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ULTRASTRUCTURAL CHANGES OF THE HUMAN ENTERIC NERVOUS SYSTEM AND INTERSTITIAL CELLS OF CAJAL IN DIVERTICULAR DISEASE

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ENS ULTRASTRUCTURE IN DIVERTICULOSIS
ABSTRACT

Background In spite of numerous advances in understanding diverticular disease, its pathogenesis remains one of the main problems to be solved. We aimed to investigate the ultrastructural changes of the enteric nervous system in unaffected individuals, in asymptomatic patients with diverticulosis and in patients with diverticular disease.

Methods Transmission electron microscopy was used to analyse samples of the myenteric, outer submucosal and inner submucosal plexuses from patients without diverticula \((n = 9)\), asymptomatic patients with diverticulosis \((n = 7)\) and in patients with complicated diverticular disease \((n = 9)\). We described the structure of ganglia, interstitial cells of Cajal and enteric nerves, as well as their relationship with each other. The distribution and size of nerve processes were analysed quantitatively.

Results In complicated diverticular disease, neurons exhibited larger lipofuscin-like inclusions, their membranous organelles had larger cisterns and the nucleus showed deeper indentations. Nerve remodeling occurred in every plexus, characterised by an increased percentage of swollen and fine neurites. Interstitial cells of Cajal had looser contacts with the surrounding cells and showed cytoplasmic depletion and proliferation of the rough endoplasmic reticulum. In asymptomatic patients with diverticulosis, alterations of enteric nerves and ICC were less pronounced.

Conclusions In conclusion, the present findings suggest that most ultrastructural changes of the enteric nervous system occur in complicated diverticular disease. The changes are compatible with damage to the enteric nervous system and reactive remodeling of enteric ganglia, nerves and interstitial cells of Cajal. Disrupted architecture of enteric plexuses might explain clinical and pathophysiological changes associated with diverticular disease.
KEYWORDS

Diverticular disease; colon; TEM; ENS; interstitial cells of Cajal.
ABBREVIATIONS

CDD – complicated diverticular disease;

ICC – interstitial cells of Cajal;

ICC-MY – interstitial cells of Cajal around the myenteric plexus;

ICC-SM – interstitial cells of Cajal around the submucosal plexus.
1. INTRODUCTION

Colonic diverticula are herniations of the mucosa and submucosa through weak points in the muscular layer of the colon (Meyers et al., 1973). In most cases, the presence of diverticula (also known as diverticulosis) causes neither symptoms nor complications (Strate et al., 2012). However, diverticulosis becomes more frequent with age and 20% of affected individuals develop symptomatic uncomplicated diverticular disease (Everhart and Ruhl, 2009; Feuerstein and Falchuk, 2016). Moreover, the disease in some patients will advance to complicated diverticular disease (CDD), causing acute or chronic diverticulitis and subsequently leading to lower quality of life, hospital admissions, surgical intervention or even death (Unit, 2003).

During the last decade a number of studies have identified that obesity, use of NSAIDs, smoking and genetic predisposition are definite risk factors for the disease (Schieffer et al., 2018). Additionally, a substantial amount of neuromuscular changes have been documented in diverticular disease. There is a decrease in the number and size of enteric ganglia (Simpson et al., 2009), a decrease in neuron numbers (Deduchovas et al., 2008) and changes in innervation patterns of the colonic musculature (Golder et al., 2003; Böttner and Wedel, 2012). Interstitial cells of Cajal (ICC), whose role is to receive regulatory inputs from the enteric nervous system and generate electrical activity and motor coordination (Sanders et al., 2014), decrease in number too (Bassotti et al., 2005a). Finally, the muscular layer of the colon shows disturbed ultrastructural architecture of smooth muscle cells (Hellwig et al., 2014), as well as increased collagen deposits in longitudinal muscle (Pantaroto et al., 2015). Based on these changes, diverticular disease is classified as a subtype of enteric myopathy characterised by muscular degeneration and smooth muscle cell myofilament deficiency (Knowles et al., 2010).
The pathogenesis of diverticular disease remains one of the main problems to be solved. A number of morphological studies have compared unaffected individuals to patients with uncomplicated diverticular disease or diverticulitis. Regardless of numerous documented changes associated with diverticular disease, the causality of these changes remains mostly unaddressed. For this reason, multiple reviews conclude that the etiology of diverticular disease has not been understood completely (Tursi, 2016; Rezapour et al., 2018; Schieffer et al., 2018).

