

Allergic Predisposition, Histamine and Histamine Receptor Expression (H1R, H2R) Are Associated with Complicated Courses of Sigmoid Diverticulitis

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Abstract

Background We aimed to evaluate our hypothesis that allergic predisposition and expression of histamine receptors might contribute to complicated courses of sigmoid diverticulitis.

Methods Expression of histamine and histamine receptors (H1R, H2R) was analysed on protein level (immunohistochemistry/immunofluorescence (IF)) as well as mRNA level (reverse transcription–PCR (RT-PCR) in surgical specimen of patients ($n=101$) having undergone resection for sigmoid diverticulitis ($n=57$ complicated diverticulitis/ $n=44$ non-complicated diverticulitis).

Results The mean number of comorbid diseases per patient was 1.76 ± 1.25 . Thirty-nine of 101 patients (38.6%) exhibited allergic predisposition (grass poll, food, drug, pets, etc.). Comorbid diseases were significantly associated with complicated diverticulitis ($p=0.027$). Complicated sigmoid diverticulitis was significantly associated with high H1R and H2R expression ($p<0.001$). Furthermore, an association of complicated diverticulitis with allergic predisposition was found (odds ratio=3.2, $p=0.0097$). IF double-labelling experiments showed a strong correlation of increased histamine expression with expression of H1R and H2R on intestinal enterocytes (histamine/H1R, $\rho=0.841$, $p<0.0001$ and histamine/H2R, $\rho=0.806$, $p<0.0001$). The results of increased H1R and H2R expression in complicated sigmoid diverticulitis were also detected on mRNA level in a subset of patients (RT-PCR, $p=0.009$).

Conclusions Our findings suggest that allergic predisposition might be another important risk factor for complicated courses of acute sigmoid diverticulitis and linked with histamine receptor expression. Supportive therapies with antihistaminic drugs might become an option. Allergic predisposition might be worth considering when indicating surgery for sigmoid diverticulitis.

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Introduction

Diverticulitis of the sigmoid colon is a very common condition in the Western world. Its increasing incidence is attributed to lifestyle issues and the ageing population. Different types of diverticulitis develop on the basis of asymptomatic diverticulosis, although the vast majority of patients with diverticulosis remain asymptomatic.^{1–4} Treatment of sigmoid diverticulitis is performed in a type-adjusted fashion. Sigmoid resection is performed for complicated types of acute diverticulitis. In most cases, a strategy with early elective resection after antibiotic pretreatment is currently chosen. On the other hand, sigmoid resections are also performed for chronically recurrent types of sigmoid diverticulitis. However, the former strategy of routine surgical resection after a second attack of sigmoid diverticulitis has been abandoned, and surgery is recommended based on risk evaluation.⁵ In this respect, immunosuppression, steroid intake, diabetes and collagen vascular diseases are well-recognized risk factors associated with complicated courses of acute diverticulitis.⁵

The underlying molecular mechanisms which lead to complicated diverticulitis types are not well understood as yet. Insights into the inflammatory process in sigmoid diverticulitis, leading to either complicated diverticulitis (with perforation) or chronically recurrent courses of the disease, would be of major interest. It is known that the pathophysiology of diverticular disease is complex and relates to abnormal colonic motility, changes in the colonic wall, mucosal inflammation, imbalance in colonic microflora and visceral hypersensitivity.⁶

Histamine occurs throughout the gastrointestinal tract, in enterochromaffin-like cells, restricted to the fundic mucosa of the stomach, mast cells, nerves and intestinal bacteria.⁷ Histamine is actively produced and released in enterochromaffin-like cells, harbouring the synthesis enzyme histidine decarboxylase, and is stored predominantly in mast cells. This enzyme is present in the intestinal mucosa of injured tissues as well.⁸

Histamine exerts its effects through H1, H2, H3 and H4 receptors. H1Rs are reported to be expressed on enterocytes, connective tissue cells, within the muscle layer, blood vessels, immune cells and ganglion cells of the myenteric plexus in the human intestine.⁹ H2Rs appear to be located on parietal cells in the fundic mucosa¹⁰ and have also been found in the intestinal epithelium, immune cells and myenteric ganglia in humans.⁹

Histamine regulates multiple functions at the gastrointestinal level. Contraction of intestinal smooth muscle and inflammatory mechanisms are described as one of the best characterized responses mediated by H1Rs. Throughout the gastrointestinal tract, contractile effects are also exerted on vascular smooth muscle and on endothelial cells, resulting in an increase in vascular permeability. H2Rs, located on parietal cells, are potent stimulants of gastric acid secretion and may inhibit contractile effects.¹¹ The role of H3Rs and H4Rs is less well defined.⁹

Our investigation presented herein deals with the hypothesis that there might be a link between allergic predisposition, histamine and histamine receptors (H1R, H2R) and complicated types of sigmoid diverticulitis. We regarded this hypothesis as promising due to the established role of histamine receptors with respect to intestinal barrier function.¹² This barrier function might be important for sigmoid diverticulitis as well, and histamine release might contribute to the massive inflammation seen in complicated types of diverticulitis.⁸

Patients and Methods

Patients and Distinction of Groups

We analysed a prospectively documented consecutive patient series ($n=101$) having undergone resection for sigmoid diverticulitis between January 1, 2008 and December 31, 2009. Patient characteristics and comorbidities were collected in a prospectively maintained database (EXCEL, Microsoft). Patients were divided into two groups (group A: complicated diverticulitis; group B: non-complicated diverticulitis).

