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DYSPLASTIC EPITHELIAL CHANGES AS A SEQUENCE OF BINARY EVENT IN GASTRIC PATHOLOGY

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ABSTRACT

A simple, semiquantitative method for evaluation of epithelial and glandular changes in gastric hyperplastic lesions is presented. The differences of morphological features of the neighboured cells are denoted as the twenty-elements series of binary digits (0 or 1). The study was carried out in 89 gastric biopsy specimens divided qualitatively into five diagnostic groups (Jarvis and Whitehead classification) 1) no hyperplastic changes 2) low grade dysplasia, 3) moderate dysplasia, 4) severe dysplasia and 5) highly differentiated carcinoma. The following cytomorphological features were assessed semiquantitively in glandular epithelia: differences in The shape of the nuclei, differences in the size of the nuclei and in the position of the the nuclei, differences in the axial direction of the nuclei as well as the differences in gland shapes. The resulting data were in the form of "percent of changes" for each case and each feature. To evaluate the usefulness in such kind of data ANOVA, Kruskall-Wallis nonparametric ANOVA, Mann-Whitney test and multiple regression analysis were performed. Intra- and interobserver variability was also assessed. We concluded that our simple method gives comparable and reproducible results. All applied statistical methods gave similar results. The most "discriminating" cell features were: position of the nucleus in the cell, size and shape of the nucleus and loss of nuclear polarisation. Significant differences were found between the control group and low grade dysplasia and between high grade dysplasia and carcinoma.

Key words: gastric dysplasia, gastric cancer, morphometry, semiquantitative methods, statistics.

INTRODUCTION

The degree of loss of anisotropy and the severity of cellular or nuclear changes are the key diagnostic indicators in dysplasia or tumor differentiation. The traditional method of evaluation of these characteristics, based on the principles of stereological analysis is associated with considerable difficulty because of the necessity to follow a systematic sampling procedure (Weibel 1979). The methods based on the principle of systematic sampling within a layer, in spite of their tediousness do not eliminate a subjective selection of the evaluated areas

Another method used to evaluate the deviations is syntactic pattern analysis, developed and applied for histopathological evaluations by Bartels et al. (1987). These authors assessed the degree of displacement of the centres of gravity of cell nuclei profiles in relation to a virtual curve (determined e.g. of the course of epithelial basal membrane). As a method of statistical analysis they proposed the ARIMA model (Autoregressive Integrated and Moving Average model) also called Box-Jenkins model (Box and Jenkins, 1976). The method was originally designed for identifying the structure of the time series, but it proved to be an effective statistical tool also in many other areas. In morphological studies, the condition of a morphological element (e.g. size, staining, etc.) was treated as an element of a time series. Despite its advantages and flexibility, ARIMA is a complex technique; it is not simple to use, it requires considerable experience and although it often yields satisfactory results, they are largely dependent on the investigator (Balis and Peppers, 1982).

In the present paper we propose a simple and non-complicated method of comparing morphological phenomena, identified qualitatively or semiquantitatively in particular cells belonging to a certain layer, using non-parametric series tests. A linear sequence of binary digits based on the presence of a certain qualitatively detectable morphological characteristics was treated as a series. The analysis was carried out on the cells of foveolar gastric mucosal epithelium demonstrating no dysplasia, various degrees of dysplasia and early stages of carcinoma. In addition to the changes in the location of the nuclear centres of gravity, the differences in shapes, sizes, staining and polarization of the cell nuclei were also evaluated, as well as the nuclear-cytoplasmic ratio.

MATERIAL

The investigated material comprised 89 biopsy specimens taken on gastroscopy from the gastric body mucosa of 65 patients (mean age 58.12; age SD = 12.35; M/F ratio 4/1).

