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The Role of Morphometry in Diagnostic of Chronic Gastritis

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1. Introduction

Chronic gastritis is one of the most frequent gastric diseases. The morphometry in diagnostic of chronic gastritis can as appear to be a potentially significant tool. The evaluation of the histoarchitectural structure of the gastric mucosa and quantification of the various neuroendocrine cells develop the new objective methods to describe changes caused by chronic gastritis. The development of specimen digitalization and computer image processing systems offer possibilities of automatization of this process on every high level.
Based on the endoscopic evaluation and mucosa biopsy analysis, variable numbers and various subtypes of the neuroendocrine (NE) cells have been described in association with different types of chronic gastritis. The neuroendocrine cell population, such as enterochromaffin-like cells (ECL), G cells, and D cells may occur as hyperplastic lesions, hypergastrinemia, hyperplasia, dysplasia, possibly type-1 gastric tumour or proliferation as an indirect effect of modern drugs, suppressing acid secretion (the post-pharmacotherapeutic hypo- or achlorhydric status). The wide range of methods used for evaluation of NE cells density in the histological slides from counting the number of cells per one microscopic field to shape diversity of gastric glands are frequently discussed in the literature. However, there is still a lack of acknowledged evidence on the participation of NE cells in stomach inflammation pathology and the relation between NE cells density and type of chronic gastritis require further investigation. The morphometry analysis of the mucosa specimen supported by the computerized automatic evaluation can be frequently taken into account in order to reflect the mechanism of stomach inflammation. In that analysis, other factors such as presence of Helicobacter pylori, sex and anatomical region of biopsy should be included and discussed.
In this chapter we focused on the following topics:

- Investigation of the role of different types of NE cells in chronic gastritis.
- Methodology of the evaluation of NE cells population in mucosa histological slides – different approaches and measures.
- Statistical investigation of the observed variations in the NE cells population with respect to diagnosis (type of chronic gastritis), anatomical region, Helicobacter pylori, sex, hyperplasia and other factors.
- Selected aspects of the computerization of automatic quantitative evaluation and space distribution of the NE cells, evaluation of the shape of mucosa glands and

morphometry analysis, for digital images of the selected field of view and virtual slides of the whole mucosa specimen.

2. General morphometric feature of chronic gastritis

The diagnosis of chronic gastritis is a complex problem which includes the evaluation of different clinical and histological features. This problem drew attention of many researchers, however, the consensus has not been reached. Following numerous classification systems, since the 90's of XX century the Sydney System has been used. In 1994 it was updated and added certain modifications to improve the criteria of atrophy evaluation. The commonly used criteria, presented in the chapter "Chronic gastritis" with the quality evaluation of the specimens does not give specific results in many diagnostic cases. In the light of the recent researches, the diagnostic system will be developed and improved in near future by the morphometric quantitative evaluation of different aspects of the observed changes.

Evaluation of neuroendocrine cells, which was introduced into diagnostic, provides new objective methods to extend the analysis of chronic gastritis. The first works are dated back to the 70's of XX century and focused mainly on the detection of neuroendocrine cells also in gastric mucosa. The gastric neuroendocrine cells represent the diffuse neuroendocrine cells system (DeLellis et al., 1984; Falkmer & Wilander, 1995; Lloyd, 1999; Osamura, 2002). This system contains also the APUD cells (Amine Precursors Uptake and Decarboxylation cells), first described and classified by Pearse (Pearse, 1969, 1974; DeLellis et al., 1984). All these cells originate from the stem cells of alimentary duct (Fenoglio-Preiser, 1999; Furth, 2002). Currently, according to Pears and Takor-Takor concept (Pearse & Takor-Takor, 1979) about 40 different types of the neuroendocrine cells can be distinguished.

The neuroendocrine cells of the gastric mucosa are normally located in the epithelium layer of the mucosal glands in prepyloric and oxyntic parts of stomach, sometimes in the stem part and never in the superficial epithelium of the glands of foveolar layer of gastric musoca (Dayal, 1992; Kozlowski et al., 1995; Nichols et al., 1974; Ohning et al., 1998; Portela-Gomes & Grimelius, 1986). The most numerous cells are ECL (entorechromaffin-like) cells, G (gastrinin) cells, D (somatostatin) cells and ECn (Enterochromaffin) cells. The ECL, G and D are more than 75 percent of neuroendocrine cells of gastric mucosa in oxyntic and pyloric parts (D'Adda et al., 1989; Dayal, 1992; Falkmer & Wilander, 1995; Fenoglio-Preiser , 1999). The ECL cells (this name was introduced by Hakanson et al. in 1967) are distributed in the deep 2/3 layers of gland epithelium in oxyntic and the fundus of the stomach (Bordi, 2000; Dayal, 1992; Falkmer & Wilander, 1995; Ohning et al., 1998; Solcia, 1988). They represent 30-44% of all neuroendocrine cells in these locations. Considering practical aspects, immunohistochemical methods are more valuable in detecting these cells by e.g. chromogranin-A reaction (Date et al., 2000; Dayal, 1992; Falkmer & Wilander, 1995; Whitehead, 1995). The G cells are localized only in the gland epithelium in pyloric part of gastric mucosa, mainly in the 1/3 middle region (Kozlowski et al., 1995). These cells give positive reaction not only with gastric stains, but also in chromogranin-A stain (Kinoshita et al., 1998). The D cells can be detected by anti-somatostatin reaction and ECn cells in anti-serotonin stain. The depicted monoclonal antibodies are the most specific and useful for the quantitative evaluation of several neuroendocrine cells for pathological evaluation of gastritis.

2.1 Morphometry of neuroendocrine cells

The relationship between hypergastrinemia and Helicobacter pylori colonization proved in the 90's initiated a renaissance of gastric mucosa neuroendocrine cells examinations. Initially there were examinations of mutual relations between G and D cells, later other gastric mucosa endocrine cell types were taken into the consideration. Up to now there is lack of acknowledged evidence on the participation of cells of this type in the stomach inflammation pathology. The dependence of the density of the neuroendocrine cells such as D, EC or ECL on the type of stomach disease was confirmed in previous publications (Bordi et al., 2000; Falkmer & Wilander, 1995; Kozlowski et al., 1995, 2001, 2003a, 2003b). An interesting recently published study (Peterson et al., 2009) presents the comparison between G-cells morphometry performed in three different manners: G-cell counting and presented as a ratio between its number and 1000 epithelial cells; image analysis gives the results as a ratio between the brown stained area to the total cytokeratin positive area and point counting in the superimposed grid. As far as now, there are significant differences in the used methods of morphometry. As far as these methods are concerned, we focused on the manners based on the cell recognition. They can be distinguished from other approaches either with or without consideration of the histoarchitectural structure.

2.1.1 Quantification the neuroendocrine cells in field of view

The most basic approach of the neuroendocrine cell quantification is count cells in the field of view without taking into account the observed histoarchitectural structure. As described in the study by Green et al., 1989, the neuroendocrine cells are counted in a few fields of view, with 200x magnification and results can be recalculate as the mean of cell quantity per one field. Generally, this method requires only the recognition of immunoreactivity of each cell in the field of view and counting them.

First, it should be noted that different distributions of the neuroendocrine cells in the area of the mucosa cross-section are observed. In the corpus of the stomach, the neuroendocrine cells are observed in the about 2/3 width of epithelium cross-section. Their distribution is approximately regular as it is presented in the Fig. 1.