Patients in *group A* ($n=57$) were operated for *complicated diverticulitis types*: there were 42 cases with ‘covered perforation’ (43%), 3 cases with ‘free perforation’ (3%) and 12 cases with ‘phlegmonous diverticulitis’ (12%). ‘Phlegmonous diverticulitis’ was characterized by histopathologic confirmation of a severe inflammatory infiltrate, which had not vanished due to antibiotic pretreatment, but without evidence of covered or free perforation. Surgery for complicated diverticulitis was performed as early elective resection, after 10 days of pretreatment with antibiotics (cephalosporine, metronidazole). Only the three cases with ‘free perforation’ presented with acute abdomen and underwent emergency surgery.

Patients in *group B* ($n=44$) were patients with *non-complicated diverticulitis*, who were either operated for chronically recurrent diverticulitis ($n=41$) or uncomplicated diverticulitis ($n=3$). The operation in the latter three patients with uncomplicated diverticulitis was indicated due to the risk profile of the patients with comorbid diseases and immunosuppression. Patients with chronically

recurrent diverticulitis underwent surgery because of the clinical course with more than one prior episode of diverticulitis. Histopathologic work-up of specimen in group B revealed scarring of the colonic wall, fibrosis, but no intensive infiltration with inflammatory cells.

The mean age of the whole population was 59 ± 12.8 years (group A, 56.9 ± 13.2 years; group B, 61.5 ± 12.1 years). Patients' characteristics and comorbid diseases are described in Table 2. Patients had given their informed consent that the surgical specimen retrieved during their operations could be used for research purposes like this current investigation, which is according to the rules of the local ethics committee.

Definition of Allergic Predisposition

Allergic predisposition of all patients is documented prospectively during routine clinical work-up. We gathered all prospectively documented information from the hospital's electronic patient chart. In detail, information about prevalence of allergies was documented—by the surgeon, during “physical and history”—by the anaesthesiologist, during “premedication visit”—by the nurse in the “central patient management” as well as the nurse at the ward. All these sources of prospectively documented information were exploited for our investigation. Although some patients had undergone testing for allergies and owned an allergy pass, this was only a minority. Testing for allergies was not part of this investigation, and information on allergic predisposition was mainly based on history.

Postoperative Pathologic Work-up and Staining Procedure

Immediately after retrieval, the specimens were fixed in 4% formalin solution for at least 8 h. Subsequently, the specimens were inspected macroscopically and cut into slices of 3–4 mm thickness. Representative layers according to the macroscopic findings were selected in a standardized fashion. Diverticula of varying depth and inflammatory alterations were selected. Tissue sections were embedded in paraffin and cut into 2- μ m thin sections using a standard microtome. Sections underwent standard processing and haematoxylin–eosin (H&E) staining. Histopathologic analysis was performed by two experienced gastrointestinal pathologists on routine H&E-stained sections (2 μ m) with special focus on stromal areas and infiltrating immune cells.

Immunolabelling against H1R (Santa Cruz, CA, USA), H2R (Acris Antibodies, Heford, Germany) and histamine (Biomol, Hamburg, Germany) was performed in additional serial sections of 2 μ m thickness. Normal colon tissues from healthy individuals served as controls ($n=10$). These were patients who had undergone colon resection for

noninflammatory diseases, especially colon cancer specimen. The tissue was taken from the sigmoid colon, where no inflammation and no tumour were present.

Serial sections were deparaffinized in xylene and ethanol and rehydrated in water. Heat-induced epitope retrieval (HIER) was performed with citrate buffer, pH 6.0 (DAKO, Hamburg, Germany). Slides were then incubated in normal serum (2%) and bovine serum albumin (0.5% BSA) at room temperature for 20 min to block nonspecific binding. For immunofluorescence (IF) double staining, slides were incubated with the histamine antibody or control antibodies overnight at 4°C in a humidified chamber and with secondary Cy3-conjugated AffiniPure donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories Inc., Suffolk, England, 1:200 dilution) for 30 min at room temperature. The slides were incubated with the second primary antibody H1R or H2R diluted in TBS plus 0.5% BSA overnight at 4°C, followed by incubation with the secondary fluorescein isothiocyanate (FITC)-conjugated AffiniPure donkey anti-goat IgG (Jackson ImmunoResearch, 1:200 dilution, 30 min, room temperature). Slides were then counterstained with DAPI (4',6-diamidino-2-phenylindoldihydrochlorid, Sigma-Aldrich), covered with polyvinyl alcohol mounting medium (DABCO, Sigma-Aldrich) and analysed using a Zeiss camera (Jena, Germany). The photographed images were processed using the Metamorph software package (Visitron Systems, Puchheim, Germany).