The qualitative diagnoses established on the basis of these biopsy specimens according to the criteria given by Jarvis and Whitehead (1985) were as follows: without pathological changes 3 cases, superficial gastritis 5 cases, chronic gastritis 31, atrophic gastritis 8, peptic ulcer 8, gastric cancer 15, other lesions 19. In non-carcinomatous cases the following coincident lesions were also diagnosed: intestinal metaplasia 17, foveolar hyperplasia 14, dysplasia 10 cases. The selected 89 specimens constituted qualitatively representative examples which were classified as belonging to the appropriate groups of diagnoses. For the needs of the present study, the following groups were distinguished: without hyperplastic lesions (group CONTR), minimal dysplasia (group DYSP1), moderate dysplasia (group DYSP2), severe dysplasia (group DYSP3), carcinoma (group CARCIN).

METHODS

Processing of tissue samples.

The cutting plane (6 sections 4μm. thick per section) was perpendicular to the plane of the mucosa. To achieve this the spatial orientation of the specimens was carefully checked under a binocular microscope during embedding in paraffin. The technical quality of the microscopic sections was high and the processing was preformed according to the recommendations of standarization committee of the Polish and European Societies of Pathology (Weber, 1992).

Semiquantitative analysis.

The used method, combined diagnostic applicability with simplicity of procedure and equipment, and reduced subjective assessment of tissue changes to the minimum.
In each section 5 microscope fields were analysed at 20x objective and 10x ocular magnification. The diameter of the field was ~20 mm. Cells were evaluated:

- in the foveolar region and
- in the glandular region section cut longitudinally along the axis of gland, or in the section cut perpendicular to the long axis of gland, if present

The results of evaluation were recorded in the form of a binary digits which produced sequence of "zeros" or "1's" (e.g. 01110011000111...) according to the principles set up for given characteristics, taking care that the number of elements of such a sequence was exactly 20. Each digit of the sequence corresponded to a single morphological object (a cell, a nucleus, a gland). The "0" value stands for the lack of qualitative difference in the cell or gland given from the next cell or gland, whereas "1" value means that there is such a difference. The fields in which the degree of abnormalities was the highest were evaluated. The basic characteristic taken into account in statistical analysis for each studied characteristics was the relative number of changes. In the present paper this index, will be referred to as the "percent of differences".

The following morphological characteristics (Collins et al., 1991; Cuello et al., 1979; Grundman, 1975; Morson et al., 1980; Ming et al., 1984; Oehlert, 1978, Tosi et al.,1990).have been adopted as the fundamental diagnostic criteria for evaluation in our study.

A. Position of cell nucleus (POSITNUC).

The position of the cell nucleus in the epithelial cells of the gastric mucous membrane was recorded. If the nucleus was situated in parabasal one third of the cell, "0" value was recorded; other positions resulted "1".

The evaluation was started from a randomly selected cell and carried out always progressed in the same direction. If on the cross-section of a glandular tubule less than 20 cells were counted, the recording was continued by repeated assessment, e.g. of the 1st cell as the 15th one, the 2nd as the 16th , etc. Untill the sequence of 20 digits was obtained.

B. Differences in size of epithelial cell nuclei (SIZENUC).

The evaluation was started from a randomly chosen cell and exactly 20 cells were evaluated in each case, progressing always in the same direction. If the nucleus of the next cell directly adjacent to the analysed one was larger and/or more intensively stained, it was recorded as "1", in the opposite case (identical or weaken staining), "0" was recorded.

C. Differences of nuclear shapes (SHAPENUC).

Oval nuclei were recorded as "0", all other shapes as "1". As a result of the analysis, the sequences consisting of 20 numbers each were obtained.

D. Nucleus/cytoplasm ratio (NUCYTRATIO).

A cell nucleus the external borders of which appeared to be in contact with the cell membrane in at least 3 points were described as "1", otherwise "0" was recorded.

E. Loss of polarisation of cell nuclei (POLARNUC).

The direction of the long axes of two adjacent cell nuclei was analysed. If the axes were parallel to each other and the cell nuclei were located at the same level, "0" was recorded, in the contrary cases "1". Of course it was difficult estimate this in histological section. Thus, the terms used above should be treated with certain tolerance, the differences to ca +/- 15^{o} were neglected, whereas more marked differences were recorded as belonging to the "1" category.

