodies against GnRH1, prevalence of which tended to be significantly elevated compared with controls (Table 2). Consecutive patients with diabetes mellitus had a higher prevalence of IgM antibodies against progonadoliberin-2 than controls (Table 2), whereas the expression of IgM and IgG antibodies against GnRH1 was equal ($p = 1.000$ and $p = 1.000$, respectively). Except for a tendency toward a shorter half-time of gastric emptying rate in patients with antibodies against progonadoliberin-2 (27.0 [26.0–50.5] and 52.5 [36.2–71.438], respectively, $p = 0.056$), no other clinical associations could be found. None of the patients with diabetes mellitus showed antibodies against the GnRH receptor, LH, or LH receptor.

The ELISA for the analysis of GnRH2 was unstable, and for this reason these data are not shown.

The distribution of antibodies was equal in men and women among controls and patients with diabetes mellitus. In patients with IBS and dysmotility, all antibodies were expressed in women.

Table 2. The expression of IgM antibodies in patients with IBS, dysmotility, and/or diabetes mellitus.

	GnRH1 <i>n</i> (%)	<i>P</i> -VALUE	PROGONADOLIBERIN-2 <i>n</i> (%)	<i>P</i> -VALUE	GnRH RECEPTOR <i>n</i> (%)	<i>P</i> -VALUE
IBS/dysmotility (<i>n</i> = 45)	6 (13)	0.007	4 (9)	0.040	4 (9)	0.087
Diabetes mellitus with GI complaints (<i>n</i> = 19)	2 (11)	0.088	0	1.000	0	1.000
Consecutive patients with diabetes mellitus (<i>n</i> = 40)	1 (2)	1.000	5 (12)	0.008	0	1.000

Notes: Fischer's exact test was used for the comparisons between the prevalence of antibodies in the different groups and healthy controls. The cut-off value to define the expression of antibodies in the healthy blood donors was set to relative unit (RU) >97.5th percentile. $P \leq 0.05$ was considered as statistically significant. **Abbreviations:** GI, gastrointestinal; GnRH, gonadotropin-releasing hormone; *n*, number.



Discussion

The major finding in our present study was that the expression in serum of IgM antibodies against GnRH1 was higher in patients with IBS or dysmotility, and tended to be higher in patients with gastrointestinal complaints related to diabetes mellitus, compared to controls. The prevalence of IgM antibodies against progonadoliberin-2 was higher in patients with IBS or dysmotility and in consecutive patients with diabetes mellitus, and the prevalence of IgM antibodies against the GnRH receptor tended to be higher in patients with IBS or dysmotility, compared to controls. Although these three diseases are classified as different entities, they have gastrointestinal pain and dysfunction in common. The difference in antibody prevalence between the two diabetes cohorts may be because of the fact that the patients with gastrointestinal complaints had a more severe gastrointestinal disorder with gastroparesis in 53% of the patients, compared to 20% in consecutive diabetes patients, and a higher prevalence of gastrointestinal symptoms.

The present study confirms previous results that IgM antibodies against GnRH1 and GnRH receptors are present in a subgroup of patients suffering from functional disorders and dysmotility,^{3,12,14} as well as in patients with diabetes mellitus.^{12,13} In the present ELISA, a titer above the 97.5th percentile of the standard curves in controls was considered positive. In the former ELISA, we classified all antibody levels above 0 in absorbance, after subtraction of background levels, as positive, and therefore the prevalence was lower in both controls and patients.^{3,12,13}

We have previously reported gastrointestinal complications and expression of GnRH antibodies in serum and a reduced number of GnRH-containing enteric neurons in patients after treatment with GnRH analogs.^{5,6,27} However, the patients who expressed antibodies against GnRH1 in the present study cohort have not been treated by any GnRH analogs. Both GnRH1 and GnRH2 are expressed in enteric neurons of the human ENS.^{5,6,8,27} The antibodies may represent a primary, autoimmune disease rendering neuronal damage and gastrointestinal complaints. Another possibility is that the antibodies are secondary to all kinds of damage of the ENS exposing GnRH to immune-reactive cells.²⁸ Our hypothesis is, taking all studies in this field into consideration,^{3,5,6,12-14,16,27,29} that the antibodies are secondary to the neuronal damage in a subgroup of patients, and not causal. Full-thickness biopsies are not considered in patients with IBS. Thus, we cannot examine the expression of GnRH in the bowel wall in these patients. However, as GnRH is expressed in enteric neurons, and the number of GnRH-containing enteric neurons is reduced in patients suffering from dysmotility with serum antibodies against GnRH and progonadoliberin-2,^{5,6,8,27} we postulate that neuronal damage exposes GnRH and progonadoliberin-2 to immune-presenting cells, resulting in antibody formation. The antibodies could thus be markers of enteric neuron damage. We know from animal trials that in spite of a marked loss of 50% of enteric neurons after treatment

