

A Prospective Study of Gastric Carcinoids and Enterochromaffin-Like Cell Changes in Multiple Endocrine Neoplasia Type 1 and Zollinger-Ellison Syndrome: Identification of Risk Factors

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Context: Multiple endocrine neoplasia type 1 (MEN1) patients frequently develop Zollinger-Ellison syndrome (ZES). These patients can develop proliferative changes of gastric enterochromaffin-like (ECL) cells and gastric carcinoids (ECL-cell tumors). ECL-cell changes have been extensively studied in sporadic ZES patients and can be precursor lesions of gastric carcinoids, but little is known about factors influencing their severity or development of carcinoids in MEN1/ZES patients.

Objectives: Our objective was to prospectively analyze ECL-cell changes and gastric carcinoids (ECL-cell tumors) in a large series of MEN1/ZES patients to detect risk factors and deduct clinical guidelines.

Setting and Patients: Fifty-seven consecutive MEN1/ZES patients participated in this prospective study at two tertiary-care research centers.

Interventions and Outcome Measures: Assessment of MEN1, gastric hypersecretion, and gastroscopy with multiple biopsies was done according to a fixed protocol and tumor status. ECL-cell changes and α -human chorionic gonadotropin staining were assessed in each biopsy and correlated with clinical, laboratory, and MEN1 features.

Results: ECL-cell proliferative changes were universally present, advanced changes in 53% and carcinoids in 23%. Gastric nodules are common and are frequently associated with carcinoids. Patients with high fasting serum gastrin levels, long disease duration, or a strong α -human chorionic gonadotropin staining in a biopsy are at higher risk for an advanced ECL-cell lesion and/or gastric carcinoid.

Conclusions: Gastric carcinoids and/or advanced ECL-cell changes are frequent in MEN1/ZES patients, and therefore, regular surveillance gastroscopy with multiple routine biopsies and biopsies of all mucosal lesions are essential. Clinical/laboratory data and biopsy results can be used to identify a subgroup of MEN1/ZES patients with a significantly increased risk for developing gastric carcinoids, allowing development of better surveillance strategies. (*J Clin Endocrinol Metab* 93: 1582–1591, 2008)

Characteristically, multiple endocrine neoplasia type 1 (MEN1) patients develop parathyroid hyperplasia (causing hyperparathyroidism) and pancreatic endocrine tumors

(PETs) as well as pituitary and adrenal adenomas (1). The most characteristic PETs are pancreatic nonfunctional tumors and duodenal gastrinomas, which arise from proliferative precursor le-

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Abbreviations: BAO, Basal acid output; DH, diffuse hyperplasia; ECL, enterochromaffin-like; FSG, fasting serum gastrin; α -hCG, α -human chorionic gonadotropin; IR, immunoreactivity; LH, linear hyperplasia; MAO, maximal acid output; MEN1, multiple endocrine neoplasia type 1; MH, micronodular hyperplasia; PET, pancreatic endocrine tumor; PPI, proton pump inhibitor; ZES, Zollinger-Ellison syndrome.

sions in the pancreas and duodenum, respectively (2, 3). Over the last few years, other tumors have been increasingly described, including foregut carcinoids (thymic, bronchial, and gastric) and nonendocrine tumors (skin, smooth muscle, and central nervous system) (4). PETs and foregut carcinoids are receiving increased attention, because with effective treatment of the parathyroid and pituitary disease, they are becoming important determinants of survival (4–6).