An ultrastructural study would offer further insight into the changes that occur on a larger level, such as enteric neuropathy (Wedel et al., 2010) or myenteric plexitis (Bassotti et al., 2015). Ultrastructural studies on Crohn’s disease have shown that enteric nerves and ICC sustain damage (Dvorak and Silen, 1985; Wang et al., 2007). Furthermore, experimental studies have also found that morphological ICC changes during and after infection are only visible using electron microscopy (Wang et al., 2002). Considering the evidence that inflammatory bowel diseases and diverticular disease show overlap (Collins and Winter, 2015), the possibility of previously undocumented changes of the enteric nervous system and ICC is likely.

We aimed to investigate the ultrastructural changes of the enteric nervous system and ICC. To better understand the timing of these changes, we investigated the enteric nervous system in unaffected individuals, in patients with asymptomatic diverticulosis and in patients with CDD. We employed transmission electron microscopy to analyse the morphology of the three main ganglionated – myenteric, outer submucosal and inner submucosal – plexuses of the enteric nervous system and the ICC around them.
2. MATERIALS AND METHODS

2.1. Patients

Colon samples from each of 25 patients were received from the Department of Surgery at the Lithuanian University of Health Sciences Kaunas Clinics between December 2015 and May 2017. 16 patients were operated on for non-obstructing colon cancer: 9 patients did not have macroscopic diverticula (control), 7 patients had macroscopic diverticula (asymptomatic diverticulosis, later referred to as diverticulosis). 9 were electively operated on for recurrent episodes of diverticulitis (complicated diverticular disease).

Patients with previous or current irritable bowel syndrome or inflammatory bowel disease were excluded from the study. One patient in the diverticulosis group had received chemotherapy six months before the operation. Among patients with complicated diverticular disease, the median duration of symptoms was 16 months (range 2–72 months).

Study group characteristics are described in Table 1.

The experimental procedures were approved by the Kaunas Regional Biomedical Research Ethics Committee, Kaunas, Lithuania (code BE-2-10). The study was performed in accordance with the ethical standards of the Declaration of Helsinki. All patients gave written informed consent prior to their inclusion in the study.

2.2. Tissue processing

1–5 cm length circular segments of the sigmoid colon were taken from the distal resection margin without any signs of macroscopic diverticula and immediately placed in a cold saline solution in the operation room. Within 30 minutes, 1 x 1 x 0.5 cm samples were cut from the main sample in cold saline and placed in a 2.5% glutaraldehyde 0.1 M PB solution (pH 7.4) at 4 °C for 2 hours for immersion prefixation.
After the serosa was removed, the samples were then dissected in a Petri dish with the same fixative into three layers: the 1st layer containing the inner submucosal plexus was made by dissecting the mucosa from the rest of the sample by cutting along the submucosa, the 2nd layer containing the outer submucosal plexus was made by cutting along the circular muscle layer, and the 3rd layer containing the myenteric plexus between the outer part of the circular muscle layer and the longitudinal muscle layer. These three layers were further dissected into 1 x 1 x 2 mm sized tissue samples using a Stemi 2000CS stereomicroscope (Zeiss, Gottingen, Germany), fine scissors and tweezers. The samples were fixed in a 2.5% glutaraldehyde 0.1 M PB (pH 7.4) solution at 4 °C overnight.

