Role of chronic atrophic gastritis of the body-fundus and achlorydria in the development of epithelial dysplasia and gastric carcinoma

G. Testino, F. De Iaco[1] and M. Cornaggia[2]

Unit of Hepato-Gastroenterology, S. Martino Hospital, Genova, Italy.
(1) Unit of Internal Medicine, Bordighera Hospital, Italy ; (2) Department of Human Pathology, University of Pavia at Varese, Italy.

Abstract

Background/ Aim : **Chronic atrophic gastritis of the body-fundus with hypo-achlorydria has been long since considered the precursor of gastric cancer (GC).**

A study has been made about the histological pattern of the body-fundic mucosa (oxyntic area) in course of preneoplastic lesions (epithelial dysplasia), associated or progressed to gastric cancer, in order to evaluate the real association with chronic atrophic gastritis and, therefore, with a reduced acid secretion.

Methodology : **The study of the histological condition of the body-fundic mucosa and of the acid secretion has been effected in 120 cases of epithelial dysplasia (ED) from January 1990 to November 1997.**

The casuistry is composed of 70 cases of low grade dysplasia (LGD) and 50 cases of high grade dysplasia (HGD).

Gastric biopsy specimens were studied for dyspepsia : for each patient, at least 8 specimens were obtained from the lesion area and in surrounding areas. Besides, at least 4 biopsies have been performed in the opposite gastric region. ED diagnosis was effected according to well defined criteria. The histological study of gastric mucosa in gastritis was effected or revised in accordance with the updated Sydney sistem (Houston).

Stimulated acid secretion was expressed as Maximal Acid Output (MAO), which is the amount of HCl produced in one hour, following stimulation with pentagastrin (6 microng/kg).

The clinical outcome subdivision of ED was made using the criteria of Rugge et al. (12).

Results : **HGD significantly associates with GC in comparison with LGD.**

The histological evaluation of the oxyntic area shows severe chronic atrophic gastritis (SCAG) in a low percentage of cases (15/120 : 12.5%) : LGD 9/70 : 12.85% ; HGD 6/50 : 12%.

Complete achlorydria has been noted in 5 cases of LGD and in 1 case of HGD only.

In case of GC (43 subjects) SCAG has been evidenced in 10 cases and complete achlorydria in 5 cases.

Conclusions : **From the data of the present experience emerges that the presence of SCAG of the oxyntic area in course of ED or early GC is limited to a low percentage of cases.**

Such concepts induce to modify some indications related to the endoscopic surveillance and, in accordance with the American Society of Gastrointestinal Endoscopy we are stating that there are no sufficient data to support subsequent endoscopic surveillance for the subjects with atrophic gastritis. (Acta gastroenterol. belg., **2004**, 67, **327-330**).

Key words : CAG : Chronic Atrophic Gastritis, GC : Gastric Cancer, NAG : Non Atrophic Gastritis, ED : Epithelial Dysplasia, MAO : Maximal Acid Output.

Chronic atrophic gastritis (CAG) has been long since considered the precursor of gastric cancer (GC). Such concept has been translated by Correa in a model which provides the following statement : non atrophic gastritis (NAG) → CAG → achlorydria with bacterial overgrowth → carcinogenic nitroso-compounds increase → preneoplastic changes → GC (1,2).

Actually, various important epidemiological studies have pointed out the association between such condition and the GC ; nevertheless, it is important to underline how most of the studies on the correlation among AG of the body-fundus (with reduction/ lack of the parietal cells)/ achlorydria and GC have involved patients with advanced neoplasia : in fact, the presence of AG and the reduction of acid secretion are often linked to the extent of the neoplasia (3,4).

Some experiences have questioned the real importance of achlorydria in favouring the development of GC (3,4). Besides, for some years, the American Society of Gastrointestinal Endoscopy has been reducing the importance of the endoscopic surveillance in case of AG, even in presence of pernicious anemia (5).

Owing to such considerations, a study has been made about the histological pattern of the body-fundic mucosa in course of preneoplastic lesions such as the epithelial dysplasia (ED) associated or progressed to GC, in order to evaluate the real association with CAG and, therefore, with a reduction of acid secretion. In fact, atrophy in the oxyntic mucosa is closely linked to the loss of acid secretion (6).

Materials and methods

The study of the body-fundic mucosa and of the acid secretion has been effected in 120 cases of ED diagnosed from January 1990 to November 1997 (Regional Hospital of Genova and Multizonal Hospital of Varese).