For immunohistochemistry (IHC), the pretreatment procedure (fixation, deparaffinization, rehydration, HIER and blocking) of the slides was the same as described for IF. A four-step immunoperoxidase labelling for single antigens in formalin-fixed paraffin-embedded sections was used as previously described.¹³

Quantification of Immunohistochemistry and Immunofluorescence

H1R, H2R and histamine IHC quantification was performed, analysing six representative individual high-power fields ($\times 400$) for each sample. Quantification was done by means of cell counting as previously described.¹⁴

Real-Time Quantitative Reverse Transcription–PCR Analysis

Using reverse transcription–PCR (RT-PCR), we examined a subset of samples ($n=10$) regarding H1R and H2R expression on mRNA level. To analyse gene expression of H1R and H2R by RT-PCR in a formalin-fixed, paraffin-embedded (FFPE) tissue, we extracted total cellular RNA and performed cDNA synthesis using the Absolutely RNA FFPE Kit and the AffinityScript QPCR cDNA Synthesis Kit (Stratagene, Waldbronn, Germany). Areas of interest for

each tissue section underwent manual microdissection. For both groups (complicated and uncomplicated diverticulitis), equal amounts of tissue areas were assessed ($2 \times 1.5 \text{ cm}^2$ surface area per section, thickness of $10 \text{ }\mu\text{m}$). The primer sequences used for RT-PCR are given in Table 1. Matched human colon cDNA was purchased by BioChain (Hayward, CA, USA) as control. The reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for relative quantification and cDNA quality control. RT-PCR reactions were carried out as described previously.¹⁴ The relative quantification value, fold difference, is expressed as $2^{-\Delta\Delta C_t}$.

Statistical Analysis

Statistical analysis was performed with MedCalc Software, version 11.4.2 (Mariakerke, Belgium). The demographic characteristics, baseline clinical parameters and outcomes of patients in the two groups were summarized and compared statistically. All values were expressed as median $\pm$ interquartile range (IQR) because the D'Agostino–Pearson test did not show a normal distribution of gene and protein expression. Therefore, the median of H1R and H2R expression levels was chosen to divide patients into two different groups with high versus low expression. Median cutoff value for either high or low expression was set at 42% for H1R and 26% for H2R expression in all cases ($n=101$). Cases with less than 10% positive cells were regarded as negative according to normal tissue expression results. Correlation between two variables was estimated based on the Spearman correlation coefficient. Pearson's chi-square test analysis or Fisher's exact test was used to investigate the relation between two categorical variables when necessary and appropriate. Odds ratios (ORs) and exact two-sided confidence intervals were used to estimate effect size of the risk factor. Data were analysed using the Student's *t* test or the Mann–Whitney *U* test when appropriate. *P* values of less than 0.05 were regarded as statistically significant.

Results

The mean number of comorbid conditions per patient was 1.76 ± 1.25 , with cardiovascular disease (hypertension/coronary artery disease) representing the most common

comorbidity (59%, see Table 2). Prevalence of comorbid diseases was significantly associated with complicated diverticulitis (group A, 54 of 57, 95% versus group B, 35 of 44, 80%, $p=0.028$).

Thirty-nine patients (39% of the whole population) showed allergic predisposition (grass poll, food, drug, pets, etc.). Allergic predisposition was associated with the probability of presenting with complicated diverticulitis (OR=3.2; 95% confidence interval [CI], 1.3271–7.8239, $p=0.0097$). Of these 39 patients with allergic predisposition, 29 patients presented with complicated diverticulitis (group A). Among these 39 patients, 23 patients had sigmoid diverticulitis with covered perforations, 4 had phlegmonous diverticulitis (with intensive inflammation, despite antibiotic pretreatment, but without evidence of perforation) and 2 patients presented with free perforation.

H1R and H2R Expression (IHC)

Normal colon epithelium (used as control) showed weak expression of H1R and H2R (each <10%). No expression of H1R and H2R was found in stromal cells. H1R and H2R expression in colon epithelium and lamina propria stromal cells of non-complicated diverticulitis (group B) was slightly increased compared with normal colon epithelium ($x < 10\%$). A strong expression of H1R and H2R was found in colon epithelium in complicated diverticulitis (group A), characterized by massive inflammatory infiltration (see Fig. 1).

Increased expression of H1R was demonstrated with IHC on intestinal enterocytes of 83 specimens (82.2%) of the whole population. There was a statistically nonsignificant difference between H1R expression in complicated diverticulitis (group A, $n=57/57$, 100.0%) and non-complicated diverticulitis (group B, $n=26/44$, 59.1%, $p=0.09$).