Afterwards, samples were postfixed for 2 h with 1% osmium tetroxide in 0.1 M PB (pH 7.4), dehydrated using a series of graded ethanol solutions and embedded into a mixture of Epon 812 and Araldite resins (Sigma-Aldrich, Steinheim, Germany) using a LYNX II automatic tissue processor (EMS, Hatfield, PA, USA). Tissues were carefully oriented in flat embedding molds for transverse sectioning. Semi-thin sections (1 µm) were stained with methylene blue according to Ridgway (Ridgway, 1968) and were analysed with a Zeiss AxioMat light microscope (Carl Zeiss, Jena, Germany) to confirm the sections contained structures of the enteric nervous system. Ultrathin sections (50–70 nm) were cut using a Leica EMUC7 ultramicrotome (Leica Mikrosysteme Handelsges.m.b.H., Vienna, Austria) and mounted on 600-mesh thin bar support nickel grids (Agar Scientific, Essex, UK). Samples were stained with uranyl acetate and lead citrate for 7 minutes each.

2.3. Transmission Electron Microscopy

Ultrathin sections were analysed using a Tecnai BioTwin Spirit G2 transmission electron microscope (FEI, Eindhoven, the Netherlands) at 100 kV. Images were collected using a bottom-mounted 16 MP Eagle 4K TEM CCD camera, using TIA software (FEI, Eindhoven,
the Netherlands) at 4800x, 6800x and 9300x magnification. Electron micrographs were analysed morphometrically with the software package Fiji (Schindelin et al., 2012; Rueden et al., 2017).

Neurons were identified based on descriptions by G. Gabella (Gabella, 1972) and compared between the three patient groups. Furthermore, the size of lipofuscin inclusions were measured when found in a neuron, and the density of lipofuscin inclusions was expressed as the ratio of lipofuscin area to neuron area, excluding the nucleus from the neuron area. Neurons with no lipofuscin inclusions were included when comparing results across the patient groups. The cross-sectional area of all completely visible neurites found in all three of the studied plexuses was measured and the resulting measurements were classified into either damaged or healthy neurites based on observations by Iijima-Ando and colleagues (Iijima-Ando et al., 2012). We based the classification on the translucency of the neurite, the presence of cytoskeleton, the shape of the neurite and the integrity of the membrane. We also created a category of fine neurites which are unmyelinated neurites with a diameter of less than 0.2 µm based on the widely recognized range of 0.2 to 1.5 µm of unmyelinated nerve fibres (Kiernan and Rajakumar, 2014). ICC were identified using the following morphological criteria: numerous mitochondria, abundant intermediate filaments, the presence of caveolae and close contact to adjacent smooth muscle cells and enteric nerves (Faussone-Pellegrini and Thuneberg, 1999). Occurrence of properties associated with abnormal ICC as described by Wang and colleagues (Wang et al., 2005) were described between the patient groups, counted and presented as proportions.

2.4. Statistical Analysis

Statistical analysis was performed using R 3.5.1 (R Core Team, 2015). Data was imported with dplyr (Wickham et al., 2016) and readr (Wickham et al., 2017) packages and was
visualised with ggplot2 (Wickham and Chang, 2016). In order to estimate the effect of diverticulosis and CDD on the percentage of fine neurites and damaged neurites, logistic regression was used, with type of neurite as the outcome variable and study group as the predictor variable. To estimate the overall change of variance of neurite profiles, linear regression was used, with the variance of measured cross-sectional areas per tissue sample as an outcome variable and layer of the colon and patient group as predictor variables. The control group was used as a reference group when fitting the models. Because the distribution of the area of neurite profiles was log-normal, logistic transformation was done before calculating the variance. For proportional data, the Chi-square test was used to estimate differences of morphological occurrences across different patient groups.

3. RESULTS

3.1. Ultrastructural changes in ganglia

Using qualitative assessment, neurons in the control group showed similar characteristics to those described in other studies (Gabella, 1972). The neurons contained numerous ribosomes, either free in the cytosol or attached to the endoplasmic reticulum (Fig. 1a). Mitochondria were usually round and distributed in groups, however, some of them were swollen. The Golgi apparatus was frequent, usually interspersed in the cytosol. Neurofilaments and microtubules were clearly visible in neuronal somata and were especially pronounced in dendrites. Cell bodies occasionally contained one or two small lipofuscin inclusions. Neurons were usually located at the periphery of ganglia. Somata either contacted the basal lamina directly or were partially covered by glial processes. Inside ganglia, varicosities and axon terminals with synaptic vesicles were found near somata. Axodendritic and axosomatic synaptic contacts were common in the neuropil. The nucleus
was usually round with few shallow indentations and the nucleoplasm largely contained euchromatin, with slight condensation near the nuclear lamina.