F. Shape of gastric mucosal glands (SHAPEGLAN).

The above characteristics of the histological texture was evaluated in the visual field under 25 x magnification (2.5x objective and 10x ocular). Straight glands with a regular contour were referred to as "0", and toryuous glands as "1". The length of the sequence recorded varied and was dependent on the number of glandular tubules found in the specimen.

Fig. 1 and Table 1 illustrate the method of characterisation of epithelial lesions.

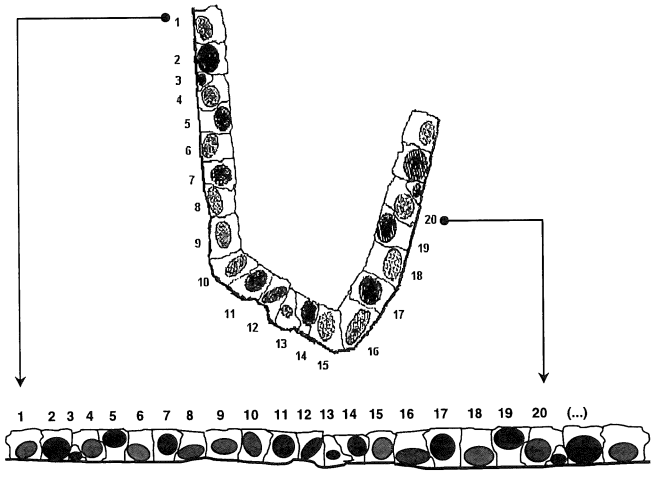

Figure 1. An example of scoring of cell features.

Table 1. Binary data from scoring of cell features (Fig.1).

Feature	Cell No.																				
CELL No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**%diff**
POSITNUC	0	0	0	0	1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	1	60
SIZENUC	1	1	1	1	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1	1	75
SHAPENUC	0	0	0	1	0	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	30
NUCYTRATIO	0	1	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1	1	1	45
POLARNUC	1	0	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	55
SHAPEGLAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

All the assessments were carried out under a light microscope, with an ocular gird which helped in locating the gland under consideration, also an ordinary electronic calculator was used.

The qualitative diagnoses were based on examination of the samples by the authors and on consultation with pathologist colleagues. For statistical analysis the Statistica for Windows® (StatSoft Inc. Tulsa, USA) software was used. The levels of significance were defined as follows: $p < 0.05$ significant and $p < 0.01$ highly significant.

STATISTICAL ANALYSIS OF RESULTS AND DISCUSSION

Descriptive data

For each investigated sample the result of semiquantitative analysis consisted of 6 sequences of numbers describing the analysed characteristics. For each sequence, "percent of differences" was calculated. The indices obtained in this way were subjected to statistical analysis using typical measures of location and dispersion (means, SD, variance). Because of the necessity to perform multiple intra-group and inter-group comparisons, and non-equal numbers of cases in groups, or significant differences between their variances, the methods of analysis of variance or median tests were used, also the modifications of popular tests of significance of the differences between the means were used. (Bahr and Mikel, 1987; Bartels and al., 1987; Domanski, 1990; Dunhill, 1985; Hellwig, 1987; Zuk, 1989). The table below (Table 2) presents the mean values of particular variables in 5 analysed diagnostic groups:

Table 2. Mean percent of differences in the position of cell nucleus (POSITNUC), differences of size (SIZENUC) and shape (SHAPENUC) of the nucleus and nuclear/cytoplasmic ratio (NUCYTRATIO) and shape of gastric mucosal glands (SHAPEGLAND). The percent of differences are presented for normal cases (No dysplasia), dysplasia (grades 1-3) and highly differentiated carcinoma.

