INTEROBSERVER VARIABILITY IN REPORTING GASTRIC DYSPLASIA

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Abstract

General background: There is still a controversy in the recognition, the terminology used, and histopathologic evaluation of two essential elements in gastric carcinogenesis: atrophy and dysplasia.

Materials and Method: 115 cases, with the slides and their histopathologic reports, from the archive of the LAP were studied for the diagnostic value, the report of dysplasia, the interobserver variability, the relation of dysplastic lesions with inflammatory, atrophic and metaplastic ones. There have been studied retrospectively the reports from the Archive with distribution of the cases according to endoscopic diagnosis, and to the biopsy report and there have been reexamined the slides. The comparison of the median values of the numeric variables was made with the Mann-Whitney test (non-parametric equivalent of the Student's "t" test).

Results: The endoscopic clinical diagnosis were: malignancy /suspicious for malignancy 88 cases (76%) and the nonneoplastic diagnosis (like ulcer or gastritis) 27 cases (24%). From all the cases sent with the clinical diagnosis of malignancy, that was not confirmed by biopsy 51% were reported as dysplasia of different grades and 49% were reported as without neoplastic changes, from 6 cases sent as suspicious for malignancy, 50% were reported as dysplasia and the rest without neoplastic lesions and, from the diagnosis sent as nonneoplastic lesions, 46% of them displayed dysplasia and the rest (54%) were nonneoplastic lesions. From the reexamination of the cases it resulted that there is no difference in reporting the malignancy, but there is a difference in the cases reported as dysplasia (p=0.001) and Negative for Neoplasia (p=0.063, bordeline).

Conclusion: The use of guidelines is to lower the interobserver subjectivity. The evaluation of dysplasia is influenced by the "interobserver variability", especially in atypical reactive lesions. The interobserver variability happens even when there are used different classifications for the evaluation of a pathological lesion.

Introduction

The development of the flexible endoscope and its wide use in gastroenterology has influenced the management of gastric cancer. Remarkable advances have been made in Japan, where, nearly 50% of the cases with gastric cancer are discovered in an "early" phase, which means confined to the mucosa and submucosa (42). However, if we see the global distribution of gastric cancer it is still one of the major health problems, despite the universal attempts to lower its mortality (17). Surgery is the treatment of choice, but in most of the cases the prognosis is not favorable, and the 5-year survival rate is lower than 20% in most of the countries (29).

Endoscopic methods permit also the identification of premalignant lesions and their diagnosis by pathologists plays an important role in the management of the patients (4,2,28,15,7). Gastric mucosa can have progressive changes that go from inflammation to multifocal atrophia and intestinal metaplasia, and further to dysplasia (8,9). There is still a controversy in the recognition, the terminology used, and histopathologic evaluation of its two essential elements: *atrophy and dysplasia* (12,13,14).

Here we present our data in reporting dysplasia, its histopathologic features, interobserver variability related to it, and the need for a standardized terminology for its reporting.

Materials and Method

There have been included the consecutive bioptic specimens of 115 cases, with the slides and their histopathologic reports, from the archive of the LAP. These bioptic specimens were prepared with the standard histopathological techniques and stained with H-E, PAS and Giemsa. There have been studied the following parameters from the bioptic materials: the adequacy of the bioptic specimen, its diagnostic value, the report of dysplasia, the interobserver variability,

the relation of dysplastic lesions with inflammatory, atrophic and metaplastic ones.

The retrospective study comprises: a-*the review of the reports from the Archive* with distribution of the cases according to endoscopic diagnosis, and to the biopsy report b-*microscopic reexamination*. The pathologist has examined the slides and made the diagnosis blinded to the results of the first examination by other pathologist, but with information on patient's clinical data. During the reexamination it is evaluated also the presence of active inflammation (PMN), chronic inflammation (MN), intestinal metaplasia (M) and atrophy (A).

The report of dysplasia in the reexamination has been made based on Padova classification and the report of inflammation, atrophy and intestinal metaplasia has been made according to the guidelines of the Modified Sydney System (MSS) (31,9). To check the distribution of inflammation, atrophy and intestinal metaplasia in dysplastic lesions we used a control group of 100 cases with chronic gastritis gastric ulcer, gastric cancer and duodenal ulcer. The above mentioned parameters were compared between the two groups.