The opposite cases are observed in the antrum where there is a high concentration of the neuroendocrine cells in the basis of foveolar region of the mucosa in cross-section (Fig. 2).

Accuracy of this approach is strongly influenced by the fact that the absolute number of the immunoreactivity cells in the field of view relates to the specimen orientation, thickness or size. As it can be seen, this quantification approach required selection of the field of view located exactly in the region with the presence of neuroendocrine cells. In the opinion of the authors, the calculation of the ratio between immunopositive and immunonegative cells in the gastric mucosa only in the region with presence of the neuroendocrine cells should be appropriate but more difficult to perform as it requires the specification of this area. A subjective opinion on location of neuroendocrine area border will have significant influence on the results.

2.1.2 Quantification the neuroendocrine cells in histoarchitectural structures.

The more complex approach, comparing to the one described above, is calculation of the number of cells per mm2 of the area of the mucosa glandular epithelium or per one gland (Aruin et al., 1984; Tzaneva & Julianov, 1999; Azzoni et al., 1996). The last proposition is particularly interesting, yet its implementation requires recognition of separate glands in the microscopic image. The benefit is additional possibilities of measuring the mucosa gland dimensionality.

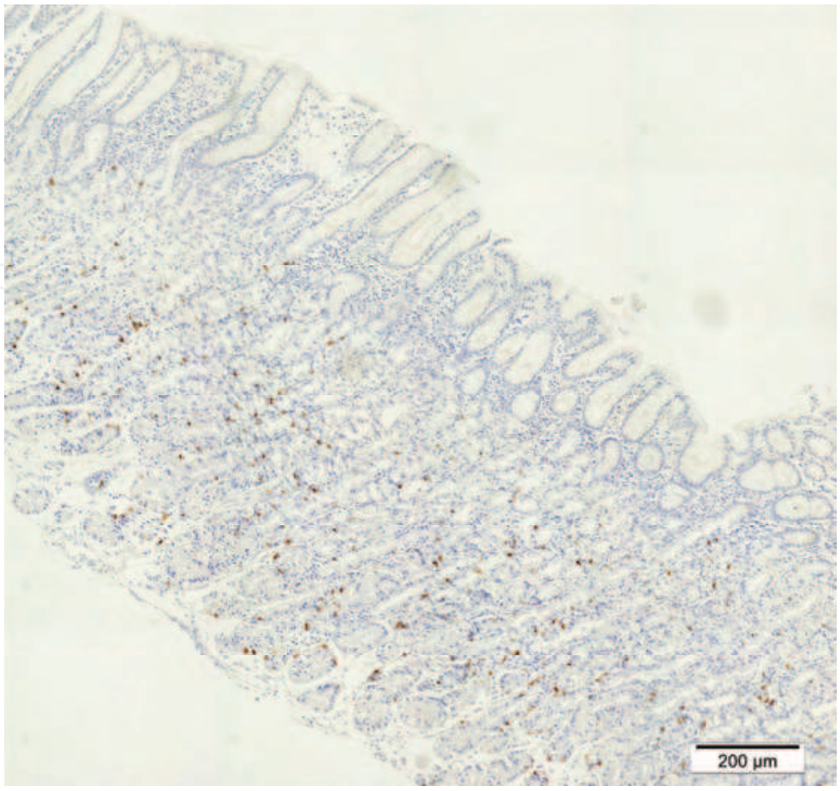


Fig. 1. The distribution of the neuroendocrine cells in the corpus mucosa (chromogranin-A, virtual slide).

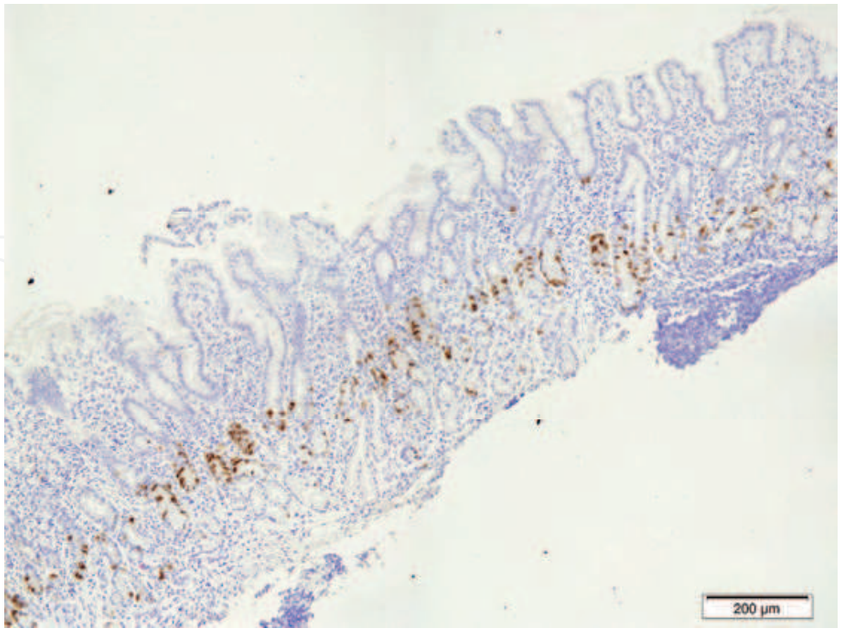


Fig. 2. The example the neuroendocrine cell localization in the antrum (chromogranin A, virtual slide)

It should be noted, that the obtained long cross-section shapes of the glands are very rare. The type of the specimen excludes efficient control over the section and as a result, the obtained shapes of gland sections located from epithelium to mucosa are rather round than ellipsoidal, as it is presented in Fig. 3.

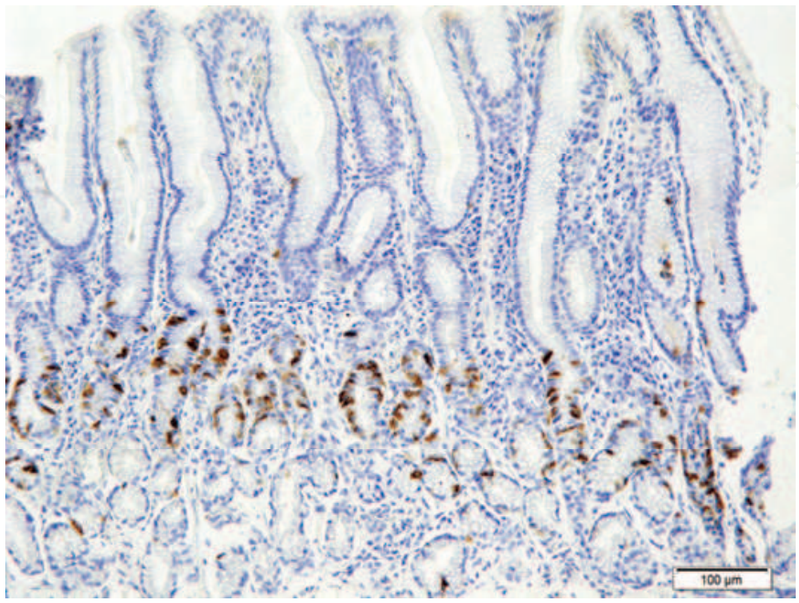


Fig. 3. Explanation of different shapes of glands (chromogranin-A, virtual slide).

Efficient evaluation of the number of neuroendocrine cells in the glands can be performed only by counting these cells in the sections of the glands. To support this evaluation, the shape of gland cross-sections should be taken into account.

