Colonic wall changes in patients with diverticular disease — Is there a predisposition for a complicated course?

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**Article Info**

**Abstract**

**Background:** The aim of this study was to evaluate colonic wall changes and enteric neuropathy in patients with either uncomplicated (UDD) or complicated diverticular disease (CDD). Furthermore, we evaluated the presence of an anatomic sphincter at the rectosigmoid junction (RSJ).

**Methods:** Samples of colonic tissue from fifteen patients with UDD, fifteen patients with CDD and fifteen patients as control were collected. Collagen quotient I/III was measured with the Sirius-red test, expression of MMP-1, MMP-13, innervation (S100), proliferation (Ki67) and apoptosis (TUNEL) in the colonic wall were investigated by immunohistochemical studies. Furthermore, measurements of the different layers were performed to investigate the RSJ.

**Results:** Patients with either UDD or CDD had lower collagen I/III quotients compared to the control group, significant for CDD (p = 0.007). For MMP-1 and MMP-13 only a slight increase for patients with CDD was found. The percentage of proliferating (Ki67) and apoptotic (TUNEL) cells was significantly higher for patients with CDD than in the control group (p = 0.016; p = 0.037). Upon investigating the S100-expression a significant reduction in glial cells density was found in the myenteric and mucosal plexus for both groups (UDD and CDD) compared to the control group. Measurements of the different colon layers oral, aboral and at the RSJ revealed equal values.

**Conclusions:** This study has shown that colonic wall changes and enteric neuropathy seem to play a role in the pathogenesis of colonic diverticulosis. None of our results suggest a predisposition for a complicated diverticular disease. Furthermore, the presence of an anatomic sphincter at the rectosigmoid junction could not be detected.

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**1. Introduction**

Diverticular disease of the colon is a common entity in the western world in the past century that affects one-third of people aged over 45 years and up to two-thirds of those older than 85 years [1]. Although the diverticular disease is a wide-spread gastrointestinal (GI) tract disease, the pathophysiology is not yet been completely understood. Different pathophysiological causes of colonic diverticulosis have been reported, from the epidemiological to the basic research level. Changes in diet [2], ageing population [3], structural changes in the colonic wall [4], a questionable sphincter at the rectosigmoid junction [5], enteric neuropathy [6] and disorders in colonic motility [7] seem to play a role in the formation of colonic diverticula. However, these previous studies have not distinguished between the uncomplicated or complicated disease.

The purpose of the present work was to examine the changes in the pathogenesis of colonic diverticular disease in respect to collagen metabolism, bowel innervation and cell proliferation. Moreover, we investigated whether these changes predispose to a complicated diverticular disease and evaluated the presence of an anatomic sphincter at the rectosigmoid junction.

**2. Material and methods**

All samples of colonic tissue analysed in the study were obtained from the Department of Surgery at the Technical University of Aachen. Patients gave written informed consent for participation in the trial. Fifteen specimens of patients without diverticulosis as control (median age 69.6 ± 8.6 years), specimens of fifteen patients with uncomplicated diverticulosis (median age 67.9 ± 7.7 years) and specimens of fifteen patients with complicated diverticulosis...
2.1. Sirius red and crosspolarisation microscopy

Furthermore, measurements of the different layers were performed. Samples for the complicated diverticular disease (UDD) specimens from patients operated during an inflammation-free interval by elective surgery were used for the analysis. Samples for the complicated group (CDD) were from those patients who underwent emergency surgery for acute diverticulitis with pericolic abscess or perforation presented with acute abdominal pain (Table 1).

The differentiation between collagen types I and III was measured by the Sirius-red test, expression of MMP-1, MMP-13, innervation, proliferation (Ki-67) and apoptosis (TUNEL) in the colonic wall were investigated by immunohistochemical studies. Furthermore, measurements of the different layers were performed to investigate the sphincter at the rectosigmoid junction.

2.2. Immunohistochemical studies

Immunohistochemical investigation was performed on paraffin embedded 3 μm sections using peroxidase conjugated, affinity-isolated goat anti-mouse immunoglobulins (1:50, Dako, Hamburg, Germany). All sections were routinely stained with haematoxylin and eosin (H&E) and processed at the same time to reduce internal staining variations. Briefly, immunohistochemistry was done according to the instructions of the manufacturer. Monoclonal rabbit anti-MMP-1 and -MMP-13 (1:1000, Biomol, Hamburg, Germany) were applied with heat exposure in a microwave. Each sample, 3 regions of interest in the submucosal layer were captured in a high power field (400×, area 100 μm x 100 μm) by a digital camera (Olympus C-3030, Hamburg, Germany). The degree of immunohistochemical staining was evaluated according to the immunoreactive score (IRS) by Remmele and Stegner [9]. Here, the percentage of positively stained cells and staining intensity were registered and each related to a defined score. Multiplication of both values resulted in the Remmele score. Microscopical analysis was performed with the help of a digital image analysing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA).