We excluded from the study the lesions that after the reexamination were considered not appropriate like superficial materials, and those composed entirely of necrotic-inflammatory tissue.

Statistical analysis:

The comparison of the median values of the numeric variables was made with the Mann-Whitney test (non-parametric equivalent of the Student's "t" test).

To compare the percentages according to the different parameter categories we used the test of *chi-square* in those cases were the expected value of every cell in the table was > 5 (*chi-square test for independent proportions*). To compare the percentages according to the different parameter categories we used the exact Fisher's test in the cases when the expected value of every cell in the table was < 5 (Fisher's exact test).

Results

The patients were 66 males and 49 females, with a median age of 45 years, ranging from 18-81 years. The distributions of the cases according to the endoscopic clinical diagnosis were: malignancy/suspicious for malignancy 88 cases (76%) and the nonneoplastic diagnosis (like ulcer or gastritis) 27 cases (24%).

Table nr.1 Bioptic diagnosis

Adenocarcinoma / Lymphoma/ Adenomatous polyp	48 (42%)
Dysplasia	33 (29%)
Inflammation	29 (25%)
Not appropriate	5 (4%)

After the histopathologic examination of these

cases, confirmation of the carcinoma is done only in 54% (48 cases) of the cases suspected and the rest, 46%, were referred as dysplastic lesions or nonneoplastic inflammatory lesions (Table nr.1).

Taking in consideration that 76% of the cases examined were materials preleveted from macroscopic lesions with high suspicion for malignancy, the possibility of discovering the dysplastic lesions that accompany them can be great. During the distribution of the biopsies according to the clinical diagnosis, we see that a definitive diagnosis is accomplished only in 42% of the cases examined and the rest have been reported as descriptive diagnosis with the conclusion for the repeat of biopsy if clinically suspected. The group of the diagnoses with the description of dysplasia and inflammation in the histopathological report were distributed according to clinical diagnosis. From all the cases sent with the clinical diagnosis of malignancy, 51% were reported as dysplasia of different grades and 49% were reported as without neoplastic changes, from 6 cases sent as suspicious for malignancy, 50% were reported as dysplasia and the rest without neoplastic lesions and, from the diagnosis sent as nonneoplastic lesions, 46% of them displayed dysplasia and the rest (54%) were nonneoplastic lesions (Table nr.2).

Table nr.2 Comparing the cases of Dysplasia and NN with the clinical diagnosis

Clinical Diagnosis	Histopathological report	
	Dysplasia	Negative for neoplasia (NN)
Neo (39)	20 (51%)	19 (49%)
For determination (6)	3 (50%)	3 (50%)
NonNeo (22)	10 (46%)	12 (54%)
Total = 67	Total = 33 49%)	Total = 34 (51%)

As we see in table nr.2, there is no significant difference (p>0.01) in the data regarding the dysplastic lesions in the group strongly suspected for malignancy in endoscopy, with the group of clinically nonneoplastic lesions and those for determination. The same thing is also with the non-neoplastic inflammatory lesions.

Taking in consideration the fact that dysplasia has the same frequency in the lesions highly suspicious for malignancy and those for nonneoplastic lesions, we raised the question: are these symptoms true dysplasia? Maybe, a part of them are atypical regenerative changes? What terminology should we use to report gastric dysplasia?

From the reexamination of the cases it resulted that

there is no difference in reporting the malignancy, but there is a difference in the cases reported as dysplasia (p=0.001) and NN (p=0.063, borderline) (Table nr.3, Table nr.4).