	Class				
Feature	No dysplasia	dysplasia grade I	dysplasia grade II	dysplasia grade III	Carcinoma
POSITNUC	0.30	0.58	0.37	0.50	0.16
SIZENUC	0.26	0.35	0.35	0.33	0.07
SHAPENUC	0.37	0.47	0.46	0.37	0.16
NUCYTRATIO	0.27	0.34	0.29	0.32	0.13
POLARNUC	0.25	0.36	0.29	0.35	0.16
SHAPEGLAN	0.30	0.40	0.27	0.36	0.28

Low values in the cases of carcinoma are notable. It is due to the fact that unlike the diagnostically dubious cases (severe dysplasia or carcinoma?), in the situations when the histological malignancy is obvious, most glands are of irregular shape, and the cytomorphology of most cells is altered. Thus, frequently no differences are detected between the individual elements, but there is rather a uniform difference from normal patterns in most of the elements.

Non-parametric Kruskall-Wallis ANOVA

The analysis of variance for ranged variables (Kruskall-Wallis ANOVA) was applied (Table 3). Variables which did not demonstrate statistically significant differences between the groups CONTR, DYSP1, DYSP2, DYSP3 and CARCIN have not been listed.

Mann-Whitney test

The median values for each of the histological features were compared between the groups using Mann-Whitney U -test for the medians. The results are based on modified analysis (Holm, 1979; James, 1989) because of multiple comparisons (Table 4).

Table III shows that statistically significant differences were observed between the CONTR and DYSP1 groups, which could be distinguished by the variables describing the position of nucleus in the cell and the loss of cell polarisation. DYSP3 and CARCIN groups differed in terms of the variables describing the position of the nucleus in the cell, the size of cell nuclei, the nucleus/cytoplasm ratio, as well as the loss of polarisation of the nuclei. The comparison of corresponding median values for all groups is shown in Fig 2

Table 3. The results of the Kruskall-Wallis ANOVA.

Feature	Kruskal-Wallis test value (H)	p-level
SIZENUC	16.32	0.0026
SHAPENUC	16.00	0.0030
POSITNUC	15.05	0.0046
POLARNUC	14.91	0.0049

Table 4. The statistical significance of intergroup differences (Mann-Whitney U-test).

Variable	CONTR vs. DYSP1	DYSP1 vs. DYSP2	DYSP2 vs. DYSP3	DYSP3 vs. CARCIN	CARCIN vs. CONTR
POSITNUC	HS	ns	ns	HS	S
SIZENUC	S	ns	ns	HS	HS
SHAPENUC	ns	ns	ns	ns	HS
NUCYTRATIO	ns	ns	ns	HS	HS
POLARNUC	HS	ns	ns	HS	S
SHAPEGLAN	S	ns	ns	ns	ns

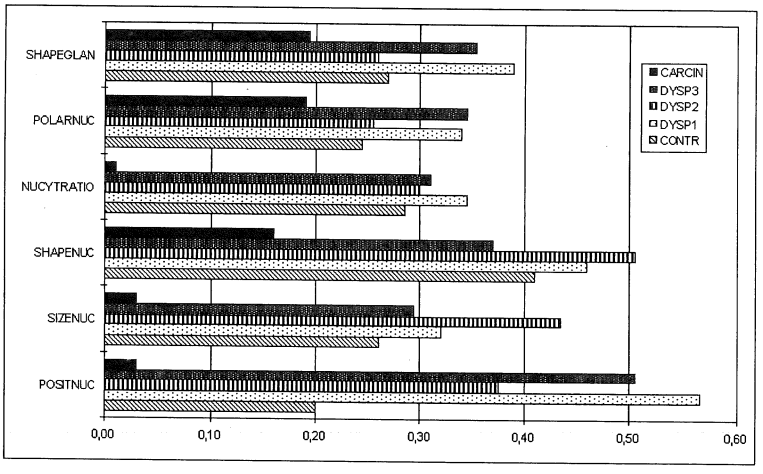

Fig. 2. Graphical presentation of the medians of the percent difference. The results on the six investigated features are shown separately for each atypia class.

Analysis of variance (ANOVA)

The calculated indices, after checking the conditions of applicability, were subjected to the analysis of variance (ANOVA). Tables 5 and 6 illustrate the test values for the main effect, as well as the R Rao values.

