„CAROL DAVILA" UNIVERSITY OF MEDICINE AND FARMACY, BUCHAREST
DOCTORAL SCHOOL
MEDICINE

Lesions of the Gastric Autonomic Nervous System in Helicobacter pylori Infection – A Morphopathological and Immunohistochemical Study
PHD THESIS SUMMARY

Doctoral tutor:
PROF.UNIV.DR. STĂNICEANU FLORICA

Doctoral student:
TUDORICĂ (CĂS. STICLARU) LIANA-CĂTĂLINA

2023

TABLE OF CONTENTS

PERSONAL CONTRIBUTION

7. Discussion

INTRODUCTION

Helicobacter pylori is the most widespread chronic bacterial agent in human, as over half of the world's population is colonized with this gram-negative bacterium. It is responsible for a variety of upper gastrointestinal disorders, including chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma or gastric carcinoma. Disease progression and severity of symptoms largely depend on sophisticated interplay between bacterial virulence factors and host immune response.

Over the past decades, major advances have occurred in better understanding the host-pathogen interactions, thanks to a variety of studies focusing on bacterial virulence factors. In addition to direct cytotoxic and proinflammatory effects that bacterial colonization has on the gastric mucoasa, it has been suggested that the pathogenic mechanisms of H. pylori infection might be much more complex than generally believed. Recently, it has become increasingly evident that H. pylori pathogenesis may also involve alterations of gastric enteric nervous system (ENS) morphology and function, due to complex neuroimmune interactions, with subsequent implications on disease outcome. However, the current state of knowledge does not yet allow detailed characterization of the underlying mechanisms of these interactions.

Aside from H. pylori-associated changes in the neurochemical (neurotransmitter/ neuropeptide) content of gastric nerve fibers, too few studies have been done to determine whether the H. pylory-induced gastric inflammation could cause neuroplastic alterations in the myenteric ganglia. Therefore, in this study, we aimed to directly assess the effects of H. pylori infection on gastric nervous system morphology and neurochemical coding, in order to shed light on the potential abnormalities that may result from it. Our hypothesis is that H. pylori infection influences the number of myenteric neurons and glial cells and disturbs neuronal homeostasis.

CURRENT STATE OF KNOWLEDGE

1. H. pylori infection: overview

Although H. pylori has infected humans for more than 58,000 years, it largely escaped notice until it was cultured by Marshal and Warren. The discovery changed the historic dogma regarding gastric disease causation and led, ten years later, to recognition of H. pylori as a type I carcinogen.

The microorganism causes one of the most common infections in humans that affect most populations throughout the world, but the prevalence is strongly correlated with socioeconomic status, so it varies greatly among countries and among population groups within the same country. Epidemiological studies state that in developing countries, among which is Romania, more than eighty percent of the adult population acquires the infection during lifetime. The bacterium is acquired by oral ingestion in early childhood and is mainly transmitted within families.

H. pylori harbors several unique properties that allow it to perfectly adapt to the unfavorable acid environment of the gastric lumen, facilitate penetration of the mucus layer, swimming and spatial orientation in the mucus, attachment to epithelial cells, evasion of the immune response and, as a result, lead to persistent colonization and life-long survival in the human stomach, its only known natural niche. Bacterianl successful strategies consist of urease secretion, multiple unipolar flagella, adhesion proteins babA and sabA on its surface, strain-specific, disease-related genes vacA and cagA.

H. pylori triggers a florid and continuous gastric inflammation consisting of neutrophils, lymphocytes (T and B cells), plasma cells, and macrophages in virtually all infected subjects. Following successful colonization of the gastric mucosa, host immune responses (both innate and acquired) are stimulated, generating specific antibodies and activated Th cells, along with varying degrees of epithelial cell degeneration and injury.

The clinical course of H. pylori infection is highly variable and is influenced by bacterial genetic diversity and extensive polymorphism in host genes responsible for directing the immune response. The specific interactions between host and microbe strongly correlate with the risk of developing gastritis, peptic ulcer, gastric carcinoma or gastric lymphoma.

2. The enteric nervous system

ENS is by far the largest and most complex part of the autonomic nervous system (ANS), consisting of glial cells and various types of neurons organized in two networks of myenteric ganglia within the gut wall. It was described as the "brain in the gut", since it has the unique ability to control gastrointestinal functions independent of the central nervous system. In the stomach, the ENS is represented mainly by the Auerbach plexus (or the myenteric plexus), which is situated between the circular and the longitudinal layers of the muscularis propria and provides motor innervation to both muscle layers and secretomotor innervation to the gastric mucosa. Only sparse submucosal ganglia, present mainly in the antrum, form the gastric Meissner plexus.

The enteric ganglia are composed of a variety of functionally distinct neurons (20 different types) including three major groups: intrinsic primary afferent neurons (IPAN), interneurons and motor neurons. Additionally, enteric ganglion cells and nerve fiber tracts are supported by numerous glial cells. Although originally thought to have only supporting role, recent data indicate that glial cells contribute to enteric nervous function and display phenotypic changes in different pathological conditions, including gastrointestinal inflammation.

3. Enteric nervous system abnormalities in H. pylori infection

The brain gut-axis is made of central (CNS) and autonomic nervous system (ANS), and modulates gastric and intestinal activity through the regulation of the gastrointestinal immune system, mucosal inflammation and intestinal microflora in response to stress, emotions and environmental influences. Multiple interactions and functions involved in this communication system work not only via the nervous system, but also through the endocrine, immunological and metabolic pathways (neuroendocrine-immune crosstalk).

The immune system and the enteric nervous system, as part of the ANS, work together in close cooperation and are able to regulate each other`s activity to maintain gastrointestinal homeostasis. Gastrointestinal inflammation leads to immune response activation, which in turn evokes enteric neuroplasticity.

Enteric neuronal plasticity comprises a wide range of changes in enteric neurons and glial cells. As a result of adaptive responses to different types of stimuli/conditions, enteric

neurons are able to change their structural, functional or chemical phenotype in order to maintain homeostasis of gut functions. In this context, the capacity of the ENS to undergo adaptive changes and/or reparative events may be viewed as an attempt to improve disturbed gut function and ameliorate symptoms.

Morphological changes of gastric neural tissue have received little attention in the pertinent literature, but structural nervous alterations have been documented in small and large bowel inflammatory disorders. In addition, numerous studies have appeared in the literature documenting the involvement of neurotransmitters in pathophysiology of gastric inflammation in animal models of H. pylori-induced gastritis or in human disease.