Although there are a number of recent studies of PETs and foregut carcinoids in MEN1 (5, 7, 8), little is known about gastric carcinoids [also called gastric neuroendocrine tumors or entero-

chromaffin-like (ECL)-cell tumors]. Although it is established that gastric carcinoids occur almost exclusively in patients with MEN1 with Zollinger-Ellison syndrome (ZES; 21–70% of MEN1 patients; type 2 carcinoids) (4, 9, 10) and that they can be malignant in 10–30% of cases (11–13), their frequency, contributing factors, or association with other features of MEN1 are largely unknown. This has occurred because previous studies are limited by small patient numbers, their retrospective nature, and the small number of gastric biopsies analyzed. Furthermore, it is proposed that gastric carcinoids develop in chronic hypergastrinemic states, such as MEN1/ZES, atrophic gastritis, and per-

TABLE 1. Demographic, clinical, and laboratory characteristics of 57 MEN1/ZES patients

ZES characteristics	no (%)	MEN1 characteristics	no (%)
No. of patients	57	Age (yr)	
Male gender	27 (47)	At onset of MEN1	31.6 ± 1.1 (12.6–60.6)
Age (yr)		At diagnosis of MEN1	38.0 ± 1.7 (12.6–72.8)
At onset of ZES	35.7 ± 1.2 (18.4–60.6)	Disease (yr)	
At biopsy	47.2 ± 1.6 (22.4–77.0)	MEN diagnosis to biopsy ^a	16.1 ± 1.5 (1.5–53.4)
Duration (yr)		HPT observed to biopsy ^b	15.0 ± 1.6 (0–53.4)
from onset of ZES	11.4 ± 1.1 (0.5–31.4)	MEN1 onset to last follow-up	21.7 ± 1.4 (6.1–58.7)
Symptoms		MEN1 features	
Pain	38 (67)	ZES	57 (100)
Diarrhea	39 (68)	HPT	55 (96)
Esophageal symptoms	28 (49)	Pituitary disease ^a	32 (60)
Ulcer history	33 (58)	Adrenal abnormality ^a	24 (45)
Antisecretory drugs at biopsy		Other functional PET ^a	2 (4)
Proton pump inhibitors	52 (91)	Bronchial carcinoid ^a	9 (17)
H ₂ antagonists	3 (5)	Thymic carcinoid ^a	5 (9)
None	3 (5)	First MEN1 symptom	
Duration of antisecretory drug		Hyperparathyroidism	24 (42)
With PPI	4.4 ± 0.5 (0–16)	ZES	25 (44)
Any	8.8 ± 0.9 (0–24.6)	Pituitary disease	3 (5)
Sustained hypochlorhydria present ^d	25 (56)	Other	1 (2)
FSG at biopsy (pg/ml) (median)	490 (57–110,000)	Asymptomatic	4 (7)
BAO at biopsy (mEq/h) ^e	38.6 ± 4.2 (2.9–144)	HPT related ^a	
Previous surgery		History of renal colic	29 (55)
Acid-reducing	2 (3.5)	Age at HPT observed (yr)	32.2 ± 1.6 (14.2–71)
Gastrinoma resection	22 (39)	PTX performed	47 (89)
Duration from surgery (yr)		Age at first PTX (yr)	36.6 ± 1.6 (17.2–59.5)
Acid-reducing	17.8 ± 7.3 (10.5–25.2)	Time first PTX to biopsy	10.3 ± 1.4 (0.2–38.8)
Gastrinoma resection	6.2 ± 1.0 (0.39–18)	PTX once	20 (38)
Primary PET location		PTX twice	17 (32)
Duodenal tumor	28 (49)	PTX more than twice	10 (19)
Pancreatic tumor	27 (47)	Pituitary related ^a	
Primary PET size		Age at diagnosis (yr) ^c	38.7 ± 2.3 (12.6–71)
0–0.9 cm	14 (25)	Prolactinoma	26 (49)
>2.9 cm	5 (9)	ACTH	3 (6)
Not detected	23 (40)	GH	1 (2)
Tumor extent		Nonfunctional	8 (15)
Localized	39 (68)	Surgical treatment	7 (13)
Liver metastases	18 (32)	Duration diagnosis biopsy	7.8 ± 1.3 (0–33.7)
Bone metastases	2 (4)	Other MEN1 features ^a	
		Active HPT at biopsy	35 (66)
		Family history of MEN1	36 (68)

Mean ± SEM (range) is indicated for continuous variables with a normal distribution, and median (range) is indicated for continuous variables that are not normally distributed (FSG). HPT, hyperparathyroidism; PTX, parathyroidectomy.