The casuistry is composed of 70 cases of low grade dysplasia (LGD) (41 men, average age, 59.2) and 50 cases of high grade dysplasia (HGD) (31 men, average age, 58 years).

Gastric biopsy specimens obtained in course of gastroscopy were studied for dyspepsia : for each patient, at least 8 specimens (range 8-16) were obtained from the lesion area and in the surrounding areas. Besides, at least 4 biopsies (range : 4-8) have been performed in the opposite gastric region.

For the correspondence : Gianni Testino, M.D., Via Ausonia 30/9, 16136 Genova, Italy. E-mail : testinogia@tiscalinet.it.

Table 1. – **Low Grade (LGD) and High Grade Dysplasia (HGD) : body-fundic/ antral localization and endoscopic patterns**

	Body-Fundus	Antrum	Endoscopic Patterns			
			Ulcer	Scar	Slightly Raised/Polip (Adenoma)	Flat Lesion
LGD	20	50	37	6	11	16
HGD	17	33	31	3	11	5

Any cellular atypia, abnormal differentiation with increased cell proliferation, and disorganized mucosal architecture were considered as histological hallmarks of dysplasia.

Epithelial dysplasia diagnosis was effected by an experienced pathologist (M.C.) according to well-defined criteria (7,8,9) (Table 1).

Cell proliferation has been effected with the study of Ki-67 immunohistochemistry (10). Routinely processed tissue sections of normal lymph node and tonsil were used as staining controls.

All tissues had been fixed in formalin and routinely processed to praraffin wax. Five-micrometre serial sections were cut and mounted on glass slides coated with 2% aminopropyltrioxylane (APES) (Sigma Corp, USA) in acetone. Sections were dewaxed in xylene and rehydrated in graded ethanols. Endogenous peroxidase activity was blocked by immersion in 0.3% methanolic peroxidase for 15 min. Immunoreactivity of the target antigens was enhanced using pressurized heat antigen retrieval (pressure cooking). The sections were placed in a pressure cooker containing 0.01 M sodium citrate buffer (pH6), heated to 130°C for 2 min, and then cooled. The percentage of positive cells was determined by counting at least 500 cells in at least 4 different areas, chosen at random. The average score was calculated by the arithmetic average of the results obtained in individual cases. A stain was considered positive when more than 20% of the HGD cells showed a positive nuclear staining (10).

The histological study of the gastric mucosa in gastritis (hematoxylin-eosin) was effected or revised in accordance with Dixon's classification (updated Sydney sistem – Houston) (11) taking into consideration both the inflammatory aspects, and the epithelial and glandular alterations. In relation with the aim of the study, the presence of atrophy has been evaluated and the cases have been subdivided in "non atrophic gastritis" (NAG) and in "atrophic gastritis" . Gastric atrophy was defined as of loss of glandular tissue and fibrous replacement of lamina propria and it was graded as mild/moderate chronic atrophic gastritis (MCAG) and severe chronic atrophic gastritis (SCAG).

In no study cases of atrophic gastritis has been diagnosed hypogammaglobulinemia.

After the diagnosis of ED, every subject has been, after permission, submitted to an evaluation of the acid secretion.

Stimulated acid secretion was expressed as Maximal Acid Output (MAO) in mEq/h (6).

After an overnight fast, a nasogastric tube was passed into the gastric antrum and its position checked by the water recovery test. After emptying the stomach, gastric juice was aspirated and collected using an intermittent suction unit that applies suction for 30 second in each 30 second cycle. Four 30 minute collections were obtained basally, then a subcutaneus injection of pentagastrin (Peptavlon, ICI, UK, 6 microg/kg) was given, and gastric juice was collected in 15 minute batches for the following hour. Saliva was aspirated by a continuous suction pump and discharged. The H+ concentration was determined by tritation to pH 7 with NaOH 0.1 N using an autotritator. MAO was calculated as the sum of the four consecutive 15 minutes outputs, after pentagastric infusion (range of normal values : 5.4 – 24 mEq/h) (6). Using a maximal stimulation, such as pentagastrin, we can excite the total population of functional parietal cells.

The clinical outcome subdivision of dysplasia was made using the criteria of Rugge et al. as follows (12) : association with gastric cancer, GC diagnosed within 1 year ; progression, GC diagnosed 1 year after ; persistence, dysplasia degree remained the same in at least two subsequent controls ; regression, not confirmed or lower degree in at least two subsequent controls.