The main effect stands for the significance of the differentiating value for each characteristics calculated globally for all the groups. R Rao statistics determines the significance of each individual characteristics in each group and its values may range from 0 (perfect discrimination) to 1 (no discrimination) (Statistica manual, 1996).

Table 5. ANOVA - Main effect (overall $p < 0.0028$).

Feature	F-Snedecor value	p-level
SIZENUC	5.92	0.0006
SHAPENUC	5.15	0.0016
POSITNUC	4.20	0.0054
POLARNUC	4.08	0.0063
NUCYTRATIO	1.32	0.2760
SHAPEGLAN	1.02	0.4050

Table 6. ANOVA - R Rao's values (overall $p < 0.0028$).

	Feature					
Class	SIZENUC	NUCYTRATIO	POLARNUC	SHAPEGLAN	SHAPENUC	POSITNUC
CONTR	0.26	0.27	0.25	0.30	0.37	0.30
DYSP1	0.35	0.34	0.36	0.40	0.47	0.58
DYSP2	0.35	0.29	0.29	0.27	0.46	0.37
DYSP3	0.33	0.32	0.35	0.36	0.37	0.50
CARCIN	0.07	0.13	0.16	0.28	0.16	0.16

Multiple regression analysis

The multiple regression analysis and calculation of Pearson's correlation coefficient (r) was performed. The following results were obtained when the dependent variable was the diagnostic group:

- multiple correlation coefficient; $R = 0.521$
- multiple determination coefficient (R^2), which is the measure of reduction of total variability of the dependent variable according to the regression equation; $R^2 = 0.27$
- adjusted multiple determination coefficient (R^2corr) which is the coefficient of multiple determinations in which the correction for degrees of freedom was taken into account; $R^2\text{corr} = 0.139$
- standard error of estimate (SE) measuring the dispersion of the determined values around the regression line; $SE = 1.266$
- level of confidence (p); $p < 0.062$

Additionally, standardised regression coefficients (B) between intercept of the regression equation and each of the diagnostic characteristics were calculated, as well as the corresponding standard error values and confidence levels (Table 7).

Table 7. The results of the multiple regression analysis.

	B	Std. error B	p-level
Intercept	3.23	0.68	
SHAPEGLAN	1.80	1.41	0.21
SHAPENUC	-2.04	1.74	0.25
SIZENUC	-2.18	2.09	0.30
NUCYTRATIO	-0.56	1.30	0.67
POSITNUC	-0.16	1.09	0.89
POLARNUC	0.25	1.86	0.89

The overall results of the analysis indicate that there is a regressive correlation between all the analysed variables and the groups of diagnoses. The above indicates that the selected variables taken together classify the investigated cases as belonging to the particular diagnostic groups. Standardized regression coefficients for each individual analysed variable do not show, however, a statistical significance. It means, that none of the analysed variables should be treated as an individual diagnostic criterion.

Autoregressive moving average model (ARIMA)

The obtained original results (i.e. the binominal sequences) were subjected to statistical processing using autoregressive moving average model - ARIMA. Unfortunately, none of the models used in our material proved to be stationary one, and so further analysis was impossible.

Sensitivity, specificity and efficiency of diagnostic distinction

On the basis of the obtained results of semiquantitative analyses all the investigated cases were reclassified and divided into groups determined by the values of the analysed variables, and then the result of this classification was compared with the reference system. On the basis of this comparison it was established that the levels of cumulative specificity, sensitivity, and diagnostic efficiency amounted to:

- specificity = 0.875
- sensitivity = 0.537
- efficiency = 0.815.

Analysis of inter- and intraobserver variability

Inter- and intraobserver variabiality was analysed (Collan et al., 1987; James, 1989). The statistical significance of differences in assessment was evaluated using Wilcoxon test.

Interobserver variability was evaluated by comparing the results obtained by two observers with respect to each characteristics in the same preparates.

Intraobserver variability was evaluated by comparing the results obtained by the same observer with respect to each characteristics in the same preparates analysed at a 6-week interval (Table 8.).