Ki-67 expression was investigated with a mouse monoclonal antibody MIB-1 from Dako (Glostrup, Denmark) and rabbit anti-mouse antibody, 1:300 from Dako (Glostrup, Denmark) as secondary antibody. Apoptotic cells were detected by the Apop Tag Peroxidase detection kit of Q-Biogene (TUNEL, Carlsbad, USA) according to the instructions provided by the manufacturer. Glial marker protein S100 detection was carried out by a 1:500 rabbit polyclonal antibody (Abcam, Cambridge, UK) pretreatment microwave one time, citrate-puffer pH 6, and as secondary antibody goat anti-rabbit 1:500 (Dako, Glostrup, Denmark).

Three well stained visual fields were randomly selected for each slide and the number of S100 positive cell was counted. For Ki67 and TUNEL extent of staining was scored according to the percentage of positive stained cells in the specimen (0–100%).

There were no significant differences (Fig. 3) between the groups for MMP-1 and MMP-13. However, a slight increase in MMP-1 and MMP-13 expression in patients with uncomplicated diverticular disease (UDD) was seen.
and apoptosis compared to the control group. No significant difference was found comparing UDD with control group and CDD regarding both Ki67 and TUNEL.

3.4. S100

A significant reduce in glial cell density (Fig. 5) was seen in both the myenteric and submucosal plexus for UDD and CDD compared to the control group.

3.5. Measurements of colonic wall at the rectosigmoid junction

Measurements of the different colon layers oral, aboral and at the rectosigmoid junction revealed equal values for all groups without significant differences comparing groups and localisations respectively (Fig. 6 and Tables 3 and 4).

4. Discussion

The prevalence of diverticular disease within our increasingly elder population is still high. Although new therapeutic strategies were developed and have led to a better patient outcome, the pathophysiology of both the uncomplicated and complicated disease still remains uncertain. Although still controversial [10], environmental and lifestyle factors like high fibre diet and physical activity seem to protect against diverticular formation [11,12]. It is well known that right-sided diverticular disease is more common (70%) in Asian people, while in the West it is nearly always left-sided (90%). Interestingly, in the Japanese Hawaiian community with western lifestyle the dominant site has remained right-sided [13], which invokes a possible hereditary component. Furthermore, some genetic syndromes are associated with diverticular disease even in adolescents. Patients with Ehlers–Danlos syndrome [14,15], Marfan syndrome [16] and Williams–Beuren syndrome [17,18] have a higher risk for diverticular deformations. In line with these findings, clinical case reports bring up familiar risk factors for diverticular disease in the general population [19]. Hence, investigations have focused on changes in the structure of colonic wall, motility and innervation in the last years [20].

4.1. Extracellular matrix

The extracellular matrix (ECM) with components such as collagen, elastin and proteoglycans plays an important role in the integrity, strength and flexibility of the intestinal wall. It could be shown that higher levels of elastin and “cross-links” might cause the rigidity and inflexibility of the colonic wall in patients with diverticular disease [4,21,22] as well as an imbalance of collagen types I and III [23]. Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases collectively capable of degrading all components of the extracellular matrix [24]. MMP-1 and -13 are the principal matrix enzymes cleaving particularly interstitial collagen types I and III [25]. Therefore disturbances in the collagen metabolism regulated by matrix metalloproteinases (MMPs) seem to be important in the pathogenesis for diverticular disease [26,27].

In light of this knowledge the purpose of this study was to determine ultrastructural changes in the colonic wall of patients with either uncomplicated or complicated diverticular disease compared to a control group.