Pathological diagnosis and classification of neoplastic tissues were made according to the Union Internationale Contre le Cancer TNM system (13).

Results

Ki-67 hyperproliferation was evidenced in 100% of cases (120/120) (average score 84+/-15.2).

HGD significantly associates with GC in comparison with LGD, while LGD significantly persists or regressed as regard HGD ($P < 0.0001$) (Table 2).

The histological evaluation of the oxyntic area shows SCAG in 9 cases of LGD (9/70 : 12.85%) and in 6 cases of HGD (6/50 : 12%) (Tables 3 and 4).

Hypochlorydria (MAO under 5.4 mEq/h) has been noted in 7 cases of LGD (7/70 : 10%) and in 8 cases of HGD (8/50 : 16%) (Table 5).

Complete achlorydria (MAO = 0 mEq/h) has been noted in 5 cases of LGD and in 1 case of HGD only (Table 5).

Table 2. — **Clinical Behaviour of Epithelial Dysplasia**

	Cases	Association with Gastric Cancer	Progression to Gastric Cancer	Persistence	Regression
Low Grade	70	4 (5.7%)	2 (2.9%)	19 (27.1%)	45 (64.3%)
High Grade	50	33(66%)*	4 (8%)	5 (10%)*	8 (16%)*

Fischer's exact test
* P < 0.0001.

Table 3. – **Low Grade Dysplasia - NAG : Non Atrophic Gastritis, MCAG : Mild-Moderate Atrophic Gastritis, SCAG : Severe Chronic Atrophic Gastritis**

Localization	Cases	Body-Fundic histology			Antral Histology		
		NAG	MCAG	SCAG	NAG	MCAG	SCAG
Body-Fundus	20	8 (40%)	7 (35%)	5 (25%)	13 (65%)	4 (20%)	3 (15%)
Antrum	50	32 (64%)	14 (28%)	4 (8%)	20 (40%)	18 (36%)	12 (24%)

Table 4. — **High grade dysplasia – NAG : Non Atrophic Gastritis, MCAG : Mild-Moderate Chronic Atrophic Gastritis, SCAG : Severe Chronic Atrophic Gastritis**

Localization	Cases	Body-Fundic histology			Antral Histology		
		NAG	MCAG	SCAG	NAG	MCAG	SCAG
Body-Fundus	17	7 (41%)	6 (35%)	4 (23.5%)	12 (70.6%)	4 (23.5%)	1 (5.8%)
Antrum	33	23 (69%)	8 (24%)	2 (6%)	13 (39.4%)	12 (36.4%)	8 (24.2%)

Table 5. — **Maximal Acid Output (MAO : 5.4-24 mEq/h) and Epithelial Dysplasia (LGD : Low Grade Dysplasia, HGD : High Grade Dysplasia, Non Atrophic Gastritis : NAG, MAG : Mild-Moderate Chronic Atrophic Gastritis, SCAG : Severe Chronic Atrophic Gastritis)**

Body-fundic Histology	LGD		HGD	
	Cases	MAO (mEq/h)	Cases	MAO (mEq/h)
SG	40	16.5 +/- 5.6	30	17.0 +/- 7.0§§§
MAG	21	6.3 +/- 0.9*§	14	7.0 +/- 1.0***§§§§
SAG	9	1.4 +/- 0.6**§§	6	1.6 +/- 0.9****§§§§§

Achlorydria (MAO = 0 mEq/h)
*1/21, **3/9, ***0/14, ****1/6

Hypochlorydria (MAO under 5.4 mEq/h)
§1/21, §§ 6/9, §§§ 1/30, §§§§ 2/14, §§§§§ 5/6.

In particular, in case of GC SCAG has been evidenced in 10 cases (10/43 : 23.25%), hypochlorydria in 7 cases (7/43 : 16%) and complete achlorydria in 5 cases (5/43 : 11.6%) (Table 6).

Discussion

The aim of the present study has been to evaluate the importance of the association of SCAG and, most of all, hypo-achlorydria with ED and GC so that the data related to the Helicobacter pylori infection have not been expressed (6).