^a Data available on 53 patients.

^b Data available on 51 patients with HPT.

^c Data available on 34 patients.

^d Data available on 45 patients with serial acid assessments on drug.

^e BAO (n = 50) is from patients without previous acid-reducing surgery.

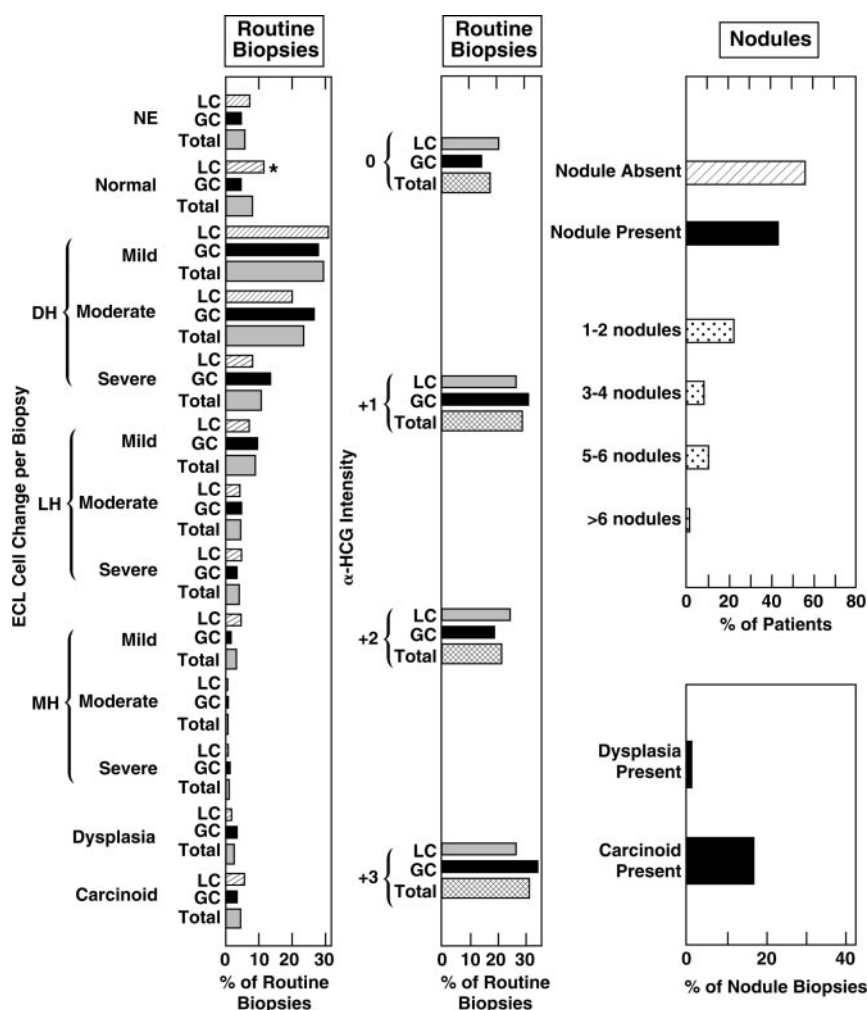


FIG. 1. Frequency of ECL-cell changes, α -hCG immunoreactivity, and ECL-cell tumors in routine gastric body and gastric nodule biopsies from the 57 prospectively studied MEN1/ZES patients. *Left panel*, The 437 routine gastric biopsies were from the greater ($n = 226$) and lesser ($n = 211$) curvature. Results are expressed as the percentage of the routine lesser-curvature (LC) or greater-curvature (GC) biopsies demonstrating the indicated ECL-cell change. *Middle panel*, Distribution of α -hCG-IR in 210 greater- and 196 lesser-curvature biopsies. *Right upper panel*, Frequency of nodules found in 25 patients. *Right lower panel*, Frequency of ECL-cell tumors and/or dysplasia in gastric nodule biopsies. NE, Not evaluable. *, $P < 0.05$ compared with greater-curvature result.

nicious anemia, due to proliferative effects of gastrin on gastric ECL cells, resulting in hyperplasia, dysplasia, and finally, carcinoids (10, 14, 15). Therefore, they are more accurately called ECL-cell tumors than gastric carcinoids or gastric neuroendocrine tumor, because the latter terms could also include tumors arising from gastrin- or serotonin-producing cells. Although ECL-cell hyperplastic changes are reported in MEN1/ZES patients (16–20), there have been no large systematic studies correlating these changes with the development of ECL-cell tumors in these patients.