This approach is connected with several problems that should be taken into account in the counting process. The first problem is to qualify particular glands for evaluation according to their cross-section. It is evident, that in the microscopic specimen some glands are cut not centrally by their lumen, but closely or exactly by their wall. In that glands the lumens are reduced to the narrow line area or only cell cluster is observed. These cases render difficult the recognition of the separate glands.

The second problem is the qualification of the glands to counting set according to its space location. This problem is illustrated in Fig 4.

Gland no. 1 is located half outside and half inside neuroendocrine region (black line outline) and its cross-section fully covers the width of this region. Gland no. 2 is fully included in the neuroendocrine area while gland no. 3 has only a small part located in this region and does not represent the full width of the measured region. For efficient counting the neuroendocrine cells in the separate glands, only glands no. 1 and 2 should be included.

The calculation of the neuroendocrine cell density based on this approach can be implemented by calculating the number of neuroendocrine cells per recognized gland, qualified for evaluation, with description of gland shape. In practice, the semi-quantitative method can be used - the glands can be divided into four groups depending on their cross-section shape: round, short ellipse, long ellipse and open. The criteria, presented in the studies (Kozlowski et al., 2009; Markiewicz et al., 2009a), are:

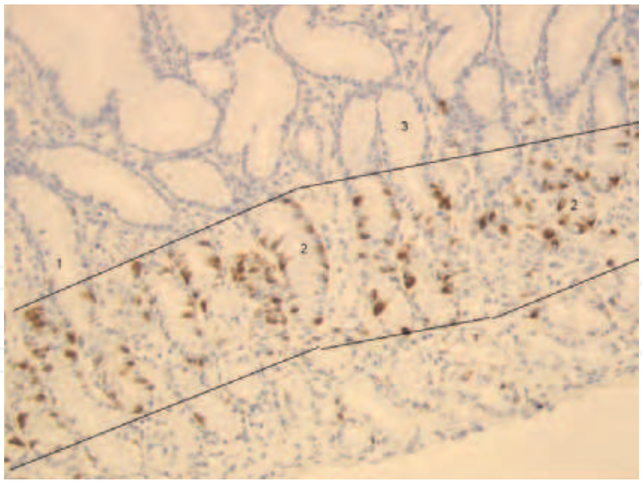


Fig. 4. Different level of inclusion of the glands in the neuroendocrine area.

- round shape – proportion between major and minor axis lengths is close to 1 (e.g. less than 1.2),
- short ellipse - proportion between major and minor axis lengths is less than 2,
- long ellipse - proportion between major and minor axis lengths is higher than 2,
- open – the gland cross-section shape is open.

The final step of the quantitative evaluation is a calculation of the number of neuroendocrine cells, detected in maximum 5 glands of any shapes described below. The recognition of the gland structures in the images gives possibility to count the ratio between numbers of neuroendocrine cells and all the cells of the mucosa glandular epithelium. Also, various geometrical features of the gland shape can be measured in these approaches.

2.2 Diagnostic influence of neuroendocrine cell morphometry

The connection between neuroendocrine cells and inflammatory disorder of the stomach is the subject of many researches. One of the observed relations deals with G cells hyperplasia in the hypochlorhydria or achlorhydria. The role of the gastrin as a regulator of the proliferation of G, ELC and D cells and their evaluation also required the morphometry of neuroendocrine cells (Calatayud et al., 2010). In another study, high correlation between age, H. pylori colonisation and atrophy of the corpus and G-cell density (Petersson et al., 2009) was reported. The degree of chronic inflammation and percentage of the G-cell was also statistically correlated. The endocrine cell hyperplasia can be developed as a consequence of functional changes and is the most prominent of autoimmune chronic gastritis. In antrum the endocrine hyperplasia is easily detected in H&E stain, but in oxyntic mucosa the immunohistochemical stains give better visualization and quantification of the changes in ECL cell population. The most detailed criteria for diagnosis and classification of gastric ECL proliferation was proposed by Solcia et al., 1995. The details are presented in table 1.

Also the changes in the number of D and G cells in atrophic gastritis were observed and several studies presented the therapy promoting the secretion of gastric acid and gastric pepsin and regulating the neuroendocrine mechanism in rats (Zhu et al., 2008; Wang et al., 2009; Czaja et al., 2008; Todorovic et al., 2008). However, as it was reported (Czaja et al., 2008), changes of serum gastrin concentration in gastric antrum in children with chronic gastritis are not statistically significantly connected with D and G cell densities.

Diagnosis	Criteria regarding increased endocrine cells	Common disorder
Simple/diffuse hyperplasia	>2x standard deviation (age/gender matched)	ZES, primary gastrin cell hyperplasia
Linear hyperplasia	Linear groups of five or more inside the glandular BM	ZES, pernicious anemia
Micronodular hyperplasia	Clusters of five or more cells within epithelium measuring <150 microns in diameter	Autoimmune atrophic gastritis
Adenomatoid hyperplasia	Aggregates of five or more micronodulares in lamina propria	Autoimmune atrophic gastritis, MEN-ZES
Dysplasias		Autoimmune atrophic gastritis, MEN-ZES
Enlarged micronodules	>150 microns	
Adenomatous micronodules	Collection(s) of at least five closely adherent micronodules, intervening BM only	
Fused micronodules	Adenomatous nicronodules with no intervening BM	
Microinfiltrative lesions	Infiltration of the lamina propria	
Carcinoids		Autoimmune atrophic gastritis, MEN-ZES
Intramucosal	Expansile/infiltrative nodules > 0.5 mm	
Invasive	Any size tumor within submucosa	

BM, Basement membrane; ZET, Zollinger-Ellison syndrome; MEN-ZET, multiple endocrine neoplasia-Zollinger-Ellison syndrome.

Table 1. Classification of ECL-cell proliferations (originally from Solcia et al., 1995).

Based on our clinical material, the authors of this chapter study the correlation between two types of chronic gastritis (superficial and deep) and neuroendocrine cells such as Chromogranin A positive cells, D cells and EC cells, identified by immunohistochemical stains. The sex and antrum/oxyntic parts are taken into account in the quantitative analysis.

3. Study of the neuroendocrine cell in chronic gastritis

3.1 Material and methods

The three types of primary antibodies has been applied:

- Monoclonal Mouse Anti-Human Chromogranin A, clone DAK-A3, code M 0869 DAKO

- Monoclonal Mouse Anti-Serotonin, clone 5HT-H209, code M 0758 DAKO
- Rabbit Polyclonal Anti-Human Somatostatin, code A0566 DAKO

Density of the following types of the cells has been further evaluated:

- Neuroendocrine cells (Chromogranin A positive cells)
- EC cells (Serotonin positive cells)
- D cells (Somatostatin positive cells)

Patients from the Military Hospital in Warsaw in Poland suffering for stomach diseases from the 1999 to 2010 were selected.

The slides used in further quantitative analysis should fulfil the following histological criteria: proper sections (automatic fixed paraffin sections, slide thickness from 3 to 5 micrometers), the right technical quality of tissue samples and the correct histo- and immunohistochemical staining, lack of any mechanical injury or any artefact (fragmentation, hemorrhages).

The morphometric evaluation of the specimens with the counting of the cell density in the field of view was performed using the acquired images of 400x magnification, at resolution of 768x576 pixels. Images were taken using Olympus BX50 microscope with the Olympus Camedia C-3030 camera and used for computer analysis. In semi-quantitative part of the studies, human experts count the number of neuroendocrine cells per one gland in five selected fields of view in each specimen.