Table nr.3 Reexamination of the cases

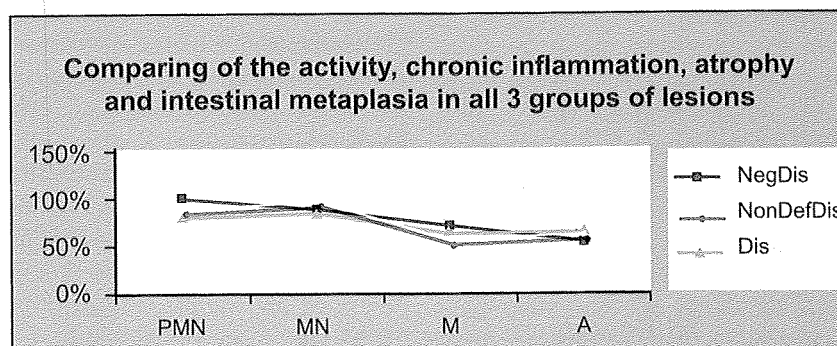|	
Negative for dysplasia (ND)	34 (51%)
Non definitive for dysplasia (NDD)	7 (10%)
Dysplasia	22 (33%)
Not appropriate	4 (6%)

Table nr.4 Interobserver variability

	Malignant neoplasia	Dysplasia		Negative for neoplasia	Not appropriate
First examination	48	33		29	5
		Dysplasia	NDD		
Reexamination	48	22	7	34	4

When comparing active inflammation, chronic inflammation, intestinal metaplasia and atrophy found in the histopathological materials of the three diagnostic groups (NN, ID and Dysplasia) we see that active inflammation (neutrophilic inflammatory cells, PMN) and chronic inflammation (mononuclear inflammatory cells, MN) are present in all three main diagnostic groups and there is no significant difference between them. These components are more expressed in the group of NN lesions and in the group of ID lesions. This expression shows the fact that the disease is active in this group of lesions and this activity can be the cause of the macroscopic changes, like ulcerative lesions, erosions, polypoid and exophytic lesions (Table nr.5, Figure nr.1).

Table nr.5 Comparison of the activity, chronic inflammation, atrophy and intestinal metaplasia in all 3 groups of lesions

	NegDys	NonDefDys	Dys
PMN	31 (91%)	7 (100%)	18(82%)
MN	32 (94%)	6 (86%)	19(86%)
M	20 (59%)	5(71%)	14(64%)
A	21 (62%)	4 (57%)	14(64%)

Figure nr.1

Discussion

The process of cancer development (cancerogenesis) is a process with a lot of steps (multistep process) that consist in the consecutive genotypic and phenotypic changes (3,6,10). The recognition of the intermediate phases of this process will help in the early identification of cancer and in the definition of the risk for malignant transformation of these lesions. According to the fact that 76% of the cases examined were materials taken from macroscopic lesions highly suspicious for neoplasia, the possibility of discovering dysplastic lesions that accompany them can be considerable, 46% are referred in the biopsy report as dysplastic lesions or non-neoplastic inflammatory lesions.

This group of lesions reflects either the changes of the mucosa adjacent to the macroscopic lesions in the cases where it was not possible to submit material from the lesion itself, or the changes of the macroscopic lesion itself. However, the morphological study of this category permits us to evaluate its connection with other precancerous lesions like dysplasia, intestinal metaplasia, gastric atrophy and the presence of the inflammation.

One of the problems with small endoscopic specimens is that not always it is possible to reach a definitive diagnosis, which helps the clinician to manage the patient. In some cases the histopathological reports are descriptive and with difficult to achieve conclusions and also difficult to manage from the clinician. This is a known problem in general for cytology and small biopsy specimens.

Their diagnostic productivity is greater with a bigger number of specimens submitted, in the form of multiple specimens (4) According to Witzeal et al (41) the diagnostic productivity in macroscopic lesions of the esophagus and the stomach with endoscopy submitted specimens was 83%. In our materials there is not a significant difference in finding dysplasia in the group that was highly suspicious for malignant neoplasia during the endoscopy, with those that were not suspicious and in the cases for determination. According to the literature data the retrospective analysis of the specimens submitted from the cases with gastric surgery for cancer have shown that dysplatic epithelium and adenocarcinoma frequently accompany each-other, suggesting the role of dysplasia as a preceding lesion (22,23).