PERSONAL CONTRIBUTION

4. Hypothesis of the research and main objectives

H. pylori infection remains a major health problem worldwide. High global prevalence, heterogeneity of clinical symptomatology, risk of developing gastric neoplasia and association with impaired central and extragastric peripheral nervous system activity are still issues having a major impact on people`s health. Moreover, given the emerging resistance of H. pylori to current therapies, alternative treatment targets must be identified. Thus, identification of all the factors involved in the pathogenesis of H. pylori infection might represent the cornerstone for effective and long-term eradication of the microorganism, which will further result in decreasing gastric cancer incidence worldwide.

This study aims to assess the effects of H. pylori infection on gastric nervous system morphology and neurochemical coding, in order to shed light on the potential abnormalities that may result from it. Our hypothesis is that H. pylori infection is able to influence the number of myenteric neurons and glial cells, to induce changes in neurotransmitter secretion in gastric mucosa and myenteric neurons and to disturb neuronal homeostasis.

The main objectives of this study are as follows:

- Investigation of morphometric parameters and histological structure of normal gastric myenteric nervous plexus;
- Morphometric analysis of gastric myenteric nervous plexus in patients with H. pylori infection, with subsequent quantitative determination of ganglionic area, identification and quantitative assessment of enteric neurons and glial cells;

- Evaluation of periganglionic inflammatory infiltrate in patients infected with H. pylori, and detailed analysis of the immune cells involved;
- Assessment of gastric myenteric neurodegeneration and neuronal apoptosis;
- Evaluation of changes in substance P and vasoactive intestinal peptide expression in myenteric neurons and gastric mucosal nerve endings;
- Assessment of correlation between the noticed changes and the degree of bacterial colonization and the intensity of the immune response to infection.

This study provides new insights into the complex mechanism underlying the pathological consequences of H. pylori infection on gastric enteric nervous tissue. A better comprehension of neural changes during H. pylori-induced inflammation could help in identifying new therapeutic options.

5. Materials and methods

We designed a retrospective cohort study carried out on archival full-thickness samples of gastric wall obtained from 40 patients with histologically proven H. pylori infection, who had undergone gastrectomy for uncomplicated non-neoplastic disease of the stomach or digestive cancer over a period of 5 years. The control group consisted of tissue samples obtained from 40 age- and sex-matched subjects with no recent history of H. pylori infection. All the samples were selected from gastric areas at least 5 cm away from macroscopically visible lesion, then immediately fixed in 10% buffered formalin and automatically processed using a Leica ASP300 S Fully Enclosed Tissue Processor. Paraffin-embedded specimens were routinely cut in 3 μm-thick serial sections and mounted on glass slides. Before use, slides were deparaffinized in xylene, rehydrated with increasing dilutions of ethanol and then with water and processed for routine haematoxylin and eosin (HE), Giemsa and PAS staining and immunohistochemistry.

Histologic features of H. pylori-induced gastritis were assessed on HE stained sections and graded according to the updated Sydney system. Neuronal damage was confirmed when cells with condensed/vacuolated cytoplasm and/or shrunken, pyknotic nuclei were identified and was described as present/absent.

Myenteric neurons and glial cells were evaluated by anti-HuC/D and anti-S100 antibodies, respectively. Ganglionic areas were measured by using anti-S100 antibody. Presence and quantification of mucosal lymphocytic infiltrate were assessed by using CD3

(T lymphocytes), CD4, CD8 and CD20 (B lymphocytes) antibodies. Apoptotic activity of myenteric neurons was examined with immunostaining using monoclonal human bcl-2 antibody. Ganglionitis, defined as myenteric plexus inflammation, was evaluated considering only T CD3-positive cells and was scored on a scale from 0 to 3 based on the appearance of the most severely inflamed ganglionic area, according to the criteria proposed by Villanacci et al. Neuropeptides expression in mucosal nerve endings and myenteric neurons, respectively, was evaluated by anti-substance P antibody and anti-VIP antibody and was quantified using a semi-quantitative scale from 0 to 3.

In order to evaluate the immunoreactive ganglionic cells, we used a slightly modified version of a previously described method by Ippolio et al. For each section, 40 sequential microscopic fields taken along the myenteric plexus were examined at 40x magnification, starting with the first ganglion present on the left side of the section. Examination of the sections and image acquisition were performed using an Olympus BX43 microscope equipped with an Olympus XC30 digital camera (Olympus Corporation, Japan) and ganglionic areas were estimated by an Image Analysis Software (cellSens Dimension, Olympus Corporation, Japan). Each microscopic field corresponded to a 0.36 mm x 0.27 mm rectangle, with an covered area of 0.0972 mm^2. Thereby, the total ganglionic length and tissue area evaluated for each section were 14.4 mm and 3.888 mm^2, respectively.

For each patient, the results were expressed as mean±SE. For groups, most data didn`t follow a parametric distribution, so they were presented using medians and interquartile ranges. The figures were designed as box-whiskers plots. Wilcoxon test for non-parametric data (two tailed) was performed to compare groups. The strength of association between variables was evaluated using γ^2 and Spearman rank correlation tests. A p value < 0.05 was considered statistically significant.

6. Results

Gastric mucosa morphology

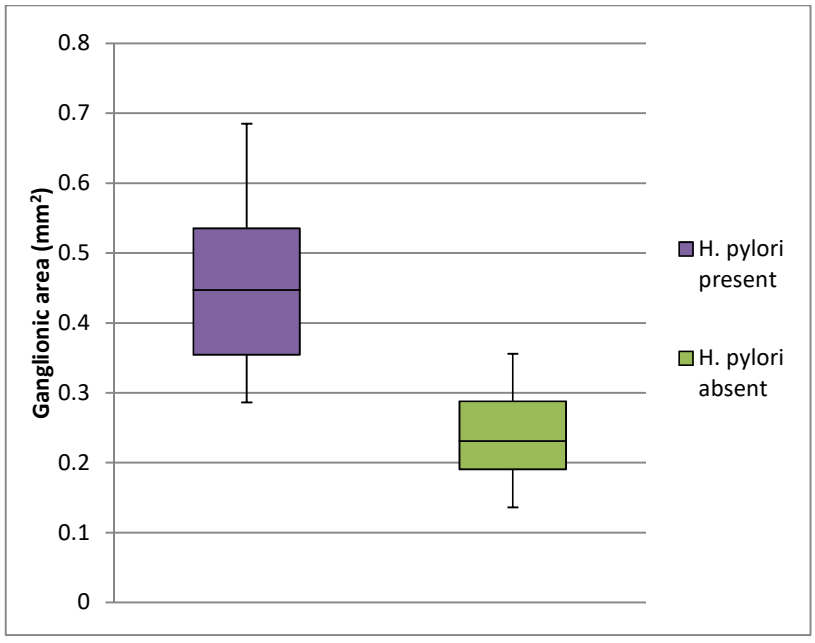
Most cases (22) showed a moderate degree of H. pylori colonization, while 13 cases had a mild bacterial density. In 5 cases, the presence of H. pylori was significant and scored as marked. All patients had chronic gastritis, and neutrophilic activity was observed in 31 (77.5%) of them. Immunohistochemical analysis revealed that the gastric mucosal

inflammatory response consisted mainly of CD3+ T cells. Intestinal metaplasia and atrophy were observed in 25 and 22 patients, respectively.