3.2 Computerized image analysis

In the last few years many computer programs for automation of quantitative analysis of microscopic specimen, based on the image processing, have been developed (Bartels et al., 1996; Lezoray et al., 2000; Markiewicz et al., 2006, 2009b, 2010). In chronic gastritis the main task of computer automatization is the quantitative evaluation (recognition and counting) of the neuroendocrine cells. In last few years we proposed various approaches and programs used in the different types of the tissues and markers (Markiewicz et al., 2006, 2008, 2009) and our experience confirmed their accuracy. The developed programs used mainly the mathematical morphology operations, such as erosion, dilation, opening, closing or watershed method (Soille, 2003; Matlab, 2010). Counting neuroendocrine cells marked by the immunohistochemistry stains, such as chromogranin-A, serotonin, somatostatin or PCNA can be performed automatically by the program in a particular field of view. The automatization of these analyses can give an answer to the methods based on counting the cells in the field of view without taking into account any specific histological structures.

For evaluation of the density of the neuroendocrine cells the authors designed the automatic program written in Matlab language. The correct recognition of the separated nuclei and reactivity of these cells is the main task in the appropriate evaluation of the distribution density of the selected stained endocrine cells. The primary antibodies used (Chromogranin A, Serotonin and Somatostatin) stains of cytoplasmic structures. In the correct stain the nuclei are blue and only cytoplasm in the immunopositive cell is brown. The problem of recognition can be solved by the following steps: extraction of the blue nuclei of the cells and their classification into two groups – immunopositive and immunonegative cells based on the neighbouring cytoplasm. However, usually in the field of view some cells are stained fully brown. This results from the fact that cutting plane of the tissue specimen may go through any possible cutting levels of the cell. Moreover, some cells in the slide are only viewed as a part of the cytoplasm. Additionally, the nuclei are sometimes covered by the

cytoplasm. According to this fact, the segmentation algorithm should posses two extraction lines: one for the cells with recognized blue nucleus and the second for recognition of the immunopositive cells without the blue nucleus.

The input image for quantitative analysis is in the form of RGB standard file. The first step is the image standardization, which means the calculation of the average RGB values of the lighter area in the field of view and linearly transformed RGB values of all pixels in the image in such a way that the lighter area will be exactly white. This step eliminates the influence of the differences in the glass transparency, lighting and other unstable values.

After standardization, the extraction of the blue nuclei as separate cells was performed. In the literature (Bartels et al., 1996; Lezoray et al., 2000; Markiewicz et al., 2006, 2008, 2009, 2010b; Kayser et al., 2006) the basic method for solving this task is the threshold operation, which is defined as follows: (Soille, 2003)

$$T_{[0,t_2]}[f(x)] = \begin{cases} 1 & \text{if} \quad 0 \le f(x) \le t_2 \\ 0 & \text{else} \end{cases} \tag{1}$$

were f(x) is the value of pixel of the image f in the x position and t_2 is the threshold value. This operation was done on the greyscale images, received by means different from the colour image, with one threshold value. Generally, this is a very difficult task because the nuclei are stained at different intensity and selecting one threshold value for this operation is problematic, and for some images even not possible.

In the papers (Markiewicz et al., 2008, 2009) the authors suggest a solution to this problem based on the threshold operation performed step by step with the increased threshold value. For the stomach specimen images we use the thresholding operation sequentially and apply an artificial neural network of Support Vector Machine (SVM) type (Vapnik, 1995; Schölkopf & Smola, 2002). The idea of this network is to create a hyperplane dividing the feature space of the input data into two separated parts with the maximum margin between them. In our case the input data are the pixels selected from the image. The input vector is composed of three colour components in RGB standard. For learning phase these data are representative for three classes: blue nuclei, brown cytoplasm and light background. Because one network recognizes only two classes, we must build three SVM networks for recognizing pixels between all pairs of classes and then use the one-against-one strategy to find the winner (Schölkopf & Smola, 2002). The learning data are manually selected from the sampled images and their quantity was 150 pixels per class. The output of the network is determined using the following formula:

$$D(\mathbf{x}) = \mathbf{w}^T \mathbf{x} + b \tag{2}$$

were w is the weight vector, $\mathbf{x}$ is the input vector and b is the bias. For learning process D(x) is defined as 1 for the first class and -1 for the alternative class in any pair of the classes. The learning of the network is understood as a task to find the optimal $\mathbf{w}$ and b values with the help of the quadratic programming with Lagrange multipliers α_k (Vapnik, 1995; Schölkopf & Smola, 2002). As a result of training we get:

$$\mathbf{w} = \sum_k \alpha_k d_k \mathbf{x}_k \tag{3}$$

and

$$b = d_k - \mathbf{w}^T \mathbf{x}_k \tag{4}$$

where d_k is 1 or -1 and α_k is the nonzero Lagrange multiplier corresponding to the k-th training data. Usually most multipliers are zero and these data points do not influence the solution.

After learning of the network the weights and bias are fixed. In the testing phase the input vectors represent the colour values of all pixels in the image. They are put to the SVM. It is evident that the output value D(**x**) will be different, depending on its relation to the respected class. For example, if the pixel is light blue, the D(**x**) signal of the SVM recognizing the nuclei will take 0<D(**x**)<1 values. For the dark blue pixels this value will be higher than 1. Based on this relevance we use D(**x**) value as an indicator of the recognized class. In the constructed algorithm we use sequential thresholding operation starting from the minimum D(**x**) value as a threshold. Then, this threshold is increased step by step until the maximum. In any step D(**x**) for the whole image is thresholded and the separated objects, whose area are in the selected range, are added to the mask of the recognized nuclei. The result of this process will be the mask of all blue nuclei for which the area is in the preselected range. This range is selected on the basis of some knowledge and the image resolution with some margin. This process is supported by the watershed operation for the bigger cells to divide them into two cells if there is a narrow space between the two or more parts of them. This will help in the case of the overlapping nuclei of the cells.

The next step is the recognition of the immunopositive cells. This is performed with the use of the SVM and mathematical morphology operations such as closing and reconstruction. First, the brown cytoplasm is extracted by using the one-against-one strategy with three SVM networks. Next, all brown areas are closed by the structural element with the disk shape of the diameter of 7 pixels long. This operation outputs the mask of the brown cytoplasm with filled internal area of them. This filled area should be only on the internal side of the cytoplasm that is on the nuclei of the cell. If the immunopositive cell is touched with the other immunonegative cell, the second nucleus should not be selected. In practice, we can tolerance some errors in the case of overlapping.

The last process of segmentation is the extraction of still unrecognized immunopositive cells without the distinct blue nuclei. They are created from a brown mask, received in the previous step of the algorithm. Any separated brown objects that did not possess the blue nucleus and lie in the selected range of the cell area are added to the set of the immunopositive cells after a watershed operation dividing the touched items.

The final task is to count the cells according to their immunoreactivity classes. This is done in the form of simple counting of any separated objects in the masks of immunopositive and immunonegative cells, independently. Figure 5a presents a sample result of recognition in the Chromogranin stain. The immunopositive cells are marked "○", the immunonegative "+", in yellow and red colours respectively. The results for the serotonin and somatostatin stains of the same patient specimens are presented in Fig. 5b and c respectively. We evaluated more than 30 patients and the achieved accuracy was on the acceptable level. Less than 5% of the cells were unrecognized or misclassified. The developed program was written in Matlab language and tested with PC Centrino Duo 1.86 MHz, 2GB RAM. The result for one image is received in less than 1 minute. It is possible to correct the classification results of the cell manually by the intervention of a human expert.