with the GnRH analog buserelin, the gastrointestinal function is well preserved with unaffected weight and healthy rats.²⁹ Thus, a subgroup of IBS patients may have enteric neuropathy, which remains undiscovered by non-sensitive, clinical examinations.

Furthermore, we know neither the time relation in the development of symptomatology and antibodies nor the difference between the formation of antibodies against GnRH1 and progonadoliberin-2. It could possibly reflect a difference in time, one antibody appearing early in the disease process and one appearing later. All blood samples were collected several years after the debut of gastrointestinal complaints, which can influence the results, as the titer declines with time.⁵ No organic changes exist that are pathognomonic for IBS, although inflammatory mediators have been discussed in recent years,³⁰ and active immune responses have been found in subpopulations of IBS patients.³¹ Antibodies against GnRH and its receptor are the first specific, organic parameter found so far, and could represent a subpopulation of IBS. That only female patients with IBS and dysmotility expressed antibodies may depend on the female predominance in this group, and does not exclude the possibility that male patients may express antibodies as well.

IBS is associated with affective disturbances and psychiatric disorders.⁴ Functional magnetic resonance imaging (fMRI) reveals significant differences in the neural processing of pain between IBS patients and controls, further underlining the importance of central mechanisms in the pathophysiology of visceral hypersensitivity in these patients.^{32,33} Both diabetes mellitus and primary Sjögren's syndrome are associated with autonomic neuropathy and gastrointestinal complaints.^{2,14,25,26} Since GnRH1 and GnRH2 are found in both the central and peripheral nervous systems,^{8,17,34} we do not know whether the antibody formation originates from a central or a peripheral neural injury. The association between autonomic neuropathy and lowered body mass index (BMI), and the occurrence of GnRH antibodies in the initial ELISA,¹³ could not be confirmed. The present and former small studies do not allow a search for clinical associations with the antibody occurrence.^{3,14}

The effect of GnRH and LH on the ENS is not thoroughly evaluated, but seems to affect motility and secretion.^{9-11,35} No patient with diabetes mellitus in the present study, or primary Sjögren's syndrome in a previous study,¹⁴ expressed antibodies against LH or its receptor, as has previously been described in patients with infertility.¹⁶ Autoantibodies against follicle-stimulating hormone (FSH), LH, and ovarian factors have also been found by others in infertile women, and could indicate an autoimmune disorder targeting the ovary.³⁶ This suggests that antibodies against LH or its receptor are more frequent in gynecological disorders than in gastrointestinal disorders. Nevertheless, the presence of LH receptors in genital organs and the gastrointestinal tract^{7,8,34} could be a plausible explanation for the observed connection between dysfunction of the digestive tract and diseases of the genital organs in women.³⁷⁻³⁹



In conclusion, we confirm previous results that IgM antibodies against GnRH1 are elevated in serum in patients with IBS, dysmotility, and/or diabetes mellitus compared to controls. Furthermore, also IgM antibodies against progesterone and GnRH receptors are elevated in these patients, whereas IgG antibodies against these peptides, or antibodies against LH or LH receptor, are not present. It remains to explain the mechanisms behind the antibody formation and to examine whether the gastrointestinal complaints, the autonomic neuropathy, or the psychological factors are associated with the formation.

Abbreviations

CIPO, Chronic intestinal pseudo-obstruction; ED, Enteric dysmotility; ENS, Enteric nervous system; GnRH, Gonadotropin-releasing hormone; IBS, Irritable bowel syndrome; IVF, In vitro fertilization; LH, Luteinizing hormone.