In diverticulosis samples, the majority of neurons were intact, except for cases where the neurons contained electrodense cytoplasm, larger lipofuscin inclusions and noticeable organelle swelling (Fig. 1b). The position of neurons and their relationship to other structures in ganglia did not differ compared to controls.

Meanwhile, neurons in CDD samples showed more pronounced changes (Fig. 1c–e). Most neurons contained larger lipofuscin inclusions, lamellar bodies and swollen mitochondria, with some of the neurons containing little to no lipofuscin inclusions. While the number of lipofuscin inclusions did not show large differences between all three patient groups, the inclusions were on average larger (Table 2, $F = 4.078$, $p = 0.018$) and occupied a larger area of the neuron body ($F = 4.501$, $p = 0.013$) in the diverticulosis and CDD groups compared to the control group. Mitochondrial vacuolisation was not only more frequent but also more pronounced than in the other groups. The rough endoplasmic reticulum was swollen and the Golgi apparatus was not discernible in the cytosol. The nucleus was usually irregular, with multiple shallow indentations of various degrees (Fig. 1e) that were uncommon in control and diverticulosis groups. The nucleolus appeared enlarged and more condensed.

### 3.2. Ultrastructural changes of neurites

The neuropil in control samples exhibited a variety of tightly packed neurite profiles (Fig. 2a). Though some of the neurites appeared swollen, most contained well contrasted microtubules. Synapses were visible in the myenteric and the outer submucosal plexuses, and some of the ganglia contained peg-and-socket junctions as well. Varicosities contained mitochondria and neurotransmitter vesicles, and lacked microtubules. The vesicles were usually small and granular with an electron-dense core or small and agranular. Neuropil in
diverticulosis samples showed a similar pattern to healthy neuropil, however, patches of swollen axons were found (Fig. 1b).

Neurites in CDD samples displayed a degree of damage not seen in control and diverticulosis samples. In neuropil of the many enteric ganglia of CDD samples, tight packing of axons was disrupted. Nerve fibres individually enveloped in glial cell processes appeared more spread apart with collagen deposits in between them (Fig. 2b). Damaged neurites were swollen, lacked microtubules and vesicles, contained translucent cytoplasm, and neurofilaments were poorly visible (Fig. 2d). Due to this, some of them impinged on adjacent neurites and glial processes. The plasma membrane was disintegrated to various degrees. Furthermore, we failed to find clearly delineated synaptic contacts between neurites, suggesting decreased synaptic transmission.

Damaged neurites were more frequent in every ganglionated plexus in complicated diverticular disease but not in diverticulosis samples (Fig. 3). In the myenteric plexus, 14.2% of neurites in control cases were damaged compared with 13.9% in diverticulosis samples (OR = 0.972, p = 0.856) and 24.6% in complicated diverticular disease (OR = 1.968, p < 0.0001). A similar tendency followed in the outer submucosal (control 8.48%, diverticulosis 8.51%; CDD 15.82%, compared to control OR = 2.027, p = 0.017) and the inner submucosal (control 15.9%, diverticulosis 13.6%; CDD 25.0%, compared to control OR = 1.756, p < 0.001) plexuses.

In addition to damaged neurites, CDD samples contained a higher percentage of fine neurites (Fig.4). Some of these neurites were closely enveloped by glial processes and were isolated from each other (Fig. 2b). These neurites contained dense axoplasm and microfilaments. Other fine neurites appeared in clusters enveloped by glial processes (Fig. 2e). A majority of these neurites were smaller than the usual lower diameter of unmyelinated axons, suggesting degeneration. The appearance of fine neurites was most pronounced in the
myenteric and inner submucosal plexuses (Fig. 4). The myenteric plexus contained 2.79% and 2.02% fine neurites in control and diverticulosis samples, respectively, as opposed to 5.02% in CDD samples (OR = 1.845, p = 0.02). In the outer submucosal plexus, fine neurites were more frequent in diverticulosis samples (control 2.42%, diverticulosis 6.96%, OR = 3.012, p = 0.039) and in CDD samples (5.63%, OR = 2.401, p = 0.099). In the inner submucosal plexus, control samples and diverticulosis samples contained a similar amount of fine neurites (8.23% and 8.67%, respectively, OR = 1.06, p = 0.829), whereas CDD samples contained higher numbers of fine neurites (11.13%, OR = 1.40, p = 0.102).