From the data of the present experience it emerges that, as a matter of fact, the presence of SCAG of the oxyntic area with the presence of preneoplastic lesions (dysplasia) or early GC is associated to a low percentage of cases (LGD 9/70 : 12.8%, HGD 6/50 : 12%, ED associated or progressed to GC 10/43 : 23%). Besides, the cases of hypo- achloridria too are limited to a low percentage (LGD 11/70 : 15.7%, HGD 9/50 : 18%, ED associated or progressed to GC 12/43 : 27.9%). However, even in a recent experience made in course of advanced gastric cancer NAG has been found in 57.9%, and SCAG in 42.1% (14). It, therefore, emerges how the Correa evolution (1, 2) is to be put, at least partially, under discussion.

Past experiences have already pointed out how not only preneoplastic changes but intestinal type early GC too can develop in a contest of normochloridria and without nitrocompounds increase. Sobala (15) has demonstrated how there is no increase of nitrocompounds in course of preneoplastic changes, Parsonnet (16) points out that the bacteria overgrowth is present in one part of the cases only, and Viani *et al.* (17) are convinced that hypochloridria is not at the origin of the formation of nitroso-compounds due to the proliferation of nitrate reducing bacteria.

Table 6. – **Cases of epithelial dysplasia associated or progressed to gastric cancer (Non Atrophic Gastritis : NAG, MCAG : Mild-Moderate Chronic Atrophic Gastritis, SCAG : Severe Chronic Atrophic Gastritis, MAO : Maximal Acid Output)**

	Body-fundic Histology				Achlorydria (MAO = 0 mEq/h)	Hypochlorydria (MAO under 5.4 mEq/h)
	Cases	NAG	MCAG	SCAG	Cases	Cases
pT1/pN0	32	17 (53%)	7 (22%)	8 (25%)	3	4
pT2/pN	9	4 (44%)	2 (22%)	1 (11%)	1	2
pT4/pN0	2	1 (50%)	– – – –	1 (50%)	1	1

Therefore, it is opportune to consider that the progression NAG → AG → hypo-achloridria/ bacterial overgrowth → preneoplastic changes → GC (1,2) is not to be considered the only one for the development of neoplastic changes and overall to be limited to few cases of SCAG of the oxyntic area. Moreover it is well known how nitrate reductase and N-nitrosatate have optimal pH between 6 and 8 (18). As a matter of fact, pH-metric evaluation has shown that pH is costantly > 5 in cases of pernicious anemia (PA) (19). In fact, only in course of severe AG of the body-fundus with normal antrum connected with PA achloridria has been found in 100% of cases (18 cases, MAO mEq/ml = 0) (20).

Even if longitudinal cohort studies have evidenced the association AG/ IM, dysplasia is not always part of this evolution : such lesion may arise in NAG (3,4,21).

The presence of multi-focal SCAG in many cases is mostly linked to the damage in the glandular necks (stem cells) stead in time, but dysplastic changes may be started in a previous period and are not linked to secretory alterations. It is possible that the continuous increase of stem cells proliferation (22) may contribute, on the one hand, to a loss of those glands with atrophic evolution, and on the other hand, by means of hyperproliferation, to the contemporary promotion / progression of dysplastic and neoplastic clones (21,22,23,24,25). Recently, Negrini et al. affirm that atrophic gastritis, preneoplastic lesions and GC could be different outcomes of a single pathogenic pathway (26).

Such concepts suggest that endoscopic surveillance program should be modified and in accordance with the American Society of Gastrointestinal Endoscopy (5) we can conclude that there are no sufficient data to support subsequent endoscopic surveillance for the subjects with atrophic gastritis.