What was considered as moderate to severe dysplasia, was accompanied in 40%-100% of cases with early gastric carcinoma, and was found in 50%-80% of advanced carcinomas, suggesting a direct role in the development of cancer (26). With the use of fiber-optic endoscopy in the late 1960 and early 1970, Nakamaura and Nagayo in Japan were the first that identified dysplasia as a possible preceding lesion of carcinoma and presented soma classification algorithms for dysplasia (or atypia as is frequently named in Japan) (24,25).

The values that express gastric dysplasia vary a lot. The diversity of these data is partially because of the differences in the studied populations and partially in the different usage of the term dysplasia. The origin of the population (different races) or a population or group with high risk (eg, the patient with chronic gastropathy) is important variables during the study. The reported dysplasia prevalence in general in western countries is from 0.5% to 3.75%, whether values from 9%-20% are reported in areas with high risk like Columbia or China (232,36,5). The prevalence of dysplasia in the patients with chronic atrophic gastritis, ulcer, or after gastrectomy, vary from 4-30% up to 40% in the patients with perinicous anemia (39,13,3).

Part of variation stands in the different risk that actually the populations studied could have, but probably one of the reasons of this difference stands in the histopathological criteria and different classifications used to report dysplasia.

For example, in one series, 92% of dysplasia in a population with 20% prevalence, were classified as mild dysplasia, what makes you think that probably those lesions were not dysplasia, but regenerative processes and non true neoplastic ones (41).

The regenerative process and especially reactive and regenerative changes that are noted in complete and incomplete intestinal metaplasia frequently are like "interpretative pitfalls" and are not described and known well. In another study, during the reviewing of the cases reported as dysplasia, some pathologist at first they reported as dysplasia the lesions that after the reevaluation were identified as regenerative changes (13). To eliminate the great variability in the histopathological/cytology reporting in general and to assure their standardization, in different specialties of anatomical pathology, there have been created reference standards, like the one of PAP test (38); of core biopsy for the breast (1), of aspirative cytology for the breast (30) or for the thyroid (11), dysplasia for Barret's oesophagus or for colon dysplasia (32).

In these reports, there is first a prescription according to the case, and at the end there is the diagnostic conclusion according to one of the categories which belongs the lesion. In the case of gastric dysplasia, there is still not a standardized language, although efforts have been made to achieve a consensus in the reporting of dysplasia, according to a pathological and therapeutical view. The actual consensuses are those of Padova (31), Hong- Kong (33), and Viena (34).
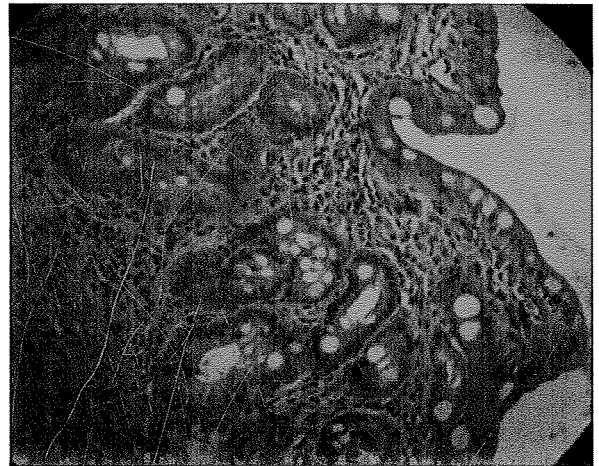
In our bioptic materials that were reported as *negative for neoplasia*, histologically specimens displayed – normal components of the mucosa, where the foveola, the glands, the neck of the gland and the stroma were well contained. The