Gastric myenteric plexus morphology

Ganglionic areas were significantly larger (median 0.447 mm^2) and the number of myenteric ganglia was higher (median 29) in H. pylori-positive patients, compared to controls (medians 0.231 mm^2 and 20.5, respectively - Fig. 1).

An important difference was also found concerning the number of myenteric neurons between patients with H. pylori-induced gastritis (median 116.5) and those without infection (median 56.5) (Fig. 2a), with a significant increment of +171% (individual values varying between 35% and 380%). In addition, more glial cells were identified in myenteric ganglia of infected patients (median 588) compared to controls (median 314) (Fig. 2b), with a mean increment of +87% (individual values varying between 19% and 172%).



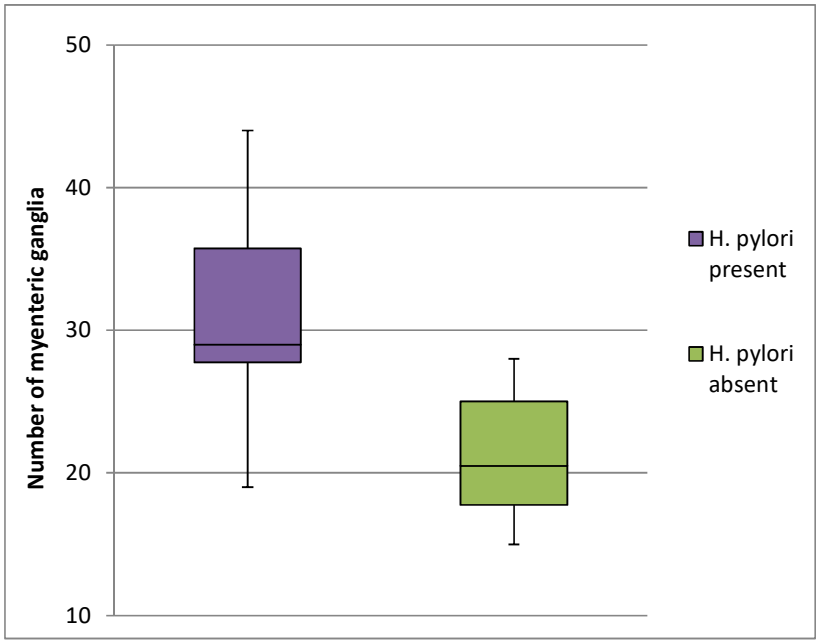
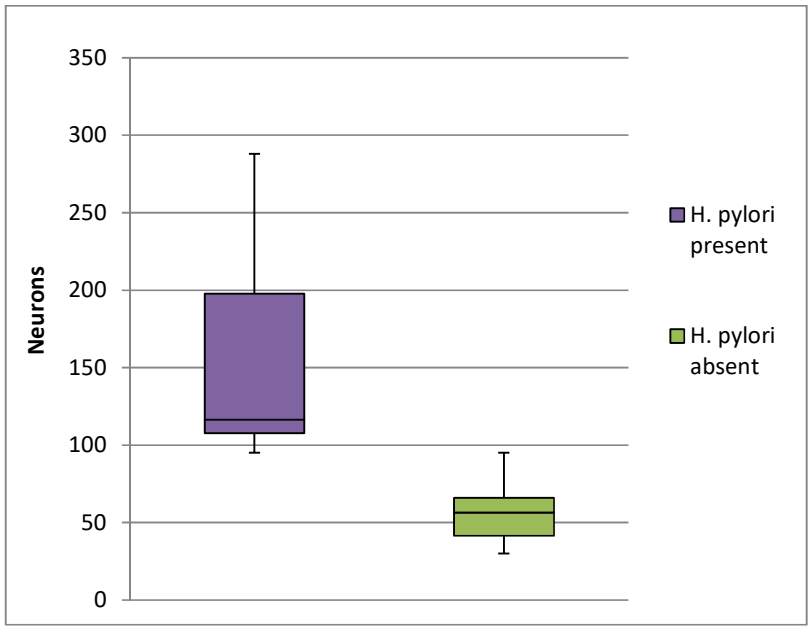


Figure 1. Number and area of myenteric ganglia in stomach. Box and whisker plots showing that gastric myenteric ganglia are larger (a, $p<0.01$) and they are increased in number (b, $p<0.01$) in H. pylori infected patients, as compared to controls.

Interestingly, in control group, the number of ganglionic areas and neuronal density didn`t correlate significantly with patients` age or with gastric region. The ratio between glial cells and neurons in myenteric plexus was fairly constant in H. pylori-negative patients, (range 5.04 - 6.87), whereas infected subjects didn`t display a correlation between glial and neuronal compartments, and the ratio was slightly decreased (range 2.06 – 6.5).