References

1. CORREA P., CUELLO C., DUQUE E., BURBANO L.C., GARCIA F.T., BOLANOS O., BROWN C., HAENSZEL W. Gastric cancer in Colombia. III : natural history of precursor lesions. *JNCI*, 1976, **57** : 1027-35.
2. Correa Phuman gastric carcinogenesis : a multistep and multifactorial process – First American Cancer Society award lecture on cancer epidemiology and prevention. *Cancer Research*, 1992, **52** : 6735-40.
3. CHELI R., TESTINO G. Chronic atrophic gastritis and gastric cancer, Verona : Cortina International, 1993.
4. CHELI R., TESTINO G., GIACOSA A., CORNAGGIA M. Chronic gastritis : its clinical and physiopathological meaning. *J. Clin. Gastroenterol.*, 1995, **21** : 193-7.
5. The role of endoscopy in the surveillance of premalignant of the upper gastrointestinal tract. Guidlines for clinical application. *American Society for Gastrointestinal Endoscopy*, 1997.
6. TESTINO G., CORNAGGIA M., CHELI R. Stimulated gastrinemia, parietal cell mass and stimulated acid secretion in autonomous chronic gastritis : Helicobacter pylori influence. *Hepato-Gastroenterol.*, 1995, **42** : 650-4.
7. GOLDSTEIN N.S., LEWIN K.L. Gastric epithelial dysplasia in adenoma : histological review and histological criteria for gading. *Human Pathol.*, 1997, **28** : 127-33.
8. TESTINO G., SUMBERAZ A., DELEHAYE E., CORNAGGIA M., VALENTINI M. Gastric high grade dysplasia. *Gastroenterology*, 1999, **117** : 286-7.
9. MISDRAJI J., LAUWERS G.Y. Gastric epithelial dysplasia. *Seminars in Diagnostic Pathology*, 2002, **19** : 20-30
10. TESTINO G., GADA D., DE IACO F., CORNAGGIA M. p53 and Ki-57 expression in epithelial gastric displasia and gastric cancer. *Panminerva Med.*, 2002, **44** : 369-71
11. DIXON M.F., GENTA R.M., YARDLEY J.H. Classification and grading of gastritis. The uptodate Sydney System. *Am. J. Surg. Pathol.*, 1996, **20** : 1161-1181.
12. RUGGE M., FARINATI F., BAFFA R. Gastric epithelial dysplasia in the natural history of gastric cancer : a multicenter prospective follow-up study. *Gastroenterology*, 1994, **107** : 1288-96
13. SOBIN L.H. TNM : evolution and relation to other prognostic factors. *Semin. Surg. Oncol.*, 2003, 21 : 3-7
14. KNAVY B.A., MORAD N.A., JAMAL A. Non-neoplastic changes in gastric antrum : are they different in distally located intestinal and diffuse-type gastric adenocarcinoma ? *Eur. J. Cancer Prev.*, 1997, **6** : 167-170.
15. SOBALA G.M., PIGNATELLI B., SCHORAH C.J. Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach. *Carcinogenesis*, 1991, **12** : 193-8.
16. PARSONNET J. Helicobacter pylori and gastric cancer. *Gastroenterol. Clin. N. Am.*, 1993, **22** : 89-104.
17. VIANI F., SIEGRIST H.H., PIGNATELLI B., CEDERBERG C., IDSTROM J.P., VERDU E.F. The effect of intra-gastric acidity and flora on the concentration of N-nitroso compounds in the stomach. *Eur. J. Gastroenterol. Hepatol.*, 2000, **12** : 165-73.
18. HILL M.J. Factors controlling endogenous N-nitrosation. *Eur. J. Cancer Prev.*, 1996, **5** (Suppl. 1) : 71-4.
19. MELA G.M., SAVARINO V., VIGNERI S., DI MARIO F. Atlas of gastric pH-metric evaluation. Berlin : Springer-Verlag, 1994.
20. TESTINO G., MENARDO G., CORNAGGIA M., PERASSO A., CHELI R. Anatomical-functional correlations in chronic atrophic gastritis with and without pernicious anemia. *Exp. Clin. Gastroenterol.*, 1993, **3** : 35-9.
21. TESTINO G., CORNAGGIA M., VALENTINI M. Helicobacter pylori, preneoplastic changes, gastric cancer : a point of view. *Eur. J. Gastroenterol. Hepatol.*, 1999, **11** : 357-9.
22. ANTI M., ARNUZZI A., GASBARRINI G. Epithelial cell turnover and apoptosis. *Ital. J. Gastroenterol. Hepatol.*, 1999, **11** : 357-9.
23. TESTINO G. Body-fundic mucopeptic cell expansion after Helicobacter pylori eradication. *Am. J. Gastroenterol.*, 1998, **93** : 2636-8.
24. HATTORI T. Development of adenocarcinoma in the stomach. *Cancer*, 1986, 1528-34.
25. TESTINO G., VALENTINI M., CORNAGGIA M., TESTINO R. Chronic atrophic gastritis and gastric cancer. *Digest. Liver Dis.*, 2000, **32** : 71-5.
26. NEGRINI R. Is autoimmunity involved in the relationship between Helicobacter pylori infection, atrophic gastritis and gastric cancer ? *Ital. J. Gastroenterol. Hepatol.*, 1999, **31** : 842-5.