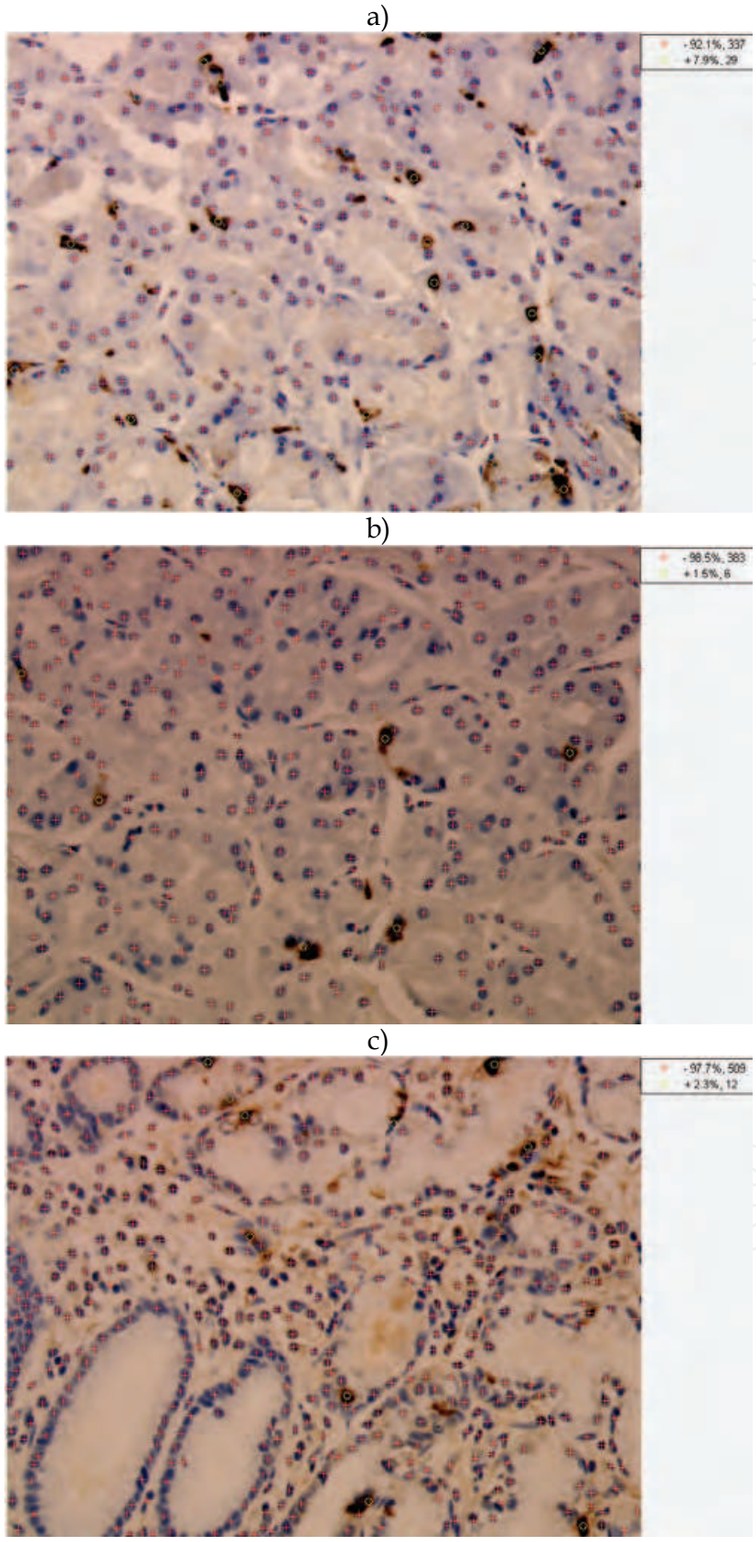


Fig. 5. The result of cell recognition in chromogranin-A (a), serotonin (b) and somatostatin (c) stains.

Calculating the number of neuroendocrine cells per one gland of the gastritis mucosa is a more complex process. The system must recognize also separate glands, measure their shape and count cells according to this histological structures. Also, based on the computerized image processing, the selected aspects of histological evaluation of the gastric mucosa can be performed. That can be gland distribution in the mucosa, proportion between gland lumen and its size. To perform the gland extraction from the image, first the cell nuclei should be recognized based on the algorithm described before. The input stage is a binary mask composed of the immunonegative and immunopositive cells. Although the contours of gland cross-section are not visible (in particular as a continuous shape), the gland face cells created the composition of the linearly bordered objects with blue stained nuclei and sometimes brown stained cytoplasm (only immunopositive cells). In comparison with other cells, the gland face cells are located more closely to each other. In practice, only gland face cells created the convex structures in the image. To reconstruct the completed gland outlining, the spaces between these cells should be filled. This task can be accomplished with the help of the Hit-or-Miss transform (HMT) with anisothropic structure element (SE) of a 35 pixels line shape (Soille, 2003; Markiewicz et al., 2009a). The based pixel of SE was a center point on the line shape, the SE foreground was formed by 3 last pixels at both ends; the rest of pixels of the line shape formed background. Because of the multipolarity and the gland contour in the image, and in the effect unconnected cell nuclei, the reconstruction process carried out by HMT should be performed in the series of SE rotation (every 5° in the scale from 0° to 175°).

Also, this operation is repeated with 23 pixels SE for obtaining the full continuous gland shape. The results of the gland face reconstruction are presented in Fig. 6. Based on the area criteria only dominant objects composed of the gland lumen and cells surrounding them should be selected as a gland cross-section planes. The recognized glands can be also categorized based on the morphometrical criteria, mainly by their major and minor axis lengths. The details of this approach were presented in the paper by Markiewicz et al., 2009a.

3.3 Results of quantitative evaluation

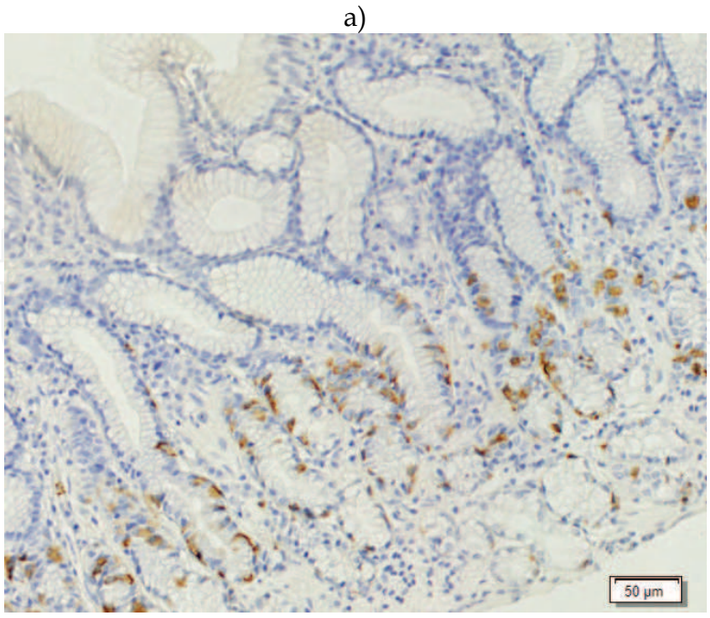
The evaluated specimens were taken from patients with the following diagnosis:

- chronic gastritis (CHG)
- chronic superficial gastritis (CHSG)

The specimens were divided based on oligobiopsy anatomical region – prepyloric or oxyntic part of stomach.