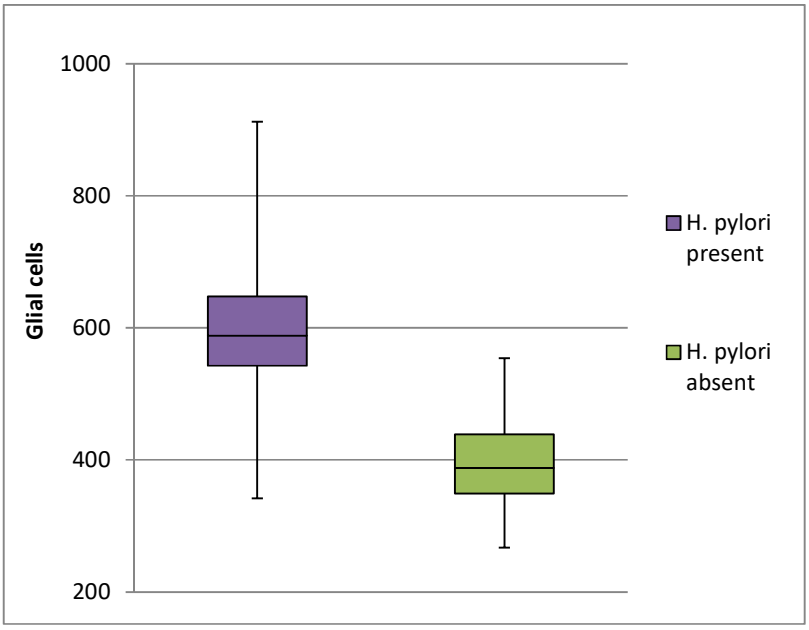
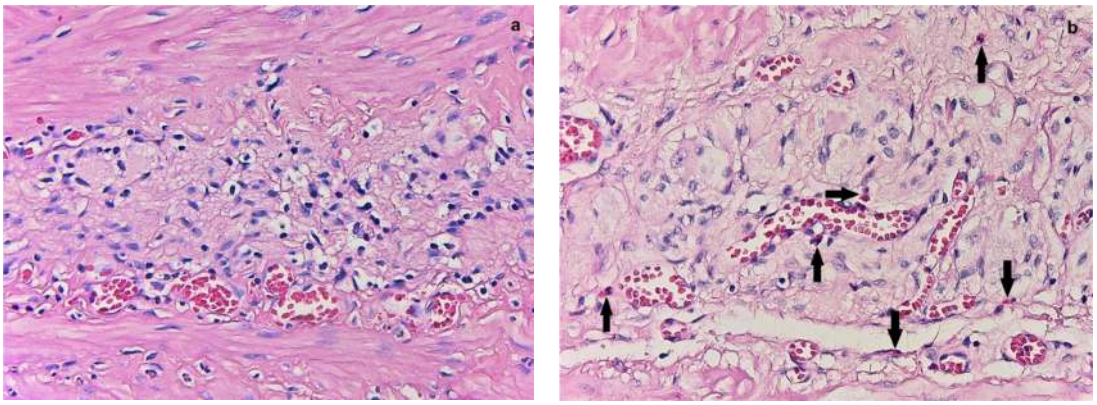


Figure 2. Number of gastric myenteric neurons and glial cells. Graphs showing that significant more myenteric neurons (a, $p<0.00001$) and glial cells (b, $p<0.00001$) were detected in H. pylori-positive group in comparison to the control group.

Ganglionitis was found in 33 (82.5%) cases with H. pylori infection. The inflammatory infiltrate was composed predominantly of CD3-positive T-cells, with a minor prevalence of B lymphocytes, plasma cells and rare eosinophils (Fig. 3). T lymphocytic infiltration of myenteric plexus was mild in 17 patients, moderate in 10 and severe in 3 of them, and correlated with T-cell density in lamina propria ($p<0.001$). Occasional inflammatory cells, most of them eosinophils, were present in the vicinity of ganglionic areas in 20 (50%) uninfected patients. Neither CD20-positive B lymphocytes nor plasma cells were observed in the control group.



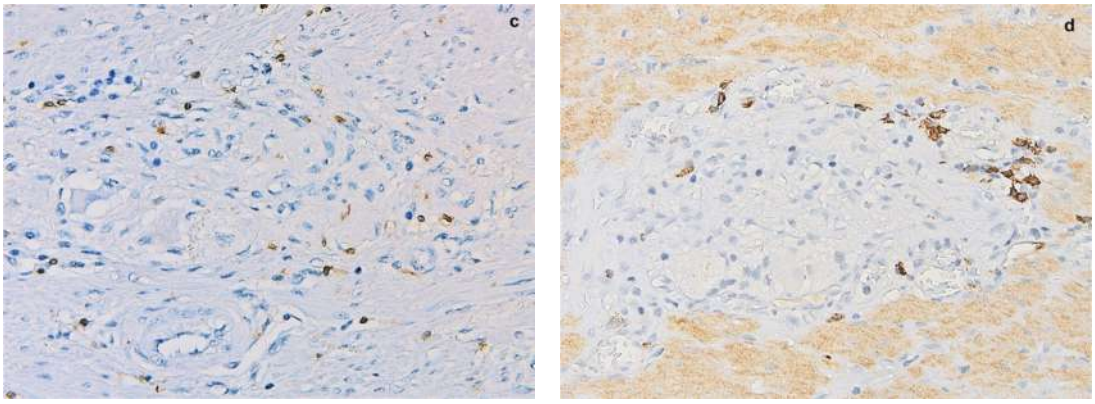


Figure 3. Representative photomicrographs showing different types of inflammatory cells around and within the myenteric plexus in H. pylori infected patients: lymphocytes (a - H&E stain, 400x); lymphocytes and eosinophils (arrows) (b - H&E stain, 400x); T lymphocytes (c - CD3 stain, 400x); B lymphocytes (d – CD20 stain, 400x).

Degeneration of neuronal cells was obviously more frequently observed in H. pylori infected patients (Fig. 4) but was modestly correlated with T-cell ganglionitis. However, a stronger association was found between neurodegenerative changes and the polymorphous inflammatory infiltrate, including T and B lymphocytes and plasma cells.

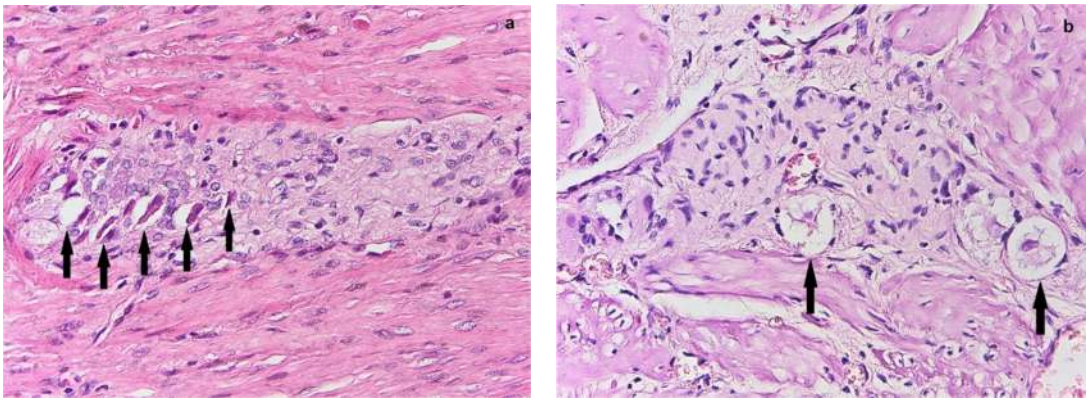


Figure 4. Representative photomicrographs illustrating signs of myenteric neuronal degeneration in H pylori-positive patients: condensed cytoplasm and pyknotic nuclei (a - H&E stain, 400x), vacuolated cytoplasm (b – H&E stain, 400x).