Therefore, the purpose of this prospective study was to assess the frequency and extent of ECL changes and ECL-cell tumors in a large number of MEN1/ZES patients and to identify for the first time possible predictive factors for each of these. This was accomplished by studying a large number of consecutive MEN1/ZES patients using an established protocol involving a large number of systematic gastric biopsies (10).

Patients and Methods

Patients

All patients admitted to the National Institutes of Health (NIH) with a confirmed diagnosis of MEN1/ZES from 1996–2005 or to La Sapienza University Hospital from 1988–1999 were included. This study was approved at the NIH by the Clinical Research Committee of the National Institute of Diabetes and Digestive and Kidney Diseases or by the local (La Sapienza) ethical committee in adherence with the declaration of Helsinki (10).

Methods

Diagnostic criteria for MEN1 syndrome and ZES were as described previously (4, 6, 21), and the onset of MEN1 and ZES was determined (4, 6, 21, 22). To establish the diagnosis of ZES, patients underwent a gastric acid analysis [basal/maximal acid output (BAO/MAO)] (23), fasting serum gastrin (FSG) measurements (10, 24), and a secretin test. To define the extent/localization of PETs and other MEN1 tumors, imaging studies were performed (5, 8, 25, 26).

Specific protocol

At the time of gastric biopsy, all patients underwent evaluation of MEN1 status, ZES diagnosis, and determination of tumor location/extent. During the initial evaluation as well as during subsequent yearly assessments, MEN1 status and ZES tumor features were assessed (21). On each follow-up, acid studies were performed to allow assessment of acid secretory control (27, 28). NIH patients were classified as having sustained hypochlorhydria if acid output was less than 0.2 mEq/h for at least 50% of all yearly assessments (10, 27, 29).

Gastroscopic biopsy and processing

Biopsies were performed using an Olympus GIF 2T100 gastroscope (Olympus America Corp., Melville, NY), and 10 Jumbo gastric biopsies (eight body and two antrum; NIH), or eight biopsies (six body and two antrum; La Sapienza) were taken (10, 30). The corporeal routine

biopsies were taken in a preset order: at the NIH, four from the greater curvature (three, La Sapienza) and four from the lesser curvature (three, La Sapienza) (10, 30). All gastric nodules were biopsied. Biopsies were processed as described previously (10, 30).

Histology and immunohistochemistry

Serial 5- μ m-thick sections perpendicular to the mucosal surface were stained with hematoxylin-eosin for evaluation of the type of mucosa, Giemsa stain for *Helicobacter pylori* evaluation, and immunostaining for endocrine cells (10). α -Human chorionic gonadotropin (α -hCG) immunoreactivity (IR) was determined as described previously (10) and graded from 0–3+ depending on the number of positive cells per linear millimeter as described previously (10).

Endocrine cell evaluation

The distribution of ECL-cell changes was investigated in sections immunostained for chromogranin A, with exclusion of areas of intestinal metaplasia. Biopsies were classified as evaluable or nonevaluable (10,