Overall, the enteric plexus change is two-fold. Since damaged neurites were usually larger than other neurites, we found that CDD neurite distributions are more dispersed compared to control and diverticulosis groups, although the effect of these changes was only slightly pronounced and was not statistically significant. The mean variance of neurite cross sectional areas was slightly higher in the CDD group compared to the control group (β = 0.165, SE 0.120, p = 0.175) and the mean variance in the diverticulosis group was similar compared to the control group (β = 0.069, SE 0.133, p = 0.605).

3.3. Alterations of interstitial cells of Cajal in diverticular disease

ICC around the myenteric plexus (ICC-MY) of control samples exhibited features of healthy ICC that have been described in previous research (Yang et al., 2012). The defining properties of ICC were the presence of caveolae and intermediate filaments (Fig. 5a). Microtubules and lysosomes were not present. In most cases, the rough endoplasmic reticulum and the Golgi appratus were poorly developed and were located around the nucleus. The cell processes, apart from intermediate filaments and caveolae, sometimes contained mitochondria. ICC-MY in diverticulosis samples exhibited similar characteristics with the exception of lamellar bodies in some of the cells and sparsely distributed caveolae.
In both samples, cell processes were closely associated with adjacent smooth muscle cells and enteric nerves or ganglia. ICC around the submucosal plexus (ICC-SM) showed similar ultrastructural characteristics with the exception of looser contact with surrounding cells and more pronounced processes.

In CDD samples, ICC-MY showed structural changes (Fig. 5c). The cells were still spindle shaped, but they showed cytoplasmic depletion. With a reduction of cytoplasm, contents of the perinuclear region resolved poorly. There was notable proliferation of ribosomes and rough endoplasmic reticulum in the cell processes (Fig. 5c and d), with most ICC-MY cells exhibiting changes (Control 2/7 cells, diverticulosis 6/13 cells, CDD 20/23 cells, \( \chi^2 = 10, p = 0.004 \)). Unlike in the control and diverticulosis samples, ICC-MY processes rarely contacted smooth muscle cells or enteric nerves but did contact other ICC-MY.

The morphology of ICC-SM in CDD samples showed changes similar to those found in affected ICC-MY, however, these changes were less consistent across the samples. Some of the processes were similar to those in control samples, but others lacked intermediate filaments and showed changes consistent with those in affected ICC-MY. In longitudinal sections of ICC-SM processes, proliferation of rough endoplasmic reticulum was notable and comparatively more frequent in CDD samples (Control 3/18, Diverticulosis 1/6, CDD 3/6, \( \chi^2 = 3, p = 0.2 \)). The processes themselves were in close contact with each other and with enteric nerves of the submucosal plexuses. Other signs of injury were absent.

3. DISCUSSION

We sought to investigate ultrastructural changes of the enteric nervous system in patients with asymptomatic diverticulosis and patients with complicated diverticular disease compared to unaffected patients. We found that the enteric nervous system in diverticulosis
does not change dramatically. Marked changes appeared in complicated diverticular disease, including degenerative changes in neurons, a higher frequency of swollen and fine neurites, and injury to ICC. All of these changes are compatible with damage and remodeling of the enteric nervous system in complicated diverticular disease.

One shortcoming of our study is that we did not investigate the enteric nervous system in symptomatic uncomplicated diverticular disease. Patients were operated on for diverticular disease due to recurrent episodes of diverticulitis, after which low grade inflammation and extensive damage to the colonic wall could occur. Because of this, complicated diverticular disease could confound the changes seen in the enteric nervous system during an uncomplicated course of the disease. This is especially important considering that alterations described in this study might play a role in symptom development. In spite of this, it is also possible that these changes could be related to exacerbated symptoms after an episode of diverticulitis (Simpson et al., 2003). The present study cannot distinguish a direct connection between the morphological changes and the course of the disease. Therefore, without uncomplicated disease samples it is hard to judge whether the changes are due to complications of the disease or due to the disease itself.