Myenteric neurons with markedly reduced or lost bcl-2 expression were observed in 23 (57.5%) infected patients, compared to only 3 (5.7%) cases in the control group ($p<0.0001$, Fig. 5). Neuronal apoptosis correlated with the presence of myenteric CD3-positive T-cell infiltrate, but didn`t correlate with signs of neurodegeneration .

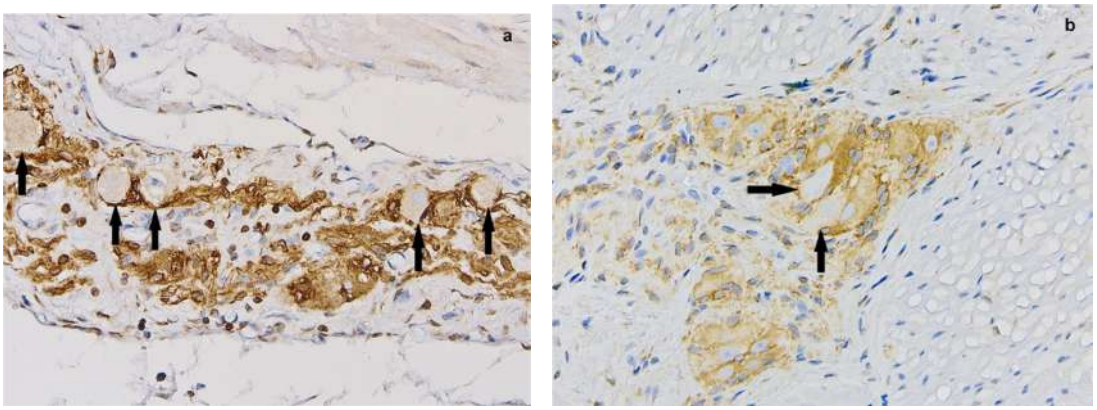


Figure 5. Bcl-2 immunohistochemical labeling of gastric myenteric ganglionic neurons: myenteric neurons with reduced or absent expression of antiapoptotic protein bcl-2 in H. pylori- positive patients (a,b, 400x)

Neurochemical changes

In H. pylori-infected patients, SP expression was significantly more pronounced in neurons than in controls (Fig. 6). Most H. pylori-infected patients (13 cases) showed a moderate SP expression in myenteric neurons, while the level of SP neuronal synthesis was low in most of the controls (15 cases, Fig. 7).

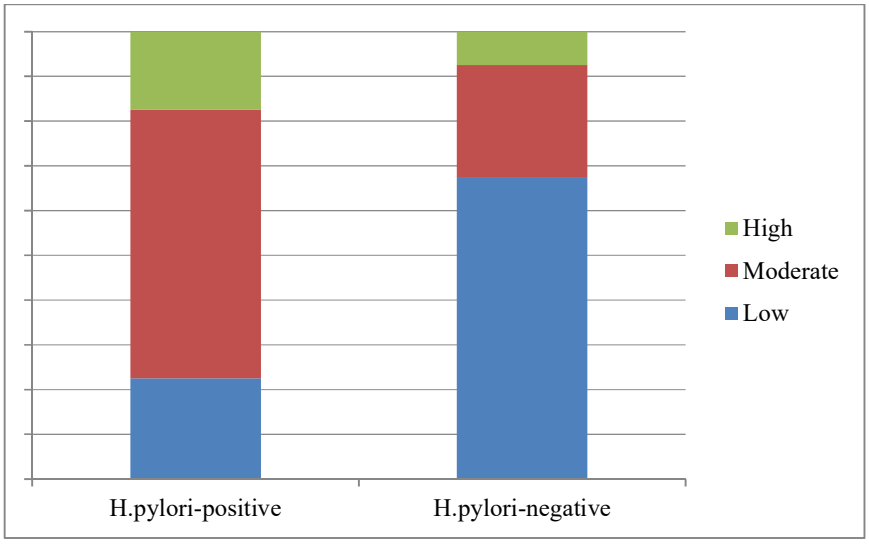


Figure 6. Differences between neuronal SP-expression in in H. pylori-infected subjects and controls

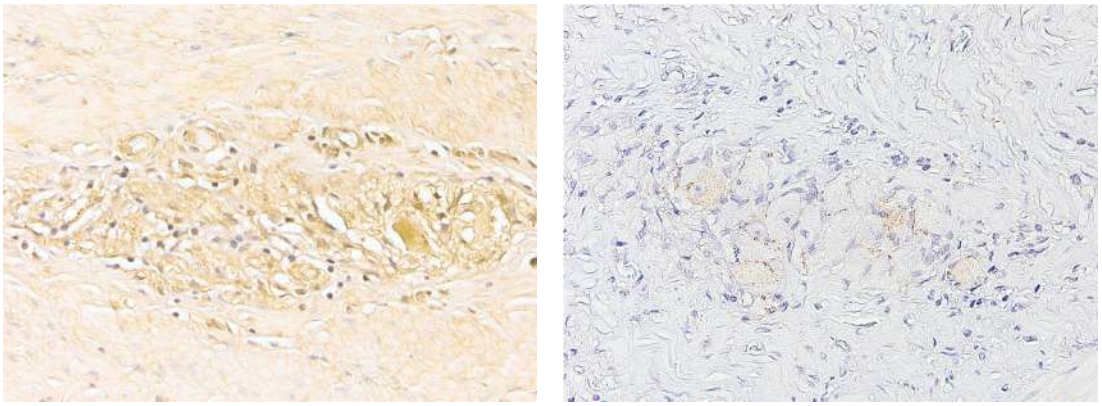


Figure 7. Moderate expression of SP in myenteric neurons in H.pylori-positive group (left image), compared to weak SP expression in controls (right image)

The density of SP immunoreactive mucosal nerve fibers was significant higher in H. pylori-infected patients than in controls. Most of them had a moderate SP expression (13 cases), while in the non-infected cases, the density of SP-positive nerve fibers was predominantly low (22 cases) - Fig.8. The correlation between SP neuronal and mucosal nerve terminations expression was statistically significant in H. pylori-positive subjects.

In H.pylori-infected group, a significant correlation was observed between the degree of ganglionitis and SP reactivity in myenteric neurons. On the other hand, the number of SP-reactive mucosal nerve fibers didn`t correlate with the degree of bacterial colonization, the intensity of mucosal inflammatory response nor with grade of neutrophilic activity.