H2R was expressed on intestinal enterocytes in 80% ($n=81/101$) of the patients. There was a statistically nonsignificant difference between H2R expression in complicated diverticulitis (group A, $n=56/57$, 98.2%) and non-complicated diverticulitis (group B, $n=25/44$, 56.8%, $p=0.08$). Only a weak expression of H1R and H2R expression was found within the lamina propria stromal cells (Fig. 1c, e).

Quantification of H1R and H2R expression by means of cell counting did also show increased H1R and H2R expression in complicated diverticulitis (group A) in comparison to non-complicated diverticulitis (group B; Table 3 and Fig. 1).

Table 1 Primer sequences used for RT-PCR

Gene	Forward primer	Reverse primer
H1R	5'-AGCCTCTCGGTGGCGGACTT-3'	5'-GAGGCAGAGAGGACGGCCCA-3'
H2R	5'-CACCGTGGTCCCTTGCGGTCC-3'	5'-AGCACCAGGAGGCCGAGGAG-3'
GAPDH	5'-ATCCCATCACCATCTTCCAGG-3'	5'-CGCCCCACTTGATTTTGG-3'

Table 2 Patient characteristics

Characteristic	Whole population <i>n</i> =101	Group A (complicated) <i>n</i> =57	Group B (non-complicated) <i>n</i> =44	<i>p</i>
Age (mean±SD)	59±12.8 years	56.9±13.2 years	61.5±12.1 years	0.075
Gender				0.548
Male	50 (50%)	30 (53%)	20 (45%)	
Female	51 (50%)	27 (47%)	24 (54%)	
Comorbidities				0.028*
No	12 (12%)	3 (5%)	9 (20%)	
Yes	54 (53%)	54 (95%)	35 (80%)	
Cardiovascular disease	61 (60%)	38 (67%)	23 (52%)	0.156
Pulmonary disease	14 (14%)	12 (21%)	2 (5%)	0.020*
Endocrine disease	31 (31%)	18 (32%)	13 (30%)	1.000
Rheumatic disease	1 (1%)	1 (2%)	0 (0%)	1.000
Dermatologic diseases	5 (5%)	4 (7%)	1 (2%)	0.383
Renal disease	14 (14%)	9 (16%)	5 (12%)	0.575
Haematological diseases	8 (8%)	5 (9%)	3 (7%)	1.000
Digestive disease	18 (18%)	8 (14%)	10 (23%)	0.300
Infectious diseases	1 (1%)	0 (0%)	1 (2%)	0.435
Smoking	11 (11%)	9 (16%)	2 (5%)	0.169
Alcohol (abuse)	7 (7%)	4 (7%)	3 (7%)	0.532
Allergic predisposition	39 (39%)	29 (51%)	10 (23%)	0.0097

**p*<0.05, significant, Fisher's exact test

Correlation of H1R and H2R with Histamine Expression (Protein Level)

To analyse whether histamine might exert its effects through H1R and H2R for the suggested pathophysiological mechanism of inflammation, we examined the correlation of H1R and H2R with tissue histamine expression. We stratified H1R and H2R into two different groups according to percentages expression of immunohistochemical analysis in serial sections. H1R and H2R expression had a strong direct correlation with histamine expression (histamine/H1R, $\rho=0.840$, $p<0.0001$; 95% CI, 0.772–0.890 and histamine/H2R, $\rho=0.842$, $p<0.0001$; 95% CI, 0.744–0.891%; Fig. 2a, b). Furthermore, there was a strong positive correlation between H1R and H2R expression (H1R/H2R, $\rho=0.828$, $p<0.0001$; 95% CI, 0.755–0.881%). Immunofluorescence double labelling showed expression of H1R (Fig. 2a, c) and H2R (data not shown) with histamine in colon mucosa cells, as indicated by correlation analysis.

H1R and H2R Gene Expression (mRNA Level)

H1R as well as H2R gene expression was significantly higher in complicated diverticulitis (group A) in comparison to non-complicated diverticulitis (group B, each $p=0.009$, see Table 4 and Fig. 1f).

Correlation of H1R and H2R Expression with Comorbid Diseases and Allergic Predisposition

Correlation of comorbid diseases and allergic predisposition with high versus low H1R and H2R expression was evaluated (see Table 5). Allergic predisposition was shown to be associated with an increased probability of high H1R and H2R expression (H1R: OR, 3.06; 95% CI, 1.303–7.187, $p=0.010$; H2R: OR, 2.92; 95% CI, 1.254–6.786, $p=0.013$). High H1R and H2R expression was associated with the probability of presenting with complicated diverticulitis (H1R: OR, 11.51; 95% CI, 4.504–29.403, $p<0.0001$; H2R: OR, 7.69; 95% CI, 3.143–18.797, $p<0.0001$). Comorbidities were associated with the probability of high H1R expression (H1R: OR, 6.158; 95% CI, 1.258–30.145, $p=0.025$).