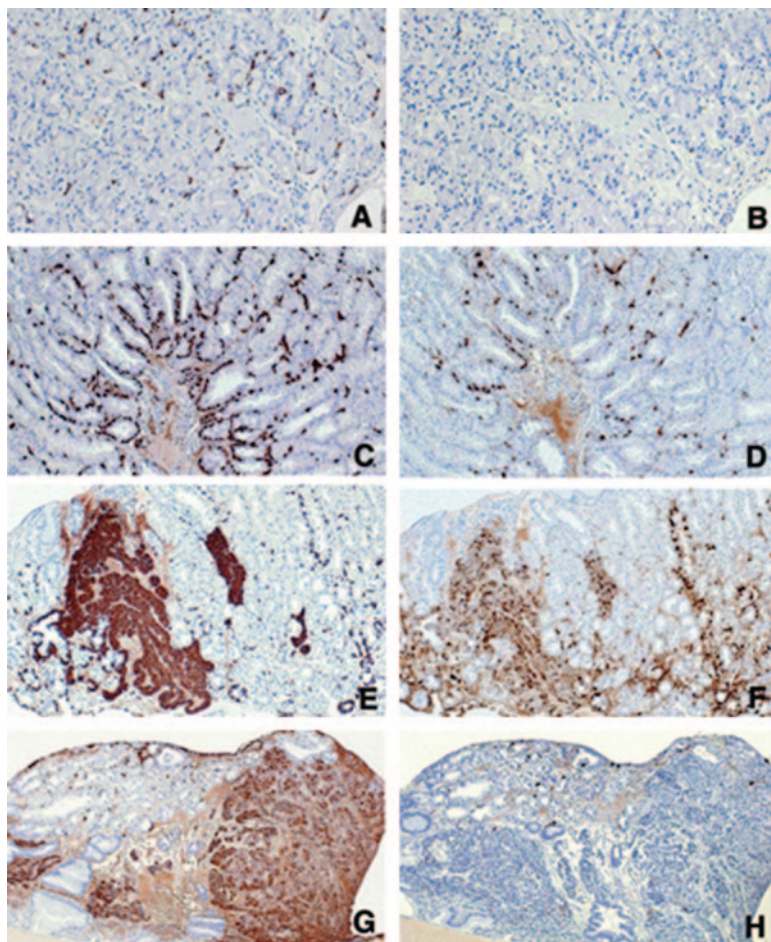


FIG. 2. Gastric biopsy specimens showing different degrees of ECL cell changes on chromogranin A staining (A, C, E, and G) or α -hCG expression (B, D, F, and H) in different patients. A, Qualitatively normal distribution of chromogranin A-positive immunoreactive cells (in brown) in the oxyntic mucosa; B, consecutive section of the same area as in A, showing no cells positive for α -hCG; C, severe LH of ECL cells (in brown); D, section of the same area as in C showing strong (3+) α -hCG expression by ECL cells; E, small intramucosal ECL-cell tumor (0.9 mm in major axis, on the left) in a routine biopsy of the oxyntic mucosa also presenting association with an ECL-cell dysplastic lesion (in the center) and severe LH of ECL cells (on the right); F, consecutive section of the same area showing strong α -hCG expression in all types of ECL-cell changes; G, ECL-cell tumor from a mucosal nodule; H, serial section of the same area as in G with the vast majority of tumor cells showing no expression of α -hCG.

30). Carcinoids were diagnosed when lesions exceeded 0.5 mm (10, 14). ECL-cell changes were qualitatively evaluated by two independent examiners as proposed by Solcia *et al.* (14). Biopsies were graded on the severity of each hyperplastic lesion. Results were classified two different ways, either according to the highest grade of ECL-cell hyperplasia (10, 30) or by compiling a patient ECL-cell index as described previously (10, 30). Briefly, this involves assigning a numerical value to each biopsy that increases with increasing grades and severity of hyperplasia (diffuse, linear, micronodular, and adenomatoid) or dysplasia and then averaged for the number of biopsies as described previously (10, 30), with the modification that each individual carcinoid was assigned a score of 14. Gastritis was assessed according to the updated Sydney System, and the expression of α -hCG was evaluated (10).

Statistics

Fisher's exact test, the Mann-Whitney *U* test, and the Cochran-Armitage test were used. Linear regression was used to find the best fit in simple regression analyses and the Spearman rank-correlation method to determine the correlation coefficients and exact *P* values. To find the factors that remain significant after adjustment for each other, the ECL-cell index was log-transformed, and linear regression with forward and

backward selection was used. The detection of carcinoids was similarly analyzed with the logistic regression model and the α -hCG-IR with extended logistic regression of ordered categories of less than or equal to 1+, 2+, and 3+. A two-tailed *P* value of <0.05 was considered significant.