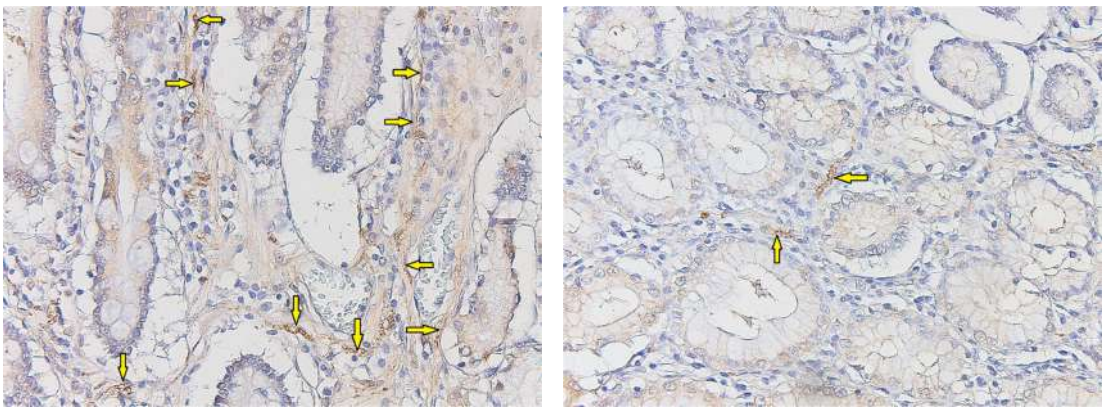


Figure 8. Moderate density of SP-immunoreactive nerve fibers in H.pylori-positive patients (left image), compared to controls (right image)

A higher level of VIP neuronal expression was also observed in H. pylori-infected subjects, compared to controls (Fig. 9). More than half of H. pylori-positive patients

presented a high expression of the neuropeptide in myenteric neurons, in contrast with the control group, where the level of VIP was predominantly low.

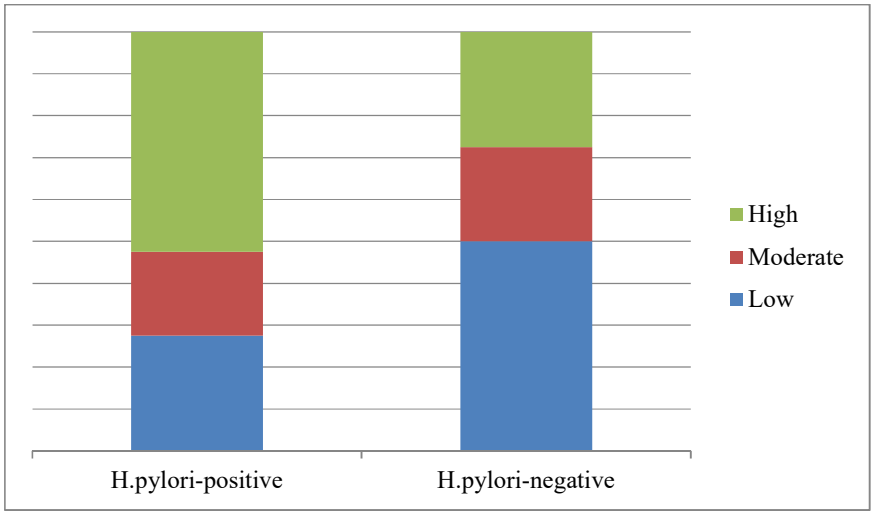


Figure 9. Differences between neuronal VIP-expression in *H. pylori*-infected subjects and controls

As expected, density of VIP expression in mucosal nerve endings was also higher in H. pylori-infected patients than in controls. The majority presented moderate (22 cases) or high VIP levels (12 cases), while in the non-infected cases, the density of VIP-positive nerve fibers was predominantly low (29 cases).

Surprisingly, we didn`t identified a statistically significant correlation between VIP neuronal and mucosal nerve endings expression in H. pylori-colonized subjects. VIP reactivity didn`t significantly correlate with the degree of bacterial colonization nor with the severity of mucosal or myenteric inflammation.

7. Discussion

Inflammation of myenteric plexus

The presence of periganglionic inflammation, referred to as enteric ganglionitis, or plexitis, reflects imbalanced neuro-immune interactions occurring within the enteric neural microenvironment. Gastric myenteric ganglionitis has received little attention in the pertinent literature, being occasionally described in association with gastroparesis or chronic intractable vomiting. In our study, the number of periganglionic inflammatory cells

was significantly increased in H.pylori-positive patients compared to controls. This is an unusual finding, as gastritis is basically a mucosal disease. The immunohistochemical analysis of the myenteric infiltrate revealed a significant component of CD3-immunoreactive T cells, in agreement with previous reports showing that in inflammatory neuropathies there is a predominant T-cytotoxic activity directed against proteins expressed by enteric neurons. However, in the present study, CD20-positive lymphocytes and plasma cells were exclusively identified in patients with H. pylori infection, indicating that, in addition to T lymphocyte activation, humoral immune response also participates in myenteric inflammation. Our results confirm previous data documenting the contribution of mature B cells to the immune response by synthesizing and releasing immunoglobulins directed against antigens expressed by myenteric neurons.

Myenteric neuronal degeneration and apoptosis

Neuronal and nerve process degeneration in myenteric plexus has been documented in patients suffering from inflammatory bowel diseases. In our study, signs of neurodegeneration, such as vacuolated or condensed cytoplasm and pyknotic nuclei, were more frequently observed in infected patients, suggesting that H. pylori can induce neuronal damage in the myenteric plexus tissue. In addition, we observed a significant relationship between injury of myenteric neurons and periganglionic lymphoplasmacytic inflammatory infiltrate. However, a weaker correlation with T-cell myenteric infiltrate was also noted, indicating that degenerative changes of gastric neurons occur as a result of a concerted action of all the inflammatory cell types (including T cells, B cells and plasma cells) recruited within myenteric plexus. Our observations confirm previous data showing the degeneration of myenteric neurons under enteric ganglionitis throughout the alimentary tract.