Discussion

Immunosuppression and especially steroid intake, diabetes and collagen-vascular disease have previously been recognized as risk factors for complicated courses of sigmoid diverticulitis since the seminal work by Jennifer Chapman from the Mayo Clinic.⁵ Our data suggest that allergic predisposition can also be regarded as a relevant risk factor in this respect. We have shown that there is a higher

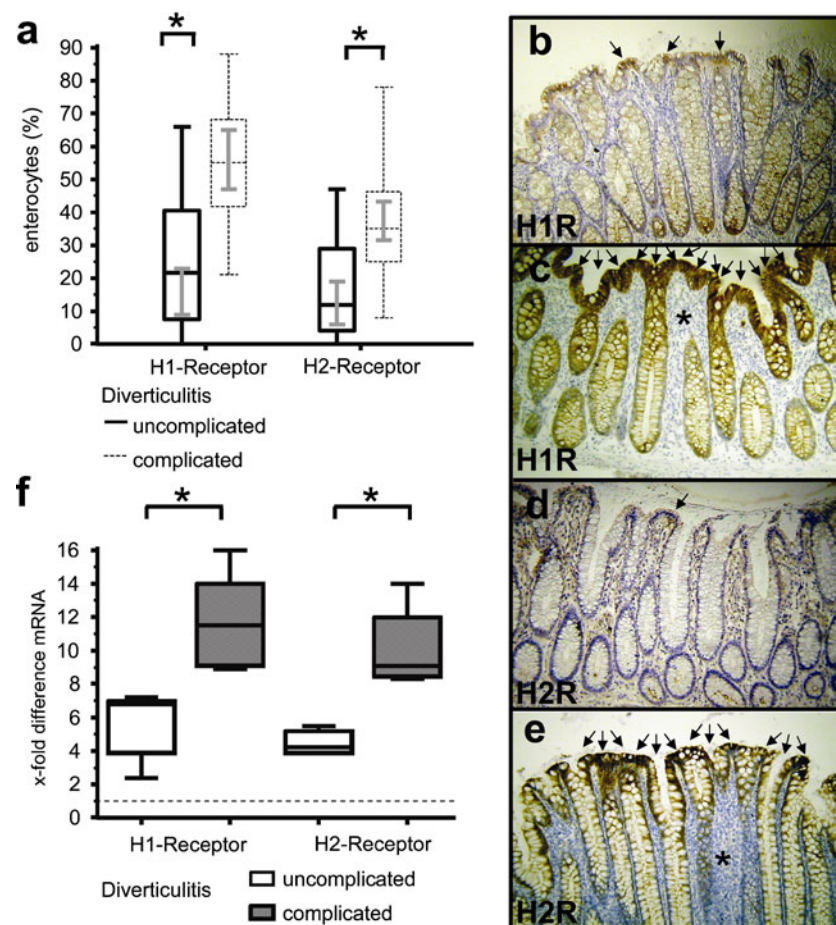


Fig. 1 Immunohistochemical analysis, staining of H1R and H2R and gene expression in sigmoid diverticulitis. Compared to non-complicated diverticulitis (group B), H1R and H2R expression is significantly (each $p < 0.0001$) upregulated in complicated diverticulitis (group A). Analysis refers to percentages of positivity of all counted cells by immunohistochemistry. Statistically significant values are indicated with *asterisks*. **a** Immunohistochemical stainings show representative expression (membranous staining pattern, *brown*) of H1R (**b, c**) and H2R (**d, e**) in intestinal enterocytes (*arrows*) of

complicated (group A) (**c, e**) and non-complicated (group B) (**b, d**) diverticulitis. *Asterisks* show increased infiltrating leucocytes due to complicated cases (**c, e**). Original magnification, $\times 100$. H1R and H2R gene expression in non-complicated diverticulitis (group B, $n=5$) is significantly (each $p=0.009$) lower in comparison to complicated diverticulitis (group A, $n=5$). Normal tissue is considered as onefold (*dotted line*). These results confirm increased H1R and H2R expression as observed by immunohistochemistry (**f**)

prevalence of allergies among patients with complicated sigmoid diverticulitis compared to patients with non-complicated diverticulitis.