Additionally, some of the morphological changes can be attributed to age-dependent degeneration as can be seen in quantitative changes in the diverticulosis and CDD groups. Specifically, the frequency of cytoplasmic lipofuscin deposits and changes to organelles (e.g. the nuclear envelope) become more pronounced with age and since both the diverticulosis and the CDD group are older than the control, degenerative changes may be attributed to age dependent changes.

Another drawback of the study was the use of human tissue received from surgical procedures. In control and diverticulosis samples, mitochondria were also swollen, and some of the neurites appeared damaged. Furthermore, as is evident in the distribution of damaged
nerve processes in Figure 3, some of the non-CDD samples contained damage equivalent to that seen in CDD samples, possibly due to ischemic damage during surgery and delayed fixation. Even though the overall degree of vacuolisation and neurite damage was higher in CDD samples, it is quite possible the ultrastructural changes seen in this study are not exclusively a correlate of the underlying disease but an artifact of the work up of the specimens.

This study is the first to describe ultrastructural changes that occur in diverticular disease. However, the idea of nerve tissue remodeling in diverticular disease has been raised previously – Simpson and colleagues showed that enteric nerves had increased in thickness and that small diameter nerves had increased in frequency in diverticular disease (Simpson et al., 2009). The authors suggested that these changes are secondary to acute diverticulitis and smooth muscle hypertrophy. Our results complement the previous findings by showing that neurites are damaged during diverticular disease. The neurites lacked microtubules, suggesting disrupted neurotransmission. The previously found increase in nerve thickness could be explained by loss of typical nerve fibre structure, swollen neurites, larger glial processes and higher collagen deposition. Usually axons are embedded in supporting glial processes (Baumgarten et al., 1970), however, in CDD, axons were found to be individually ensheathed in glial processes. Furthermore, we have shown an increase in fine neurites which coincides with new nerve sprouting but also shows regeneration of damaged nerves. Considering that ganglionic nerve cells and enteric nerves decrease in number during diverticular disease (Deduchovas et al., 2008; Wedel et al., 2010), it is most likely that fine neurites appear in response to damage to the enteric nervous system. There have been suggestions that nerve regeneration and subsequent hyperinnervation could be a cause of hypersensitivity (Stead, 1992), and the same could be the case in diverticular disease. Taking
into account the absence of remarkable ultrastructural remodeling in asymptomatic patients, it is not unlikely that the changes developed as a result of acute diverticulitis.

Nonetheless, despite being less pronounced, we were able to detect changes in asymptomatic patients which were similar to changes in CDD. This calls into question whether morphological changes in complicated diverticular disease are caused exclusively by complications of the disease. Previous studies have suggested enteric neuropathy as a potential etiological factor (Wedel et al., 2015; Barrenschee et al., 2017) and the present study provides additional evidence for the hypothesis.

Morphological changes seen in ICC are similar to those found in previous studies on Crohn’s disease (Wang et al., 2007). Wang and colleagues investigated ICC during and after inflammation. Their morphological description of ICC 60 days after infection would most likely correspond to the findings of the present study. The proliferation of the rough endoplasmic reticulum is a sign of new protein synthesis and regeneration of cell processes. The presence could be explained by the fact that CDD patients for this study were not operated urgently and suffered episodes of diverticulitis in the past. However, ICC-MY failed to show close contact with smooth muscle cells and enteric nerves. In light of a previously found decreased density of ICC (Bassotti et al., 2005a), this shows that the ICC network remains impaired even after overt inflammation has subsided. However, it is not yet possible to discern whether these changes are primary or secondary to the disease, warranting further research.