According to the statistical method for the ratio of immunopositive cell, the Chromogranin A, Serotonin and Somatostatin antibodies stain specimens should be evaluated. The images of them have been acquired in the manually selected regions, with the higher number of immunopositive cells in the center, mainly three per slide. As these cells are significanly more numerous in the Chromogranin A antibody stain, the regions were selected on the basis of this stain. In the Serotonin and Somatostatin stains we got images from too same regions. Based on the statistical results we found the threshold of the ratio of immunopositive cells for the selected antibody type. This threshold divided the set of patients into two groups. We evaluated the relation between this division and diagnosis (chronic gastritis and chronic superficial gastritis). Our suggestion on the dependence significance was based on the Fisher exact test (Fisher, 1922). If the returned p –value was

a)



b)

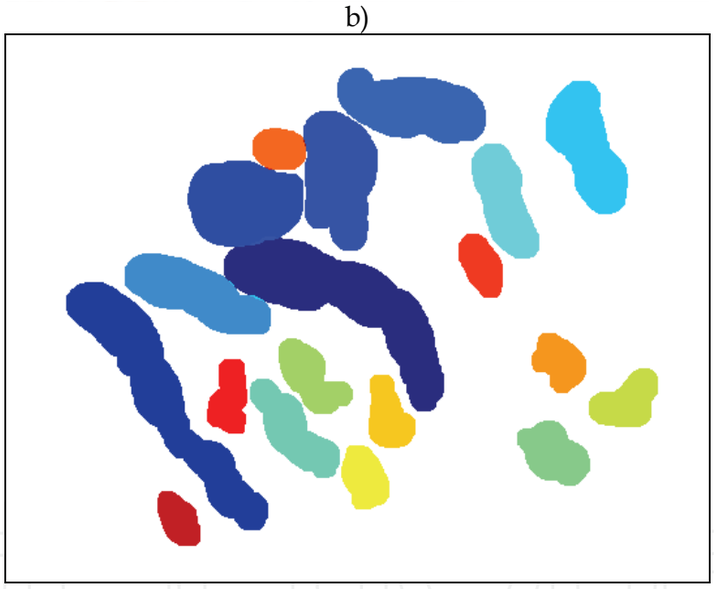


Fig. 6. The sample result of the gland cross-section plane recognition in the image.

less than 0.05, it is justified to reject the null hypothesis on lack of statistical dependence. We signed these cases as positive. The cases with p –value less than 0.07 were signed as conditionally positive with regard to restricted number of items in the data base.

To same items were evaluated with cell divided into the glands of the gastric mucosa, as was defined. The numbers of the positive cells in the recognized gland with respect to their cross-section were countered. The analysis was performed in five view fields. The returned value was calculated as the sum of the mean numbers of the positive cells in the four types of gland cross-section if they were present. Only glands with correct cutting plane were evaluated. If a field was fully packed with the cells in the gland light, this item was not useful for counting. They were divided into a set with a threshold value, similarly to the

previous manner. A difference between the number of items is the effect of the fact that some stains were too bad for automatic system but acceptable for human experts. We calculated the mean value for both examinations to illustrate scale of the difference in the cell density between types of the disease.

The results for the whole neuroendocrine cells (Chromogranin-A positive cells) are presented in table 2. The ratio of the whole neuroendocrine cells in the field of view shows that the significant dependencies with the type of disease are when:

		Ratio between NE and all cells [%]					NE cell quantity calculated in the glands			
Type of specimen	Type of disease	Threshold value and no of items		Mean of ratio	Median of ratio	Fisher test	Threshold value and no of items		Mean of cell number	Fisher test
All patients and both regions		< 5 %	>= 5 %			p=0.0965 negative	< 5.1	>=5.1		p=0.0769 negative
	CHSG	20	11	4.61	4.18		14	16	6.65	
	CHG	17	22	5.12	5.04		10	30	8.94	
Only women, both regions		< 5 %	>= 5 %			p=0.0105 positive	< 5.1	>=5.1		p=0.3175 negative
	CHSG	12	5	4.58	4.27		8	9	7.55	
	CHG	6	16	5.42	5.73		6	15	7.76	
Only men, both regions		<4.49%	>=4.49%			p=1 negative	< 6.4	>=6.4		p=0.1492 negative
	CHSG	7	6	4.65	4.18		8	5	7.35	
	CHG	10	9	4.79	3.88		6	13	10.24	
All patients, only prepy-loric		< 5 %	>= 5 %			p=0.6946 negative	< 9.6	>=9.6		p=0.4328 negative
	CHSG	6	4	5.60	4.88		6	4	10.78	
	CHG	8	10	5.26	5.0		7	11	12.29	
All patients, oxyntic		< 5.07 %	>= 5.07 %			p=0.0618 conditio nally positive	< 5	>=5		p=0.0600 condi-tionally positive
	CHSG	16	5	4.14	4.18		12	8	4.58	
	CHG	10	12	5	5.15		6	16	6.19	
Only women, prepyloric		< 5 %	>= 5 %			p=0.6224 negative	< 7.8	>=7.8		p=0.3287 negative
	CHSG	3	3	5.83	5.79		3	3	8.77	
	CHG	3	6	5.03	5.00		2	7	10.68	
Only women, oxyntic		<5.07%	>=5.07%			p=0.0033 positive	< 5.1	>=5.1		p=0.2203 negative
	CHSG	10	2	3.96	3.71		7	4	4.66	
	CHG	2	10	5.71	6.02		4	8	5.57	
Only men, prepyloric		< 4%	>= 4%			p=0.5622 negative	<13.4	>=13.4		p=0.5638 negative
	CHSG	3	1	5.25	3.89		3	1	13.8	
	CHG	4	6	5.50	4.50		4	5	13.9	
Only men, oxyntic		<4.18%	>=4.18%			p=0.3698 negative	< 5	>=5		p=0.0698 condi-tionally positive
	CHSG	3	6	4.39	4.73		6	3	4.48	
	CHG	6	4	4.15	3.71		2	8	6.94	

Table 2. The dependence between the type of chronic gastritis and the neuroendocrine cells

- patient is a woman, both regions
- region is oxyntic (conditionally)
- patient is a woman and region is oxyntic

However, the results of the Fisher test synonymously indicate, that only for women and oligobiopsy from oxyntic should be considered a significant dependence. The rest of the listed cases have the positive return of the test because they included them. The evaluation of the ratio of the neuroendocrine cells in the other cases has dubious diagnostic value or does not have it.

Comparison of these results counted only in the glands epithelium of the gastric mucosa shows that there are significant differences. A conditionally positive relation was obtained in both evaluation methods only for oxyntic region and patient gender totally. By the use of this type of examination of the specimens we received additional conditionally positive dependence for male patients and mucosa oligobiopsy from oxyntic part of the stomach. A significant dependence for female patients did not reach such significance level as in the ratio method. However, some similar relations exist and this fact suggests that for the recognition of the type of gastritis diseases the ratio method is more adequate for this evaluation than the counting only in the glands epithelium of the gastric mucosa.