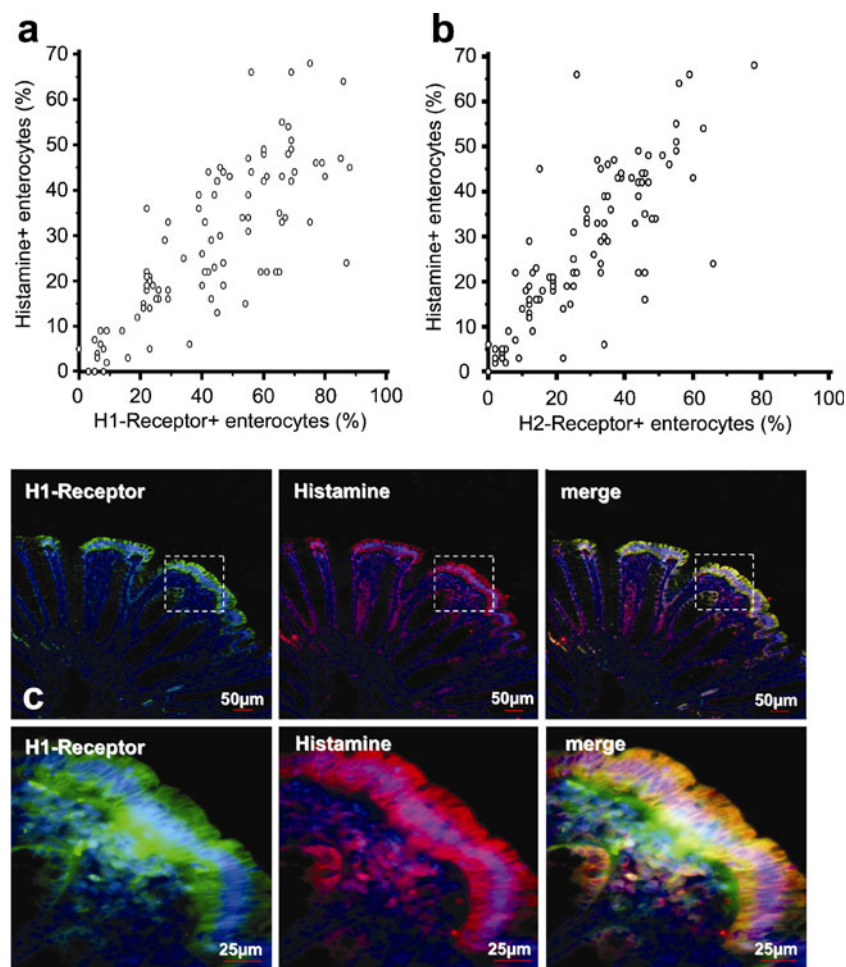
We have also shown that histamine and H1R and H2R expression in enterocytes of the colon mucosa are increased on protein as well as mRNA level. This remarkable association suggests a functional relationship.

In the Western world, the prevalence of allergies has increased two- to threefold over the past 40 years. There is an estimated 30% to 50% prevalence of allergies (including hay fever, drug and food allergy) in the general population.^{15,16} The 39% prevalence of allergies in our study population is within this range. However, the proportion of patients with allergies was

Table 3 Quantification of H1R and H2R expression (immunohistochemistry)

	Group A (complicated) $n=57$			Group B (non-complicated) $n=44$			p
	Median (%)	IQR (%)	95% CI (%)	Median (%)	IQR (%)	95% CI (%)	
H1R	55.0	41.8–68.3	47.0–65.0	21.5	7.5–40.5	9.0–23.0	$p < 0.0001$
H2R	35.0	25.0–46.3	31.6–43.4	12.5	4.0–29.0	6.0–19.0	$p < 0.0001$

Fig. 2 Correlation analysis and immunofluorescence double staining. Correlation analysis based on quantified immunohistochemical staining results of H1R and H2R in diverticulitis with histamine shows that H1R (a) and H2R (b) expression has a strong direct correlation with expression of histamine (histamine/H1R, $\rho=0.840$, $p<0.0001$ and histamine/H2R, $\rho=0.842$, $p<0.0001$). Immunofluorescence double staining (c) shows H1R with histamine coexpressing intestinal enterocytes in complicated diverticulitis (group A). (FITC) green fluorescein isothiocyanate, Cy3 red, and (DAPI) 4',6-diamidino-2-phenylindol dihydrochloride blue. Top, calibration bar represents 50 μm . Bottom, calibration bar represents 25 μm . The square box at the top demonstrates the area which is also shown in larger magnification at the bottom



higher among patients undergoing surgery for complicated diverticulitis types.

We were furthermore able to show that overexpression of H1R and H2R was associated with pulmonary disease such as allergic asthma which may be mediated by histamine.¹⁷ Histamine is known as a regulator of gastrointestinal functions, such as gastric acid production, intestinal motility and mucosal ion secretion. In humans, expression and distribution of histamine receptors within the human gastrointestinal tract are known to be rare.^{9,17}

An impaired histamine metabolism has been suggested to play an important role in the pathogenesis of inflammatory and neoplastic diseases such as Crohn's disease,¹⁸

ulcerative colitis,¹⁹ allergic enteropathy,²⁰ food allergy²¹ as well as colorectal neoplasms.²² Our results of increased H1R and H2R expression within intestinal enterocytes are in accordance to a previous study where analysis of endoscopic biopsies from patients with food allergy and irritable bowel syndrome revealed significantly elevated H1R and H2R mRNA levels compared with healthy controls. Immunohistochemistry showed expression of both receptors by epithelial cells as well.⁹ Based on expression analysis of H1R and H2R, we confirmed expression of these receptors in the gastrointestinal tract.⁹

It is well known that histamine is involved in the regulation of intestinal secretion and motility processes,

Table 4 H1R and H2R gene expression (mRNA level)

	Group A (complicated) $n=5$	Group B (non-complicated) $n=5$
H1R	Median 6.8-fold difference compared to normal tissue IQR 3.9–6.9-fold difference	Median 11.5-fold difference compared to normal tissue IQR 9.1–13.9-fold difference
H2R	Median 4.2-fold difference compared to normal tissue IQR 3.9–5.2-fold difference	9.1-fold difference compared to normal tissue IQR 8.5–11.9-fold difference