Consistent injury to ICC-MY and ICC-SM only in CDD samples has multiple possible implications for understanding the disease. Since the ICC network is highly connected, it is believed that patchy injury to ICC should not impair normal peristalsis (Wei et al., 2017). Pathological changes of both ICC-MY and ICC-SM were rare in diverticulosis samples. If these changes represent actual damage to the ICC network, then it is possible they could play
a role in the pathogenesis of the disease given sufficient injury. Such a hypothesis would require a driving force in the presence of diverticulosis, but a recent study failed to find mucosal inflammation in the presence of diverticulosis (Peery et al., 2017). However, if these changes are spurious, it could be assumed that asymptomatic individuals with diverticula do not have a damaged ICC network and, therefore, ICC might potentially be ruled out as a pathogenetic step of uncomplicated diverticular disease. This hypothesis is limited by the small number of morphological studies performed on ICC in diverticular disease which have yet to uncover whether there is a causal relationship between ICC and diverticular disease exists.

Additionally, previous physiological studies have found abnormal motor patterns in patients with diverticular disease (Bassotti et al., 2005b). Slow-wave activity of the colon is generated by ICC around the myenteric plexus (Koh et al., 1998) and it has been hypothesised that loss of normal ICC function may disturb normal motility (Strate et al., 2012). In our study, even though new terminal processes were present, they did not form the same connectivity as seen in healthy tissue. In fact, previous experimental studies have been able to show that abnormal motor patterns in the colon persist in spite of ICC plasticity (Wang et al., 2005). Considering such evidence, disturbed ICC might be a source of aggravated symptoms in patients who had experienced an episode of diverticulitis (Simpson et al., 2003; Lahat et al., 2018). Further mechanical and electrophysiological studies with a focus on ICC could help identify their exact role in diverticular disease.

In summary, the ultrastructural changes seen in the enteric nervous system are compatible with structural remodeling and injury to the ICC network in complicated diverticular disease that persist even after acute complications. Most changes appeared only during complicated diverticular disease and are absent in unaffected individuals and patients with diverticulosis. The current evidence suggests that damage occurs during a complicated course of the
disease, provoking changes and adaptations that might be related to long-term effects on disease development. It is likely that diverticular disease has multiple forces that drive the generation of symptoms. Understanding how each of them influences the course of the disease individually may be crucial for future studies and treatment options.
4. ACKNOWLEDGMENTS

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5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
6. REFERENCES


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Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Age (mean)</th>
<th>Age (SD)</th>
<th>BMI (mean)</th>
<th>BMI (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9 (5 F / 4 M)</td>
<td>58.2</td>
<td>13.3</td>
<td>27.5</td>
<td>8.25</td>
</tr>
<tr>
<td>Diverticulosis</td>
<td>7 (4 F / 2 M)</td>
<td>60.9</td>
<td>10.4</td>
<td>28.3</td>
<td>4.18</td>
</tr>
<tr>
<td>CDD</td>
<td>9 (6 F / 3 M)</td>
<td>67.6</td>
<td>11.4</td>
<td>27.1</td>
<td>2.93</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>62.3</td>
<td>12.1</td>
<td>27.6</td>
<td>5.79</td>
</tr>
</tbody>
</table>

CDD – complicated diverticular disease, F – Female, M – Male, SD – standard deviation, BMI – body mass index.

Table 2. Lipofuscin inclusion characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diverticulosis</th>
<th>CDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of lipofuscin granules (µm²)*</td>
<td>2.25 µm² (0.02–14.67)</td>
<td>3.90 µm² (0.03–25.56)</td>
<td>4.84 µm² (0.01–47.71)</td>
</tr>
<tr>
<td>Relative lipofuscin area to neuron area (%)**</td>
<td>2.66% (0–22.20)</td>
<td>4.77% (0–20.26)</td>
<td>4.91% (0–31.80)</td>
</tr>
</tbody>
</table>

* F = 4.078, p = 0.018, control as reference; Control vs Diverticulosis, t = 2.225, p = 0.027; Control vs CDD, t = 2.526, p = 0.012; Diverticulosis vs CDD, t = 0.284, p = 0.777; ** F = 4.501, p = 0.013, control as reference; Control vs Diverticulosis, t = 0.59, p = 0.556; Control vs CDD, t = 2.914, p = 0.004; Diverticulosis vs CDD, t = 1.76, p = 0.080. Values are expressed as means (min–max). CDD – complicated diverticular disease.