The next results for the EC cells (serotonin positive cells) are presented in table 3. The ratio of the EC cells in the field of view shows that the significant dependencies with the type of disease are when:

- patient is a woman, both regions
- patient is a man, both regions
- all patients and regions
- patient is a woman and region is oxyntic

However, the results of the Fisher test for all patients are not precise because there is a high inequality in the number of items in selected division. Additionally, there are significant differences in the level of the ratio between oligobiopsy region that influence the received results. Practically, only in the case of oxyntic region can we say that there are some disease dependencies, but patients must be divided in respect to their sex. For women oligobiopsy from oxyntic gastric mucosa can be considered as a significant dependence, for men it is not unequal. The results received on the set of gland shapes did not show any significant dependencies and for the recognition of the type of the disease they are not useful.

The results for the last considered type of the cells D (somatostatin positive cells) are presented in table 4. The ratio of the D cells in the field of view shows that the significant dependencies with the type of disease are when:

- patient is a woman, both regions
- all patients and regions (conditionally)
- patient is a woman and region is oxyntic

The results of the Fisher test for all patients is conditionally positive and practically only woman can speak about dependence of the ratio of the cell D with the disease. Unfortunately, in our database we have too small number of biopsies from the prepyloric for female patients (with correct somatostatin stain) to decide that this relation is for both regions. We can only think that a certain dependence is for woman patients and in the examination of the oxyntic region. We noted that for all types of staining the difference between median values for both diseases were near 100% of the level in the CHSG. This confirms well recognizable chronic gastritis and chronic superficial gastritis for this case.

		Ratio between EC and all cells [%]					EC cell quantity calculated in the glands			
Type of specimen	Type of diseases	Threshold value and no of items		Mean of ratio	Median of ratio	Fisher test	Threshold value and no of items		Mean of cell number	Fisher test
All patients and both regions		< 0.4 %	>= 0.4 %			p=0.0060 positive	< 3	>=3		p= 0.4533 negative
	CHSG	17	17	0.83	0.60		13	15	3.58	
	CHG	1	31	1.00	0.80		14	25	5.06	
Only women, both region s		< 0.4 %	>= 0.4 %			p=0.0424 positive	< 3	>=3		p= 0.3208 negative
	CHSG	10	8	0.82	0.38		8	7	3.44	
	CHG	4	16	0.8	0.70		7	13	3.89	
Only men, both regions		< 1 %	>= 1%			p=0.0325 positive	< 3	>=3		p=1 negative
	CHSG	9	4	0.86	0.70		5	8	3.76	
	CHG	5	13	1.23	1.26		7	12	3.62	
All patients, only prepyloric		< 1 %	>= 1 %			p=0.4153 negative	< 4	>=4		p= 0.6828 negative
	CHSG	5	5	1.38	1.11		5	4	5.91	
	CHG	5	12	1.29	1.37		7	10	5.03	
All patients, oxyntic		< 0.4 %	>= 0.4 %			p=0.1180 negative	< 3	>=3		p= 0.5308 negative
	CHSG	12	9	0.58	0.39		12	7	2.49	
	CHG	6	15	0.77	0.68		11	11	2.78	
Only women, prepyloric		< 1.5 %	>= 1.5 %			p=0.5805 negative	< 4	>=4		p= 0.2929 negative
	CHSG	3	3	1.52	1.31		3	2	5.69	
	CHG	6	2	1.17	1.12		2	6	5.94	
Only women, oxyntic		< 0.4 %	>= 0.4 %			p=0.0391 positive	< 3	>=3		p= 0.3811 negative
	CHSG	9	3	0.47	0.37		8	2	2.31	
	CHG	3	9	0.55	0.60		7	5	2.53	
Only men, prepyloric		< 1 %	>= 1 %			p=0.2028 negative	<3	>=3		p=1 negative
	CHSG	2	2	1.17	1.11		1	3	6.20	
	CHG	1	8	1.40	1.37		3	6	4.23	
Only men, oxyntic		< 1 %	>= 1 %			p=0.3349 negative	< 5	>=5		p=1 negative
	CHSG	7	2	0.72	0.60		4	5	2.68	
	CHG	4	5	1.05	1.11		4	6	3.07	

Table 3. The dependence between the type of chronic gastritis and the EC cells

Based on the results of morphometry of the neuroendocrine cells with recognition of the gland cross-section shape, the analysis of the correlation between the used measurements and quantity of the cells in one specific gland shape can be performed. Probably, the evaluation of the neuroendocrine cells in short ellipse-shape or long ellipse-shape glands will be representative for the whole population of the cells in a specimen. The analysis of this linear correlation gives the r coefficient equalled 0.726 and 0.809 for the short and long

		Ratio between D and all cells					D cell quantity calculated in the glands			
Type of specimen	Type of diseases	Threshold value and no of items		Mean of ratio	Median of ratio	Fisher test	Threshold value and no of items		Mean of cell nr	Fisher test
All patients and both regions		< 1.2 %	>= 1.2%			p=0.0674 conditionally positive	< 3.1	>=3.1		p= 0.0876 negative
	CHSG	18	9	1.14	0.95		21	9	3.09	
	CHG	13	19	1.51	1.33		19	21	3.50	
Only women, both regions		< 0.83%	>= 0.83%			p=0.0155 positive	< 2.1	>=2.1		p= 0.0873 negative
	CHSG	12	3	0.97	0.80		8	9	3.12	
	CHG	6	11	1.41	1.35		4	17	3.53	
Only men, both regions		< 1 %	>= 1%			p=0.7063 negative	< 3.1	>=3.1		p= 0.0751 negative
	CHSG	5	7	1.37	1.21		10	3	3.04	
	CHG	5	10	1.63	1.20		8	11	3.47	
All patients, only prepyloric		< 1 %	>= 1 %			p=0.4153 negative	< 3.1	>=3.1		p= 0.0410 positive
	CHSG	5	5	1.38	1.11		4	6	3.92	
	CHG	5	12	1.29	1.37		1	17	4.80	
All patients, oxyntic		< 0.92 %	>= 0.92 %			p=0.2049 negative	< 2.1	>=2.1		p= 0.5335 negative
	CHSG	12	7	0.96	0.80		10	10	2.67	
	CHG	8	12	1.16	1.00		8	14	2.44	
Only women, prepyloric		< 2.1 %	>= 2.1 %			p=0.1738 negative	< 4.4	>=4.4		p= 0.6084 negative
	CHSG	5	0	1.63	1.96		2	4	3.77	
	CHG	2	3	2.17	2.36		5	4	4.15	
Only women, oxyntic		< 0.83 %	>= 0.83 %			p=0.0427 positive	< 2.1	>=2.1		p= 0.4136 negative
	CHSG	8	2	0.62	0.55		6	5	2.76	
	CHG	4	8	1.09	1.12		4	8	2.37	
Only men, prepyloric		< 1.68 %	>= 1.68 %			p=0.5012 negative	<3.1	>=3.1		p= 0.0769 negative
	CHSG	2	1	1.47	1.52		2	2	4.15	
	CHG	2	5	2.03	1.83		0	9	4.53	
Only men, oxyntic		< 1 %	>= 1 %			p= 1 negative	< 3	>=3		p=1 negative
	CHSG	4	5	1.33	1.12		4	5	2.55	
	CHG	4	4	1.27	1.00		5	5	2.51	

Table 4. The dependence between the type of chronic gastritis and the D cells

ellipse-shapes respectively. Statistically, both correlations are significant (p-value less than 0.05) with R^2 equals 0.73 and 0.81 and F-statistic equals 73 and 110. The graphical illustrations of this correlations are presented in Fig. 7a and b.