No statistically significant interobserver variability was noted in the results of semiquantitative evaluation (for each of the analysed variables $p > 0.05$). Also no statistically significant differences of results obtained by the same observer evaluating the same cases on two subsequent trials divided by a considerable time interval were noted ($p > 0.05$).

The Table 9. summarises the results of statistical analyses performed according to different methods. From this table it follows that the most potent statistical tests (parametric ANOVA) allow to determine the inter-group statistical differences which are higher than the

dispersity of results within the particular groups. This method, however, does not determine the direction of these differences, which means that parametric ANOVA differentiates all the groups without their detailed specification. Thus, ANOVA evaluates the correctness of the method but does not allow for the classification of individual cases, which makes it practically useless for histopathological diagnostics.

Table 8. Results of the inter- and intraobserver variabiality analyses.

Interobserver variability		Intraobserver variability	
Feature	p-level	Feature	p-level
NUCYTRATIO	0.59	NUCYTRATIO	0.72
POSITNUC	0.10	POSITNUC	0.79
NUPOLAR	0.44	NUPOLAR	0.13
SIZENUC	0.25	SIZENUC	0.74
SHAPENUC	0.11	SHAPENUC	0.60
GLSHAPE	0.53	GLSHAPE	0.76

Table 9. Comparison of usefulness of various methods of statististical analysis for semiquantitative data gathered by histomorphometry of gastric hyperplasia.

	Statistical method or model of data analysis				
	Kruskall-Wallis ANOVA	**Parametric ANOVA**	**Mann-Whitney U-Test**	**Regression analysis**	**ARIMA**
p-level	**p<0,05**	**p<0,05**	**p<0,05 (modified)**	**p<0,05**	**-**
variables with "discriminant " value	POSITNUC SIZENUC SHAPENUC POLARNUC	POSITNUC SIZENUC SHAPENUC POLARNUC	POSITNUC SIZENUC NUCYTRATIO POLARNUC	NO SIGNIFICANT REGRESSION	no stationary models
Distinction between groups	CONTR vs. DYSP1 CONTR vs. DYSP2 CONTR vs. DYSP3 CONTR vs.CARCIN DYSP3 vs.CARCIN	CONTR vs. DYSP1 CONTR vs. DYSP2 CONTR vs. DYSP3 CONTR vs.CARCIN DYSP1 vs. DYSP2 DYSP1 vs. DYSP3 DYSP1 vs.CARCIN DYSP2 vs. DYSP3 DYSP2 vs.CARCIN DYSP3 vs.CARCIN	CONTR vs. DYSP1 DYSP3 vs.CARCIN	-	-

The analyses of regression and correlation (multiple regression analysis) allow to find equations classifying an individual case as belonging to a given group, but only if we take into consideration all the variables together. Because of non-stationary character of the "process", ARIMA was not applicable for the evaluation of our data. The best method of analysis proved to be non-parametric Mann-Whitney test, which, with the modification of the significance level due to multiple comparisons between the groups, allows to obtain not only the evaluation of the investigated variables, but also discrimination between the groups. It is also important that this test is one of the potent nonparametric tests, so the results of the analyses are highly reliable. Additionally, because of its simplicity, easy interpretation of results and the fact that most of popular statistical software has the implementation of this test (Wyrostek, 1995), it

seems that it can be used in practice for the presented method of binary notation of epithelial changes.

CONCLUSIONS

From the above presented we learn that the following features are the most useful for the evaluation of the severity of dysplasia: the position of the nucleus in the cell, variations of nuclear size, shape, and the loss of polarisation of cell nuclei. From the practical point of view it is very important that the variables distinguish in statistically significant way the groups CONTR and DYSP1, as well as DYSP3 and CARCIN.

The applied methods of statistical analysis do not have significant influence on the result of assessment of the diagnostic value of the studied features in dysplasia. All these methods detect the same types of correlation for the same variables. ARIMA, recommended by Bartels et al. (1987), could not be applied. The analysis of regression and correlation, did not seen to work satisfactory either.