In line with our preliminary investigations where no differentiation between the uncomplicated and complicated disease was made [28], we could demonstrate lower collagen type I/III ratios in patients with diverticular disease compared to controls, significant for the complicated group (CDD), whereas no difference was found comparing patients with uncomplicated (UDD) or complicated diverticular disease. Disturbances in the collagen texture seem to be important in the pathogenesis of diverticular disease. It is not clear yet if the stronger decrease of collagen type I/III ratio seen in the CDD might be the result rather than a reason for inflammation. In the present study no significant differences in the expressions of MMP-1 and MMP-13 were found comparing the three groups. Maybe this is due to patients in the control group having colorectal cancer, which is known to initiate the overexpression of several MMPs [29–31]. Notably, we did not find altered values comparing UDD with CDD.

4.2. Proliferation activity

To investigate whether a disturbance in the cell cycle or inflammation leads to diverticula, proliferation (Ki-67) and apoptosis (TUNEL) were observed in all groups. Earlier studies [32,33] have shown a higher proliferative index in patients with diverticular disease even with asymptomatic diverticulosis. In contrary to these findings we found significant differences comparing CDD with the control group, but we did not detect alterations for the patients with uncomplicated diverticular disease (UDD) compared with our control group or with CDD. These data must be interpreted with caution because higher proliferative activity is
reported even in normal mucosa [34] of patients with colorectal neoplasms [35–38].

Although we found higher levels in our group with complicated diverticular disease (CDD), we suggest that it is more likely an expression of the inflamed intestinal wall than a predisposing factor for a complicated disease. However, further work is needed to establish this.

4.3. Enteric neuropathy

Disorders in the enteric nervous system (ENS) and consequently in the intestinal motility may contribute to the development of diverticular disease [6,7,39–42]. In these studies a precise distinction between uncomplicated and complicated disease was not made. Therefore, we investigated whether there is an alteration of glial cells (5–100 positive cells) in the myenteric plexus (Auerbach’s plexus) or submucosal plexus (Meissner’s plexus) comparing our groups. We observed a reduction of glia cells in both the myenteric and submucosal plexus up to nearly 25% for both groups (UDD and CDD).

It remains elusive whether the enteric neuropathy is rather a primary event than an effect of connective tissue pathologies, mechanical stress (e.g. increased intraluminal pressure) or inflammation. In our study we could not see a significant difference comparing the uncomplicated disease with the complicated disease. Hence we hypothesize that the reduction of glial cells in the myenteric and submucosal plexus is not caused by the inflammation of the intestine wall. But a cautionary note has sounded by Wedel et al. [6] Alterations of the ENS could be a postinflammatory effect even in patients with uncomplicated diverticular disease. This was also seen in other conditions such as in postinfectious irritable bowel syndrome.

Taken together, our findings suggest that the loss of glial cells could play a role in the pathogenesis of the diverticular disease and is probably not an effect of an inflamed intestine wall.
4.4. Rectosigmoidal sphincter

The presence of a previously described anatomic sphincter in the rectosigmoidal area in cadavers [5,43] may contribute to a higher intraluminal pressure. However, the described up to 2.5 times higher muscle thickness in that area was not seen in our study. We could not find any significant differences of layer thickness in the mucosa, submucosa and circular or longitudinal muscle layer between the sigma, sigmorectoidal area and rectum in all specimens.

In a recently published study [44] using dynamic imaging (MR-defecography) in 24 patients a higher wall thickness in the rectosigmoidal junction (RSJ) was detected. Furthermore, Wadhwa et al. [45] found in manometric measurements higher intraluminal pressures for the RSJ compared to the rectum and sigma. The discrepancy to our findings could corroborate the hypothesis that the rectosigmoidal junction is more a functional sphincter rather than an anatomical structure. Further research with regard to particular muscle activation needs to be undertaken as the presence of a functional sphincter could be relevant for the distal resection margin.

The present study has limitations inherent to its relative small sample size and a control group of patients with colon cancer. However, the aim of the current study was to investigate the whole colon wall of a segment and not bioptic samples of colonic mucosa only. Thus, obtaining samples of healthy subjects are not possible.

5. Conclusion

The results of this study suggest that colonic wall changes and enteric neuropathy could play a role in the pathogenesis of colonic diverticulosis. None of our results indicate a predisposition for a complicated diverticular disease. Furthermore, the presence of an anatomic sphincter at the rectosigmoidal junction could not be detected.

Finally, it still remains difficult to determine one key parameter in the development of the disease. Probably, its pathogenesis is multifactorial including genetic, environmental and lifestyle factors with divergent emphasis for different patient populations.

Ethical approval

Ethical Approval was given by the ethical committee of the University Hospital RWTH Aachen (EK 2090).

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