Results

In this study, 57 consecutive patients with MEN1/ZES were enrolled. This cohort resembled other large series of patients with MEN1/ZES (4, 16, 22, 24, 31) with a mean age in the fifth decade and most of their clinical ZES and MEN1 characteristics. None of the patients were disease free, consistent with reports of the rarity of cure in these patients (8, 32). Therefore, more than 96% of patients were taking antisecretory drugs with proton pump inhibitors (PPIs) the most commonly used (91%). All patients were treated with antisecretory drugs at some point, and the duration of treatment with any drug or with PPIs, which are currently the drugs of choice (33), was long. For the NIH patients, 56% showed sustained hypochlorhydria (Table 1). Similar to most recent reports, almost all patients had hyperparathyroidism (Table 1) (4, 21, 34).

Overall, 559 gastric body biopsies were taken. Of these, 437 (78%) were routine with 211 lesser-curvature biopsies (38%) and 226 from the greater curvature (40%) (Fig. 1, left panel). In 25 patients, gastric nodules were detected and biopsied for a total of 122 nodule biopsies (Fig. 1, right upper panel). Of the 411 evaluable routine and 122 nodule biopsies, 41 carcinoids

(Fig. 2, E–H, and Fig. 3) were detected in 13 patients (23% of patients). Of these, 21 were in nodules (Fig. 2, G and H) and 20 in routine (Fig. 2, E and F) biopsies. A higher percentage of nodules rather than routine biopsies demonstrated a carcinoid (17.2 vs. 4.6%, $P < 0.0001$) (Fig. 1, left and right panels). Sixty-three percent of routine biopsies showed diffuse hyperplasia (DH), 17% linear hyperplasia (LH) (Fig. 2, C and D), and 5.5% micronodular hyperplasia (MH) (Fig. 1, left panel). Twelve routine (2.9%) and two nodule biopsies (1.64%) showed dysplasia (Figs. 1 and 2, E and F). Significantly fewer nodule biopsies showed dysplasia rather than carcinoids (1.6 vs. 17.2%, $P < 0.0001$) (Fig. 1, right lower panel).

No patient had all biopsies with a normal pattern (Fig. 3). Forty-seven percent had DH as the most advanced ECL-cell change, 25% LH, 3.5% MH, and 1.8% dysplasia, and in 23%, a carcinoid was found. Thirty patients (53%) had advanced ECL-cell changes (at least LH mild) (Fig. 3). α -hCG-IR was pro-