inflammatory infiltrate was minimal or absent in the cases where the mucosa was normal. These changes do not represent interpretative difficulties. In this category were included also the inflammatory and hyperproliferative lesions of the mucosa. In those materials the general architecture of the mucosa is well maintained, the foveola's can be elongated and tortuous, hyperplastic epithelial alterations become less prominent, until they disappear gradually when passing from the area with hyperplastic changes in normal areas of the mucosa. The nuclei are enlarged and sometimes hyperchromatic. There can be mitotic figures also. The pathologist does not have doubts about their nonneoplastic meaning (Figure nr.3A,B). The presence of intestinal metaplasia is also reported in this category when it does not display hyperproliferative phenomena, atypia and is not of incomplete, colonic type.
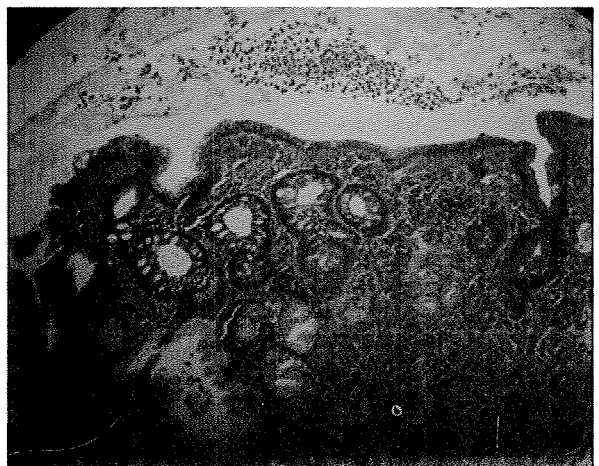
Indefinite for dysplasia is another diagnostic category reported during the reexamination of our cases and as part of the actual classifications for dysplasia. The difference in reporting dysplasia from the first and second exam can be explained with the new category introduced. This category has similar morphological features with dysplasia and is frequently difficult to differ from it. In this category are included lesions of different groups (table), which have in common the reaction or the response of the epithelium to the injury as an essential part of the organism homeostasis. In some cases, reactive changes have a special aspect. Often, this kind of specimen raises the problem of true dysplasia or reactive-regenerative changes, which are termed before as "regenerative dysplasia" or *"regenerative atypia"* (5,14). These regenerative changes are seen on the edges of gastric ulcers, on the erosions of atrophic gastritis, or lymphocytic gastritis, or in the cases of gastropathy from billiar reflux or the use of NSAID (21).

The glands show irregular architecture, hyperchromatic and stratified nuclei; the "atypical" glandular structures are lined by epithelial cells without mucus, with nuclei that have prominent nucleoli. The mitosis can be frequent. However, the maturation towards the surface, *"maturation gradient"*, (Figure nr.2), dense neutrophil infiltrate, and being close to an ulcerous lesions, suggest that these are mainly reactive-regenerative changes (Figure nr.2, nr.3). Even in the foci with intestinal metaplasia, of incomplete type, can be seen "hyperplastic" or "hyperproliferative" lesions of the glandular crypts deep in the mucosa. The expressed proliferative activity of the glands differs from complete

metaplasia, in which the nuclei are in the base, small and normochromatic.

Figure nr.2 On atrophy and epithelial metaplasia, the glands are located deeper in the mucosa and have proliferant epithelium, with mitosis even above the basal level, but with a "maturation gradient" towards the surface (H-E, 20X).

Figure nr.3 Fragments from gastric mucosa with expressed active inflammation and intestinal metaplasia. Some of the gastric crypts in the center of the picture are hyperproliferative, with elongated and pseudostratified nuclei. On the grounds of inflammation it is difficult to differ it from an atypical reactive lesion (H-E, 20X)

This situation can happen when the bioptic specimen is not appropriate or when the architectural irregularities of the glands and nuclear atypia are present at a level that is suspicious for the possibility of dysplastic changes in the proliferative cells. These doubts can be clarified with a new appropriate bioptic specimen, or eliminating the possible causes of the

hyperproliferative state, like H pylori, NSAIDs, treatment with antibiotics, treatment of GERD, etc.

In all these cases it is not easy to put the diagnosis of negative for neoplasia and to subject the patient to difficult surgery interventions. The differentiation of reactive changes like foveolar hyperplasia and metaplastic changes are a challenge for most of the pathologists (10,12). According to a series of 51% of cases reported as hyperplastic changes, and metaplastic lesions from the specialists pathologists, were reported first as moderate dysplasia from general histopathologists (10).