Author Contributions

Conceived and designed the experiments: BR, KB, and BO. Analyzed the data: BR and BO. Wrote the first draft of the manuscript: BO. Contributed to the writing of the manuscript: BR and KB. Agree with manuscript results and conclusions: BR, KB, and BO. Jointly developed the structure and arguments for the paper: BR, KB, and BO. Made critical revisions and approved final version: BR, KB, and BO. All authors reviewed and approved of the final manuscript.

REFERENCES

- Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology*. 2006;130:1480–1491.
- Ohlsson B, Melander O, Thorson O, Olsson R, Ekberg O, Sundkvist G. Oesophageal dysmotility, delayed gastric emptying and autonomic neuropathy correlate to disturbed glucose homeostasis. *Diabetologia*. 2006;49:2010–2014.
- Ohlsson B, Scheja A, Janciauskiene S, Mandl T. GnRH-antibodies and gastrointestinal complaints in patients with Sjögren's syndrome and progressive systemic sclerosis. *Scand J Rheumatol*. 2009;23:1–2.
- Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol*. 2014;6:71–80.
- Ohlsson B, Veréss B, Janciauskiene S, Montgomery A, Haglund M, Wallmark A. Chronic intestinal pseudo-obstruction due to busserelin-induced formation of anti-GnRH antibodies. *Gastroenterology*. 2007;132:45–51.
- Hammar O, Ohlsson B, Veréss B, Nordin Fredrikson G, Alm R, Montgomery A. Depletion of enteric gonadotropin-releasing hormone is found in a few patients suffering from severe gastrointestinal dysmotility. *Scand J Gastroenterol*. 2012;47:1165–1173.
- Hammar O, Veréss B, Montgomery A, Ohlsson B. Expression of luteinizing hormone receptor in the gastrointestinal tract in patients with and without dysmotility. *DTI*. 2012;6:13–18.
- Sand E, Bergvall M, Ekblad E, D'Amato M, Ohlsson B. Expression and distribution of GnRH, LH, and FSH and their receptors in gastrointestinal tract of man and rat. *Regul Pept*. 2013;10:24–28.
- Khanna R, Browne RM, Heiner AD, Clench MH, Mathias JR. Leuprolide acetate affects intestinal motility in female rats before and after ovariectomy. *Am J Physiol*. 1992;262:G185–G190.
- Mathias JR, Baskin GS, Reeves-Darby VG, Clench MH, Smith LL, Calhoon JH. Chronic intestinal pseudoobstruction in a patient with heart-lung transplant. Therapeutic effect of leuprolide acetate. *Dig Dis Sci*. 1992;37:1761–1768.
- Ducker TE, Boss JW, Altug SA, et al. Luteinizing hormone and human chorionic gonadotropin fragment the migrating myoelectric complex in rat small intestine. *Neurogastroenterol Motil*. 1996;8:95–100.
- Ohlsson B, Sjöberg K, Alm R, Nordin Fredrikson G. Patients with irritable bowel syndrome and dysmotility express antibodies against gonadotropin-releasing hormone in serum. *Neurogastroenterol Motil*. 2011;23:1000–1006.
- Berntorp K, Frid A, Alm R, Fredrikson GN, Sjöberg K, Ohlsson B. Antibodies against gonadotropin-releasing hormone (GnRH) in patients with diabetes mellitus is associated with lower body weight and autonomic neuropathy. *BMC Res Notes*. 2013;6:329.
- Mandl T, Roth B, Ohlsson B. Antibodies against GnRH and its receptor in patients with primary Sjögren's syndrome. *Scand J Rheumatol*. 2014;43:338–348.
- Roth B, Ohlsson B. Gastrointestinal symptoms and psychological well-being in patients with microscopic colitis. *Scand J Gastroenterol*. 2013;48:27–34.
- Hammar O, Roth B, Bengtsson M, Mandl T, Ohlsson B. Autoantibodies and gastrointestinal symptoms in infertile women in relation to in vitro fertilization. *BMC Pregnancy Childbirth*. 2013;13:201.
- Pawson AJ, Morgan K, Maudsley SR, Millar RP. Type II gonadotropin-releasing hormone (GnRH-II) in reproductive biology. *Reproduction*. 2003;126:271–278.