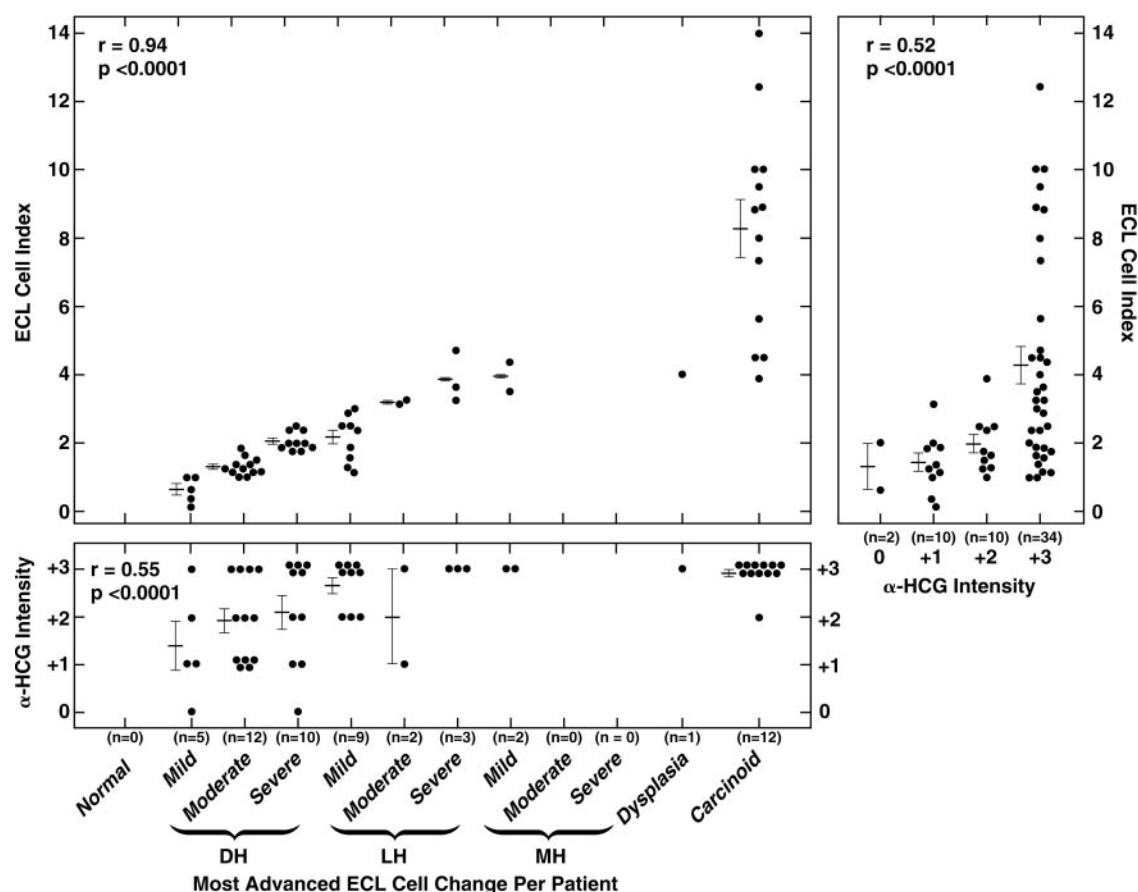


FIG. 3. Correlation of the magnitude of the ECL-cell index, the most advanced ECL-cell change, and the α -hCG-IR in 533 biopsies from 57 patients with MEN1/ZES. Each point represents data from one patient. The mean and SEM are indicated by horizontal and vertical bars, respectively. The n refers to the number of patients in each category. The upper left panel shows the correlation between the most advanced cell change per patient and the ECL-cell index computed for each patient. The upper right panel shows the correlation between the most intense α -hCG staining and the ECL-cell index in each patient. The lower left panel shows the correlation between the most advanced cell change per patient and the most intense α -hCG staining. ECL-cell index was available for 13 patients with ECL-cell tumors, whereas α -hCG staining intensity was available for only 12 patients with ECL-cell tumors.

nounced in the whole spectrum of ECL-cell changes, whereas it tended to be sparse or absent in dysplasia and carcinoids (Fig. 2).

The ECL-cell index varied from 0.13–14 (mean 3.44). The ECL-cell index was less than 3 in 61%, 3–9 in 30%, and more than 9 in 9% of patients. The ECL-cell index correlated well with the most advanced ECL-cell change per patient ($r = 0.94$; $P < 0.0001$) (Fig. 3).

The mean α -hCG score for all patients was 2.36 ± 0.12 (Fig. 1, middle panel). Seventeen percent of routine biopsies were graded 0, 29% 1+, 22% 2+, and 31% 3+ (Fig. 1, middle panel). The majority of patients (34 patients) showed strong α -hCG-IR (3+) in at least one biopsy (Fig. 3, right panel). The α -hCG-IR correlated significantly with the most advanced ECL-cell change ($r = 0.55$; $P < 0.0001$) and the ECL-cell index ($r = 0.52$; $P < 0.0001$) (Fig. 3).

Patients with a higher ECL-cell index had a longer ZES disease duration, had a higher ECL-cell index (Tables 2 and 3 and Fig. 4B1), longer duration of medical ZES therapy (Tables 2 and 3 and Fig. 4D1), a longer duration of omeprazole treatment (Tables 2 and 3 and Fig. 4C1), and a higher FSG (Tables 2 and 3 and Fig. 4). There was a very strong correlation ($r = 0.58$; $P < 0.0001$) between the ECL-cell index and the FSG level (Fig. 4A1).