Dysplasia is another reporting category. The difference in reporting dysplasia stands not only in including the category of Lesion Non-definitive for dysplasia, as discussed above, but also in another change in reporting dysplasia, *its classification in two grades,* like *low grade dysplasia* and *high grade dysplasia,* in comparison to the previous dysplasia classification in three grades: mild, moderate, severe.
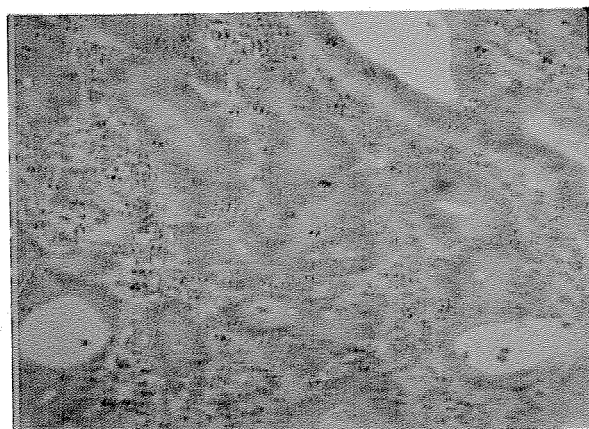
Non standardized diagnostic criteria can cause an inappropriate interobserver variability, a factor that influences the patient's care, also the evaluation of clinical guidelines. Often, pathologists find it difficult to interpret a lesion as atrophy and atrophic gastritis, and the interobserver variability in this parameter is high. As a result, even the treatment effects and patient's follow-up differ often a lot from one clinic to another (43,44).

A similar classification of dysplasia (with two grades: low and severe) is well standardized about the reporting of PAP testing or colonic dysplasia (32,33). Usually, we classify dysplasia in three grades according to cytologic and architectural characteristics of the epithelial tissue examined (27). Classifying dysplasia in two grades is easier and more reproducible. During the grading of dysplasia in three grades, often we report an intermediate grade, for example, low to moderate or moderate to severe, making it a system of three to five grades. The existence of the high and low grade alone does not permit us to find intermediate terms. In 1984, Ming (45) and an international panel recommended that moderate and severe dysplasia to be grouped in one category because they cannot be separated sharply from one-another and often they co-exist in the lesions.

In high grade dysplasia is included also the so-called "in situ carcinoma", which is a noninvasive lesion, with similar cellular changes to carcinoma, but without invasion (Figure nr.4).

The histological diagnosis seems full of undisputable "data" and the pathologists based in the content of their slides, seem that they have always applied "evidence based medicine" much earlier than this term came in to use. To minimize the subjective components (interobserver variability) of the histopathological diagnosis,

further attempts are done to assure *standardized diagnostic criteria,* which will be applied in the diagnostic process (*guidelines*).

Figure nr.4. In this material, the nuclei extend to the luminal surface of the cell; they are amphophylic, with prominent nucleoli, with increased number of mitosis and highly disorganized glands. High grade dysplasia (H-E,40X).

The consequences of non validated histopathological classifications can influence the management of some patients, consequences that aggravate even more when the criteria are not sufficient and supposed as accurate, are applied to verify the clinical protocols. An example of this is taken from the studies that are done to see the effects of eradication of H pylori in the regression of atrophy. Because the agreements for the evaluation of atrophy are not sufficient, the results taken in a clinical center are not reproducible in another center (40). In our materials, the variability exists only in the group of dysplasia; meanwhile, in the diagnosis of carcinoma, there is no variability. According to Plummer M et al (46), one study for histopathological diagnosis of precancerous lesions in gastric mucosa had an acceptable compatibility for the diagnosis in general, and a perfect compatibility for advanced lesions, meanwhile the compatibility was low for low grade lesions.

Conclusion

Although Pathological Anatomy is considered a very objective discipline and based at the "evidence", it gets influenced by a subjective parameter that is the *Histopathologist* himself. The use of guidelines is to lower the interobserver subjectivity. The evaluation of dysplasia is

influenced by the "interobserver variability", especially in atypical reactive lesions, cases that can display interpretative difficulties in differencing them from true dysplasia; in these cases the specimen can be considered as Non Definitive for Dysplasia. The interobserver variability happens even when there are used different classifications for the evaluation of a pathological lesion. The use of Guidelines will cause a lowering of this variability.

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