The presented results suggest that it is possible to perform an appropriate evaluation of the neuroendoctine cell quantity in the gastric mucosa taking into account only one type of the gland cross-section shape. The most representative aspect for this evaluation is a long ellipse shape of the glands.

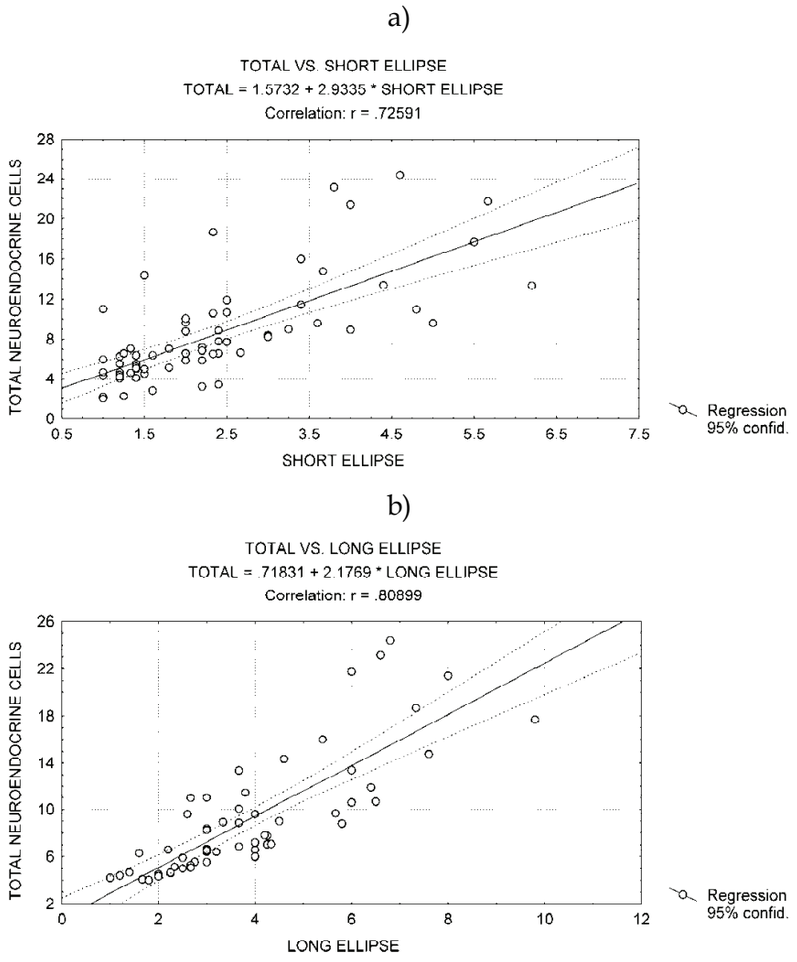


Fig. 7. Correlations between number of the neuroendocrine cells in the a) short ellipse-shape glands, or b) long ellipse-shape glands and the total number of cells in the specimen.

4. Conclusion

The presented results deal with statistical results of the density of the neuroendocrine cells in the gastric chronic diseases. The two used methods reflect some significant dependencies and possibilities of using them in the medical diagnosis. They confirm the dependence of the density of the neuroendocrine cells such as D, EC or ECL on the type of gastric disease, referred to in the previous researches. We received significant differences in the results of the used counting methods in dependence on sex of patient and oligobiopsy region. As far as recognition of type of chronic gastric diseases is concerned, we found the most significant dependence in the total neuroendocrine cells in female patients and oligobiopsy from the oxyntic part of the stomach. The Fisher test value for this case was p=0.0033 in the counting of all the cells in the field of view. For the EC and D cells this value was p= 0.0391 and p=0.0427 respectively. The other positive dependence received for the other groups of patients were negative or if there were positive, the evaluated group included women patients with oxyntic oligobiopsy specimens. We think that the positive test values in these

cases (indicating possible recognition of disease type) were caused only by the cases of women oxyntic specimens. This fact confirmed the negative recognition results received for the other types of specimens included in this evaluated group.

For the semi-quantitative method, based on the cell counting only in glandular epithelium of the stomach, the received results confirmed the significant dependence between number of the positive cells and type of gastritis only for D cells in the women oxyntic specimens. Additionally, very interesting results were obtained as regards counting all neuroendocrine cells in men oxyntic specimens. Contrary to the statistical counting, in this method we received conditionally positive test value p=0.0698. This dependence should be verified on the high number of patients.

The presented algorithm for computerized image analysis combines the mathematical morphology operations with artificial neural network of SVM type for evaluation of the ratio of the positive cells in the field of view. It can be helpful for researches in checking the endocrine cells in gastric disease and can be easily adapted to the other cytoplasm immunohistoreactivity stains. In difference to the other approaches, it imitates the human view strategy in recognition of the separated nuclei of the cells. The received accuracy is on good level and fully repeatable. In the case where we cannot use this method, the other solution is the use of semi-quantitative method with counting made manually by human experts. However, this evaluation is restricted to only few cases with a less precise method than the automatic one.

In the literature other approaches to counting neuroendocrine cell, based on grid graph, can be found (Ozkan et al., 2007). In such methods a randomly oriented parallel line-and-dot graph (grid) is superimposed on the analyzed image. The positive cells (marks in nuclear or cytoplasm staining) and negative cells coinciding with the dots on the grid are counted. This approach is oriented more on the statistics rather than on real cell recognition and in our opinion includes higher risk of counting error than full cell recognition process.

The most significant dependence between the ratio of the positive cells and type of the gastritis was in the total neuroendocrine cells in female patients and oligobiopsy from the oxyntic part of the stomach. Similar relations were found in the EC and D types of the cells. In the other type of specimens significant relations, useful for disease types recognition, were not confirmed. In the semi-quantitative evaluation performed by the human expert the diseases recognizable in female patients and oligobiopsy from the oxyntic part of the stomach were confirmed only in D cell counting. The ratio method is more effective for the recognition of the type of the disease than semi-quantitative evaluation.

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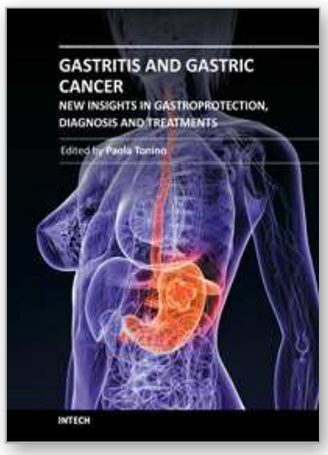

Gastritis and Gastric Cancer - New Insights in Gastroprotection, Diagnosis and Treatments

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This book is a comprehensive overview of invited contributions on Helicobacter pylori infection in gastritis and gastric carcinogenesis. The first part of the book covers topics related to the pathophysiology of gastric mucosal defense system and gastritis including the gastroprotective function of the mucus, the capsaicin-sensitive afferent nerves and the oxidative stress pathway involved in inflammation, apoptosis and autophagy in H. pylori related gastritis. The next chapters deal with molecular pathogenesis and treatment, which consider the role of neuroendocrine cells in gastric disease, DNA methylation in H. pylori infection, the role of antioxidants and phytotherapy in gastric disease. The final part presents the effects of cancer risk factors associated with H. pylori infection. These chapters discuss the serum pepsinogen test, K-ras mutations, cell kinetics, and H. pylori lipopolysaccharide, as well as the roles of several bacterial genes (cagA, cagT, vacA and dupA) as virulence factors in gastric cancer, and the gastrokine-1 protein in cancer progression.

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