- Collins PJ, Horowitz M, Cook DJ, Harding PE, Shearman DJ. Gastric emptying in normal subjects: a reproducible technique using a single scintillation camera and computer system. *Gut*. 1983;24:1117–1125.
- Hanson M, Lilja B. Gastric emptying in smokers. *Scand J Gastroenterol*. 1987;22:1102–1104.
- Castell JA, Dalton CB, Castell DO. On-line computer analysis of human lower esophageal sphincter relaxation. *Am J Physiol*. 1988;255:G794–G799.
- Abrahamsson H, Antov S, Bosaeus I. Gastrointestinal and colonic segmental transit time evaluated by a single abdominal X-ray in healthy subjects and constipated patients. *Scand J Gastroenterol*. 1988;23:72–80.
- Spechler SJ, Castell DO. Classification of oesophageal motility abnormalities. *Gut*. 2001;49:145–151.
- De Giorgio R, Sarnelli G, Corinaldesi R, Stanghellini V. Advances in our understanding of the pathology of chronic intestinal pseudo-obstruction. *Gut*. 2004;53:1549–1552.
- Wingate D, Hongo M, Kellow J, Lindberg G, Smout A. Disorders of gastrointestinal motility: towards a new classification. *J Gastroenterol Hepatol*. 2002;17(suppl):S1–S14.
- Faraj J, Melander O, Sundkvist G, et al. Oesophageal dysmotility, delayed gastric emptying and gastrointestinal symptoms in patients with diabetes mellitus. *Diabet Med*. 2007;24:1235–1239.
- Gustafsson RJ, Littorin B, Berntorp K, et al. Esophageal dysmotility is more common than gastroparesis in diabetes mellitus and is associated with retinopathy. *Rev Diabet Stud*. 2011;8:258–265.
- Cordeddu L, Bergvall M, Sand M, Roth B, Papadaki E, Li L, D'Amato M, Ohlsson B. Severe, gastrointestinal dysmotility developed after treatment with gonadotropin-releasing hormone analogs. *Scand J Gastroenterol*. In press 2014.
- Klooker TK, Braak B, Koopman KE, et al. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut*. 2010;59:1213–1221.
- Sand E, Voss U, Hammar O, et al. Gonadotropin-releasing hormone analog busserelin causes neuronal loss in rat gastrointestinal tract. *Cell Tissue Res*. 2013;351:521–534.
- De Giorgio R, Barbara G. Is irritable bowel syndrome an inflammatory disorder? *Curr Gastroenterol Rep*. 2008;10:385–390.
- Öhman L, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol*. 2010;7:163–173.
- Elsenbruch S, Rosenberger C, Enck P, Forsting M, Schedlowski M, Gizewski ER. Affective disturbances modulate the neural processing of visceral pain stimuli in irritable bowel syndrome: an fMRI study. *Gut*. 2010;59:489–495.
- Larsson MB, Tillisch K, Craig AD, et al. Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with irritable bowel syndrome. *Gastroenterology*. 2012;142:463.e–472.e.
- Naor Z. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. *Front Neuroendocrinol*. 2009;30:10–29.
- Soldani G, Del Tacca MM, Bambini G, et al. Effects of gonadotropin-releasing hormone (GnRH) on gastric secretion and gastrin release in the dog. *Drug Metab Dispos*. 1993;21:818–822.
- Meyer WR, Lavy G, DeCherney AH, Visintin I, Economy K, Luborsky JL. Evidence of gonadal and gonadotropin antibodies in women with a suboptimal ovarian response to exogenous gonadotropin. *Obstet Gynecol*. 1990;75:795–799.
- Wald A, Van Thiel DH, Hoehstetter L, et al. Gastrointestinal transit: the effect of the menstrual cycle. *Gastroenterology*. 1981;80:1497–1500.
- Mathias JR, Franklin R, Quast DC, et al. Relation of endometriosis and neuromuscular disease of the gastrointestinal tract: new insights. *Fertil Steril*. 1998;70:81–88.
- Gonenne J, Esfandyari T, Camilleri M, et al. Effect of female sex hormone supplementation and withdrawal on gastrointestinal and colonic transit in postmenopausal women. *Neurogastroenterol Motil*. 2006;18:911–918.