Bcl-2 antiapoptotic protein plays an essential role in protecting neurons from programmed cell death, promoting their survival in different types of neural injury. In contrast to the temporal pattern exhibited by neurons in the brain, it is considered that bcl-2 expression remains constant throughout adult life in peripheral neurons. Although apoptosis of myenteric neurons was documented in patients with gastrointestinal motor non-inflammatory disorders, it has not been investigated in inflammatory diseases. Our results showed, for the first time, that H. pylori is able to induce programmed cell death in myenteric gastric neurons. This finding is consistent with previous studies showing that H. pylori is able to promote apoptosis in infected gastric epithelial cells and leads to the

conclusion that the bacteria might induce apoptosis dysregulation in different cell types of gastric wall. Moreover, we found a significant association between loss of bcl-2 expression in gastric neurons and periganglionic CD3-positive T lymphocytic infiltrate. This finding suggests that T-cell mediated immune response can trigger activation of the apoptotic pathways in myenteric neurons. This hypothesis is supported by similar observations in central nervous system. Aktas et al. showed that CD4+ T lymphocytes are able to kill neurons in a passive way, independent of antigen presentation, releasing interferon gamma and other cytokines, whereas CD8+ T cells induce neuronal death directly, by recognizing antigens presented by major histocompatibility complex (MHC) class I molecules on neurons in the inflamed brain. Further investigation is required to determine if myenteric neuronal apoptosis in gastric inflammation occurs through similar mechanisms.

Neuronal and glial cell hyperplasia

A very interesting and surprising finding in this study was the neuronal cell hyperplasia observed in patients with H. pylori infection. Variation in the number of enteric neurons was previously described by some authors in inflammatory bowel diseases, while other studies failed to demonstrate any significant difference regarding neuron counting. In the context of increased neuronal damage and apoptosis noted in infected patients, we are presently unable to explain the neuronal cell hyperplasia. In our opinion, the most reasonable hypothesis is that the increased number of gastric myenteric neurons could represents a compensatory response to neuronal injury induced by ganglionic inflammation.

However, there is an even more intriguing question raised by the same observation: how can there be more neurons if they never undergo cell division? For a long time, enteric neurogenesis was thought to occur exclusively during embryonic and early postnatal stages. Nevertheless, an increasing body of evidence has accumulated in recent years, clearly suggesting that postnatal intestinal neurogenesis can occur in some circumstances, although the nature of this neurogenic cell type and the stimuli that activate this process remain largely unknown. One possible explanation could be that human adult gut harbors a pool of enteric neuronal stem cells. These enteric neuronal precursors are probably in a quiescent state of cell dormancy under physiological conditions, but they can be activated and stimulated to proliferate and differentiate in response to neuronal injury. It has been suggested that in the setting of enteric inflammation, bacterial lipopolysaccharides (LPS) could trigger activation of the enteric neural progenitor cells *via* toll-like receptor 4

(TLR4), enhancing their proliferation and differentiation into new neurons and glial cells. In a similar manner, H. pylori could directly act *via* LPS on gastric enteric nervous system, since several studies demonstrated that LPS stimulation of the human myenteric plexus from the stomach *in vitro* leads to proteomic changes of the myenteric plexus tissue.

The second possibility is that enteric glial cells can give rise to neurons through direct transdifferentiation in a serotonin-mediated mechanism. According to some authors, in induced chemical and infectious colitis, glial cell proliferation increased dramatically, yet glial cell numbers remained the same. Concurently, neuron numbers increased and these newly generated neurons strongly expressed glial markers (Sox2, Nestin, and CD49b), but without any evidence of DNA replication, thus providing evidence for a new and unexpected mechanism of enteric neurogenesis. However, further studies are necessary to elucidate if this neurogenic cell population represents mature enteric glia or neural crest-derived progenitors in the gut.

A significant increase in glial compartment was also detected by our analysis. Beside their traditional trophic and supportive functions for enteric neurons, glial cells are involved in enteric neurotransmission, neurogenesis and immune signaling, therefore their number could be influenced by the immune response in the gastro-intestinal tract. Changes in enteric glial cell number were previously reported in patients with inflammatory bowel disease, but were not evaluated in gastric inflammations. In our study, the level of neuronal hyperplasia was twice as great as glial cell hyperplasia degree, suggesting that neurons rather than glial cells were more affected in the H. pylori-positive patients herein examined. However, it is not clear if the proliferation of gastric glial cells precedes or follows neuronal hyperplasia, although some studies support the first hypothesis.

Neurotransmitters expression

SP and VIP are among the most studied neuropeptides in GI inflammation. Besides their classical involvement in the regulation of motility, blood flow and secretory activity, they are also able to play an important role in gut inflammation. In our study, we found an increased density of nerve fibers expressing SP within mucosa during H. pylori-induced chronic inflammation. This is in accordance with previous findings that SP has pro-inflammatory effects by stimulating lymphocytes, monocytes and neutrophils chemotaxis, inducing lymphocyte differentiation and activating macrophages. All these immune cells release a variety of cytokines which can produce local tissue injury described in chronic inflammation.

Additionally, in our study we identified an increased SP expression in myenteric neurons, which correlated with the level of the peptide in the mucosa. These data suggest that SP mucosal expression is due to an increased synthesis in myenteric neurons. On the other hand, a strong correlation was observed between SP neuronal expression and the degree of myenteric lymphocytic infiltrate, which in turn, correlated with T-cell mucosal density. Hence, we hypothesize that the observed augmentation in SP neuronal expression may occur as a result of the inflammatory mediators released locally by lymphocytes recruited within myenteric plexus as a result of mucosal chronic inflammation. In conclusion, SP and the immune response seem to form, in the presence of H. pylori infection, a vicious circle, with each condition promoting the other and thus perpetuating gastric chronic inflammation. This neuroimmune cross-talk hypothesis may explain persistent or recurrent symptoms observed in some patients after bacterial eradication.

Our study identified an increased density of VIP nerve endings in gastric mucosa colonized by H. pylori, data consistent with previous reports. Considering the well documented anti-inflammatory effects of VIP, this finding suggests that the neuropeptide expression may be the result of gastric ENS attempt to control mucosal inflammation by restoring the functional balance between protective and harmfull mediators. In addition, neuronal VIP level was also increased in H. pylori-subjects, but unexpectedly, didn`t correlate with mucosal expression. This lack of association may be explained by the fact that mucosal VIP originates not only from myenteric neurons, but also from a local additional source. In accordance with such an explanation are the previous findings that VIP is produced by immunocompetent cells in the gastrointestinal tract. The second possibility may be the fact that inflammation cause altered storage, transmission or release of the neuropeptide, but further investigation is required to confirm this hypothesis.

Conclusion

In summary, the data presented provide what we believe is the first evidence that gastric nervous system can be morphologically altered by host immune response in the setting of H. pylori infection. We have demonstrated for the first time that neurogenesis and gliogenesis can occur in gastric myenteric plexus during H. pylori-induced inflammation. These findings advance our knowledge of the complex mechanisms of

interaction between pathogen and host and will hopefully pave the road to a more vast scientific investigation in the area of gastric neural plasticity.