Table 5 Correlation of low versus high H1R and low versus high H2R expression (as quantified by the number of positive cases) with patient characteristics and comorbid diseases

Characteristic	H1R low (<42%) (n=47)	H1R high (≥42%) (n=54)	p value	H2R low (<26%) (n=49)	H2R high (≥26%) (n=52)	p value
Age (mean)			0.315			0.071
≤59 years	18 (38%)	27 (50%)		17 (35%)	28 (54%)	
>59 years	29 (62%)	27 (50%)		32 (65%)	24 (46%)	
Gender			1.000			0.692
Male	23 (49%)	27 (50%)		23 (47%)	27 (52%)	
Female	24 (51%)	27 (50%)		26 (53%)	25 (48%)	
Comorbidities			0.022*			1.000
No	9 (19%)	2 (4%)		6 (12%)	5 (10%)	
Yes	38 (81%)	52 (96%)		43 (88%)	47 (90%)	
Cardiovascular disease	25 (53%)	36 (67%)	0.225	30 (61%)	31 (60%)	1.000
Pulmonary disease	2 (4%)	12 (22%)	0.009*	4 (8%)	10 (20%)	0.147
Endocrine disease	13 (28%)	18 (32%)	0.666	15 (31%)	16 (31%)	1.000
Rheumatic disease	0 (0%)	1 (2%)	1.000	0 (0%)	1 (2%)	1.000
Dermatology disease	1 (2%)	4 (8%)	0.370	1 (2%)	4 (8%)	0.362
Nephrology disease	4 (8%)	10 (19%)	0.247	4 (8%)	10 (20%)	0.149
Haematological diseases	2 (4%)	6 (11%)	0.281	3 (6%)	5 (10%)	0.715
Digestive disease	9 (19%)	9 (8%)	0.796	9 (18%)	9 (17%)	1.000
Infection disease	0 (0%)	1 (17%)	1.000	0 (0%)	1 (2%)	1.000
Allergic predisposition	12 (25%)	27 (50%)	0.013*	13 (27%)	26 (50%)	0.014*
Smoking	5 (11%)	6 (11%)	0.556	2 (4%)	9 (17%)	0.029*
Alcohol (abuse)	5 (11%)	2 (4%)	0.323	5 (10%)	2 (4%)	0.172
Complicated diverticulitis	13 (28%)	44 (81%)	<0.001*	16 (33%)	41 (79%)	<0.001*

* $p < 0.05$, significant, Fisher's exact test

dysregulation of which may cause clinical symptoms such as diarrhoea and abdominal pain.²³ Therefore, we were interested to know if histamine receptor expression might be altered in patients with diverticulitis. Increased H1R and H2R in patients with complicated diverticulitis may be related to the irritable bowel syndrome (IBS)-like symptoms which these patients are frequently suffering from.²⁴ A broad spectrum of intestinal symptoms that can be evoked by histamine has been described.²⁵ This histamine intolerance was attributed to impaired histamine metabolism.^{26,27} Because of the multifaceted symptoms, the existence of histamine intolerance is frequently underestimated, or its symptoms are misinterpreted. Moreover, histamine intolerance is seldom accompanied with allergic disorders at all. Alternatively, such symptoms could be a result of elevated histamine receptor expression, as found in patients of our cohort and in patients with IBS and food allergies (FA)⁹ which—similar to reduced histamine degradation—may cause histamine hypersensitivity. This hypothesis needs to be confirmed by showing that not only diverticulitis but also other gastrointestinal diseases like FA and IBS characterized by secretory and motility disorders

improve by treatment with antihistamines, a histamine-free diet and a supplementary therapy. Diamine oxidase (DAO) is the main enzyme for the metabolism of histamine. Supplementation of this enzyme or related cofactors like vitamin B6, copper and vitamin C, which are thought to support histamine degradation,²⁸ have been discussed in this respect.²⁹

From our results, we are unable to exactly define the origin of histamine found in the colonic wall. Several sources such as the intestinal mucosa of injured tissues⁸ or intestinal bacteria^{6,7} may be considered. Indeed, the pathophysiology of diverticular disease is complex and relates to a lot of systemic and local changes such as acute and chronic mucosal inflammation as well as imbalance in colonic microflora.^{6,24} By means of co-labelling experiments, we have been able to show co-localization of histamine with H1R and H2R. This may indicate that histamine signalling is responsible for the massive inflammation seen in complicated diverticulitis. However, the functional relationship needs to be substantiated in future studies.

Finally, histamine receptor signalling could play an important role during pathogenesis of complicated sigmoid

diverticulitis in terms of damaging the intestinal barrier function.¹² Experimental studies regarding this intestinal barrier function in complicated sigmoid diverticulitis using antihistaminic drugs might be promising. However, further research would be required to support such a strategy.