The presented method of binary notation of qualitative characteristics does not require complex specialist equipment. But it allows for reliable and reproductible assessment of dysplasia of gastric mucous membrane, and can be subjected to statistical analysis using relatively simple statistical techniques. The method of Kruskall-Wallis non-parametric analysis of variance proved equally effective as the other more sophisticated methods.

REFERENCES

Bahr GF, Mikel UV. Mass, volume and dimensional distributions in biology, with special reference to cells. Anal Quant Cytol Histol 1987; 9:341-353.

Bails DG, Peppers LC. Business fluctuations: Forecasting techniques and applications. Englewood Cliffs: Prentice Hall, 1982.

Bartels PH, Weber JE., Paplanus SH, Graham AR. Detection of diagnostic clues in statistical histometry. Anal Quant Cytol Histol 1987; 9:355-368.

Box GEP, Jenkins GM. Time series analysis: Forecasting and control. San Francisco: Holden-Day, 1976.

Collan Y, Torkkeli T, Kosma VM. Personen E, Kosunen O, Jantunen E Mariuzzi GM, Montirioni R, Marinelli F, Collina G. Sampling in diagnostic morphometry: The influence of variation sources. Path Res Pract 1987; 182:401-406.

Collins JS, Watt PCH, Hamilton PW, Sloan JM, Love AHG. Grading of superficial antral gastritis: Comparision of cell-counting and photographic-based methods. J Pathol 1991; 158:303-310.

Cuello C, Correa P, Zarama G, Lopez J, Murray J, Gordillo G. Histopathology of gastric dysplasias. Correlation with gastric juce chemistry. Am J Surg Pathol 1979; 3:491-500.

Domanski C. Statistical tests (in Polish). Warszawa: PWE, 1990.

Dunhill MS. Some statistical aspects of sampling in morphometry. Anal Quant Cytol Histol 1985; 7:250-255.

Grundman E. Histologic types and possible initial stages in early gastric carcinoma. Beitr Path Bd 1975; 154:256-280.

Hellwig Z. The elements of probability estimation and mathematical statistics (in Polish). Warszawa: PWN, 1987.

Holm S. A simple sequentially rejective multiple test procedure. Scand J Statist 1979; 6:65-73.
James NT. Common statistical errors in morphometry. Path Res Pract 1989; 185:764-768.
Jarvis LR, Whitehead R. Morphometric analysis of gastric dysplasia. J Pathol 1985; 147:133-138.
Ming S, Bajtaj A, Correa P, Elster K, Jarvi O, Munoz N, Nagayo T, Stemmerman GN. Gastric dysplasia. significance and pathologic criteria. Cancer 1984; 54:1794-1801.
Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, Serck-Hansen A. Precancerous conditions and epithelial dysplasia in the stomach. J Clin Pathol 1980; 33:711-721.
Oehlert W. Dysplasien des Oberflachenepithels der Magenschleimhaut. In: Oehlert W ed. Klinische Pathologie des Magen-Darm-Traktes. Histologische Diagnose und Differentialdiagnose am gastroenterologischen Material. Stuttgart: Schattauer, 1978:80-90.
Paplanus SH, Graham AR, Layton JM, Bartels PH. Statistical histometry in the diagnostic assessment of tissue sections. Anal Quant Cytol Histol 1985; 7:32-37.
STATISTICA for Windows - Computer program manual. Tulsa: StatSoft Inc, 1996.
Tosi P, Filipe MI, Baak JPA, Luzi P, Santopietro R, Miracco C, Sforza V, Megha T. Morphometric definition and grading of gastric intestinal metaplasia. J Pathol 1990; 161:201-208.
Weber GF. Current methods in histopathology. Polish J Pathol 1992; 43:87-91.
Weibel ER. Stereological Methods. v.1. Practical Methods for Biological Morphometry & v.2. Theoretical Foundations. London: Academic Press, 1979-1980.
Wyrostek A. A revue of scientific software. III. Statistics (in Polish). Acta Bio-Optica et Informatica Medica (Wroclaw) 1995; 1:169-172.
Zuk B. Applied biometrics (in Polish). Warszawa: PWN, 1989.