Moreover, this study proves that H. pylori infection can trigger altered neuropeptide expression in both mucosal nerve endings and myenteric neurons. SP and VIP act as endogenous mediators with antagonistic effects, modulating and, in turn, being influenced by the immune system. Although the exact role of SP and VIP in H. pylori-induced gastric inflammation needs to be further elucidated, our study suggests a potential future role for SP-receptor antagonists in the therapeutic management of chronic gastritis and gastric cancer.

Fourty years after its discovery, H. pylori still remains an enigmatic pathogen with many secrets yet to be revealed and many unsolved questions to be answered. Our research provides new insights that allow us to gain a better understanding of the pathogen. In addition, this knowledge may enhance the development new personalized and efficient therapeutic strategies of H. pylori-related gastrointestinal disease.

Selective Bibliography

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori infection: Systematic Review and meta-analysis. Gastroenterology 2017; 153(2):420-429.

2. Malfertheiner P, Megraud F, O`Morain CA, Atherton J, Axon ATR, Bazzoli F, et al. Management of Helicobacter pylori infection – the Maastricht IV/Florence Consensus Report. Gut 2012; 61: 646-664.

3. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev 2006; 19(3): 449-490.

4. Budzyński J, Kłopocka M. Brain-gut axis in the pathogenesis of Helicobacter pylori infection. World J Gastroeneterol. 2014; 20(18): 5212-5225.

5. Furness JB. The enteric nervous system. Blackwell Publishing. Oxford. 2006.

6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1(8390): 1311-1315.

7. Costa AC, Figueiredo C, Touati E. Pathogenesis of Helicobacter Pylori infection. Helicobacter 2009; 14(Suppl 1): 15-20.

8. Charitos IA, D'Agostino D, Topi S, Bottalico L. 40 Years of Helicobacter pylori: A Revolution in Biomedical Thought. Gastroenterology Insights. 2021; 12(2):111-135.
9. World Health Organisation. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IACR Monogr Eval Carcinog Risks Hum 1994; 61: 1-241.
10. Lehours P, Yilmaz O. Epidemiology of Helicobacter pylori infection. Helicobacter 2007; 12(Suppl 1): 1-3.
11. Abadi ATB. Strategies used by helicobacter pylori to establish persistent infection. World J Gastroenterol. 2017; 23(16): 2870-2882.
12. Testerman TL, Morris J. Beyond the stomach: an updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. World J Gastroenterol. 2014;20(36):12781-808.
13. Amieva MR, El-Omar EM. Host-Bacterial Interactions in Helicobacter pylori Infection. Gastroenterology. 2008; 134: 306-323.
14. Konturek PC, Konturek SJ, Brzozowski T. Helicobacter pylori infection in gastric cancerogenesis. J Physiol Pharmacol 2009; 60: 3-21.
15. Gershon MD. The enteric nervous system: a second brain. Hosp Pract (1995). 1999; 34(7): 31-2, 35-8, 41-2 passim.
16. Progatzky F, Pachnis V. The role of enteric glia in intestinal immunity. Curr Opin Immunol. 2022; 77: 102183.
17. Jakob MO, Kofoed-Branzk M, Deshpande D, Murugan S, Klose CSN. An Integrated View on Neuronal Subsets in the Peripheral Nervous System and Their Role in Immunoregulation. Front Immunol. 2021; 12: 679055.
18. Mandić P, Filipović T, Gasić M, Djukić-Macut N, Filipović M, Bogosavljević I. Quantitative morphometric analysis of the myenteric nervous plexus ganglion structures along the human digestive tract. Vojnosanit Pregl. 2016 ; 73(6): 559-65.
19. Ippolito C, Segnani C, De Giorgio R, Blandizzi C, Mattii L, Castagna M, et al. Quantitative evaluation of myenteric ganglion cells in normal human left colon: implications for histopathological analysis. Cell Tissue Res. 2009; 336(2): 191-201.
20. Villanacci V, Bassotti G, Nascimbeni R, Antonelli E, Cadei M, Fisogni S, et al. Enteric nervous system abnormalities in inflammatory bowel diseases. Neurogastroenterol Motil. 2008 ; 20(9): 1009-16.
21. Berčík P, De Giorgio R, Blennerhassett P, Verdú EF, Barbara G, Collins SM. Immune-mediated neural dysfunction in a murine model of chronic Helicobacter pylori infection. Gastroenterology. 2002; 123: 1205-1215.

22. Sipos G, Altdorfer K, Pongor E, Chen LP, Feher E. Neuroimmune link in the mucosa of chronic gastritis with Helicobacter pylori infection. Dig Dis Sci. 2006; 51: 1810–1817.
23. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. Cell Mol Life Sci. 2016; 73(22): 4249-4264.
24. Ganea D, Hooper KM, Kong W. The neuropeptide vasoactive intestinal peptide: direct effects on immune cells and involvement in inflammatory and autoimmune diseases. Acta Physiol (Oxf). 2015; 213(2): 442-52.
25. Kulkarni S, Micci MA, Leser J, Shin C, Tang SC, Fu YY, et al. Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis. Proc Natl Acad Sci U S A. 2017; 114(18): E3709-E3718.

PREVIOUSLY PUBLISHED WORKS

- **Sticlaru L**, Stăniceanu F, Cioplea M, Nichita L, Bastian A, Micu G, Popp C. Dangerous Liaison: Helicobacter pylori, Ganglionitis, and Myenteric Gastric Neurons: A Histopathological Study. Anal Cell Pathol (Amst). 2019 Dec 30; 2019:3085181. doi: 10.1155/2019/3085181. PMID: 32082967; PMCID: PMC7012220 – lucrare publicată în revista *Analytical Cellular Pathology*, ISSN / eISSN 2210-7177 / 2210-7185, ISI impact factor 4,133;
https://www.hindawi.com/journals/acp/2019/3085181/

- **Sticlaru L**, Stăniceanu F, Cioplea M, Nichita L, Bastian A, Micu G, Popp C. Neuroimmune cross-talk in Helicobacter pylori infection: analysis of substance P and vasoactive intestinal peptide expression in gastric enteric nervous system. J Immunoassay Immunochem. 2018; 39(6):660-671. doi: 10.1080/15321819.2018.1529683. Epub 2018 Oct 16. PMID: 30325259 – PubMed indexed;
https://www.tandfonline.com/doi/abs/10.1080/15321819.2018.1529683?journalCode=ljii20

- **Sticlaru L**, Bastian A, Micu G, Stăniceanu F, Popp C. Functional and morphological alterations induced by Helicobacter pylori infection in gastric nerve supply. Rom J Intern Med. 2014;52(3):192-7. PMID: 25509565 – PubMed indexed.
https://pubmed.ncbi.nlm.nih.gov/25509565/