Our findings suggest that allergies, histamine and histamine receptor expression (H1R and H2R) should be considered in future molecular and clinical investigations regarding sigmoid diverticulitis. Some lines of evidence are already available regarding the importance of histamine intolerance,¹⁷ food allergies³⁰ and histamine-producing intestinal microflora,²⁴ which should be taken into account during further research regarding sigmoid diverticulitis.

The most important aspect for the surgeon is that allergic predisposition might be worth considering—similar to immunosuppression and steroid intake⁵—when indicating surgery for sigmoid diverticulitis.

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Discussant

Dr. Kirk A. Ludwig (Milwaukee, WI): First, let me say thank you for trying to bring some science to the study of diverticular disease. The clinical and basic science literature is generally not very good, and there are far too many knowledge gaps for what is such a common condition.

Diverticulitis is a disease of Western society with an increase in incidence seen as the first cohort of children raised on white flour and refined sugar came of age about 40 years after the start of the Industrial Revolution. Clearly, there is a dietary relationship, and there may also be an environmental relationship that may coincide with the two- to threefold increase in environmental allergies seen in the last 40 years. Perhaps, there is some common mechanism at work here.

My major concern with this study is whether what we see in terms of the strong H1R and H2R expression in the complicated diverticulitis patients by both IHC and RT-PCR is not simply a result of the intense inflammation seen in this group of patients as opposed to the cause of the intense inflammation.

Is it not possible that the patients in the non-complicated group might at some point have been part of the complicated group, and given sufficient treatment, they simply recovered and became part of the chronic symptom, but not terribly inflamed non-complicated group? If so, then finding high histamine receptor levels in the complicated group may simply be a result of the inflammation, not the cause of the inflammation.

Finally, how exactly was “allergic predisposition” defined and how was it determined? This assessment could be biased if it was made retrospectively. How does one define and document what many people would not think twice about... an environmental allergy? These are just so common, as to barely warrant mentioning as part of a medical history? Also, were the investigators blinded as to the group designation when examining the IHC slides so that bias was avoided?

Closing Discussant

Dr. Burkhard H. A. von Rahden: Thank you very much for your kind acknowledgement of our efforts regarding sigmoid diverticulitis. We totally agree with you that diverticulitis is somehow a “neglected disease”—and an incoming congress scheduled for autumn in Cologne/Germany is going to cover this interesting topic subsumed under this title: “Diverticulitis—a fresh approach to a neglected

disease”. Many questions are open regarding the appropriate conservative and surgical treatment and about the underlying molecular mechanisms of the inflammatory process and its implications for clinical practice. Initiating this work about histamine metabolism in this context, we found it rather astonishing that there are no data regarding this issue in diverticulitis—although the histamine story is already ongoing for some time.

Regarding your question concerning the cause-and-effect chain and whether histamine receptor expression seen on enterocytes in complicated diverticulitis might be merely epiphenomenalistic, actually, we cannot exclude this from our current data. Larger and preferably prospectively arranged investigations should answer this question. However, our results of H1R and H2R expression on protein and mRNA level suggest that it is definitely worth to take a closer look and initiate more research. The reason why this is appealing is the vast variety of potential treatment options in the modulation of histamine metabolism—e.g. with antihistaminics, supplementation of DAO, probiotics, vitamins A and D, etc.). Even if histamine receptors might merely be a bystander, it could nevertheless be that targeting might have some effect in the modulation of the disease process.

The current data suggest that complicated diverticulitis and chronically recurrent diverticulitis are different types of the disease and explicitly *not* different stages of the disease. This view seems well supported by what we see in clinical practice and what can be derived from current literature. I would like to refer to the work by Jennifer Chapman from the Mayo Clinic, who published two important papers, one about complicated diverticulitis (*Ann Surg* 242(4):576–81) and one about chronically recurrent diverticulitis (*Ann Surg* 2006, 243(6):876–830). The major findings in these studies were that complicated diverticulitis occurs mostly during the first episode, whereas a chronically recurrent course of the disease is usually not associated with an increased risk of complicated courses. We have addressed diverticulitis as a complicated type when a covered or free perforation is proven and also when there is a severe inflammatory infiltrate, which did not disappear even after antibiotic pretreatment. The latter is addressed as phlegmonous diverticulitis. Chronically recurrent diverticulitis types—by contrast—are characterized by their clinical course, and on histology, one can see evidence of chronic scarring, but no severe inflammatory infiltrate.

Concerning the definition of “allergic predisposition” and the mode of acquisition of these data, the data regarding prevalence of allergies in these patients were actually gathered prospectively. We exploited several sources of prospectively documented data—by the anesthesiologist (during premedication visit)—by the nurses (during preclinical preparation of the patient and during admission to the ward)—and by the surgeon (during history taking and physical examination). We did not stratify our results regarding different types of allergies. We did not think that the number of patients in our study would allow us to go deeper into subgroup analysis. Investigators were indeed blinded during the analysis of the IHC slides, and correlation analyses were performed during work-up of the database.