Patients with a duodenal primary had a higher ECL-cell index (Tables 2 and 3), and those with localized disease had a lower ECL-cell index. Multivariate analysis showed serum gastrin level (67% increase in ECL-cell index for each factor 10 increase in gastrin, $P < 0.0001$), the presence of a proven duodenal primary (70% increase, $P = 0.0060$), and the duration of ZES (27% increase for every 10 yr, $P = 0.023$) are independently associated with a higher ECL-cell index (Table 2).

FSG levels strongly correlated with the α -hCG-IR and the presence of a duodenal primary (Tables 2 and 3 and Fig. 4A2). The duration of omeprazole treatment or any medical antisecretory treatment was associated with a higher ECL-cell index (Tables 2 and 3). Furthermore, there was a significant correlation between α -hCG-IR and the duration of ZES (Fig. 4B2) and of omeprazole treatment (Fig. 4C2). Multivariate analysis demonstrated that the log-transformed serum gastrin (odds ratio = 18; $P = 0.0010$) and the square-root transformed duration of omeprazole treatment (odds ratio = 3.1; $P = 0.0030$) are independently associated with a higher α -hCG-IR.

In general, clinical, laboratory, or tumor variables factors associated with a high ECL-cell index and a high α -hCG-IR are also associated with carcinoids (Tables 2 and 3). Especially im-

TABLE 2. Effect of presence or absence of various clinical, tumor, biochemical, and mucosal variables on ECL-cell index, α -hCG intensity, and incidence of ECL-cell tumors (gastric carcinoids) in 57 patients with MEN1/ZES

Variable	ECL-cell index (mean \pm SEM)			α -hCG intensity (mean \pm SEM)			ECL-cell tumors detected (%)		
	Absent	Present	P	Absent	Present	P	Absent	Present	P
Onset ZES to biopsy > 10 yr	2.21 \pm 0.34	4.72 \pm 0.70	0.0029	2.03 \pm 0.19	2.70 \pm 0.12	0.007	6.90	39.28	0.0046
Diagnosis of ZES to biopsy > 5 yr	2.19 \pm 0.34	4.74 \pm 0.69	0.0012	2.10 \pm 0.17	2.63 \pm 0.15	0.016	6.90	39.28	0.0046
Duration of omeprazole > 4 yr	3.14 \pm 0.59	3.83 \pm 0.58	0.103	2.10 \pm 0.17	2.68 \pm 0.15	0.008	15.62	32.00	0.21
Duration of medical treatment > 7.4 yr	2.08 \pm 0.34	4.85 \pm 0.68	<0.0001	2.07 \pm 0.17	2.67 \pm 0.15	0.004	6.90	39.28	0.0046
FSG > 490 pg/ml	2.00 \pm 0.22	4.94 \pm 0.72	0.0013	1.93 \pm 0.18	2.81 \pm 0.09	0.0002	3.45	42.86	0.0004
MAO > 63 mEq/h	1.82 \pm 0.29	3.99 \pm 0.58	0.0005	2.41 \pm 0.23	2.47 \pm 0.21	0.92	5.88	26.67	0.16
Time since PET surgery > 5yr	2.94 \pm 0.38	5.53 \pm 1.34	0.022	2.28 \pm 0.14	2.70 \pm 0.21	0.20	17.39	45.45	0.10
Duodenal primary	1.81 \pm 0.20	5.13 \pm 0.70	<0.0001	2.00 \pm 0.18	2.74 \pm 0.14	0.001	3.70	42.86	0.0009
Localized disease	5.30 \pm 0.91	2.59 \pm 0.37	0.0018	2.65 \pm 0.17	2.23 \pm 0.15	0.12	55.56	7.69	0.0002

For continuous variables, the median value is used to divide patients into two groups. Multivariate analysis shows that FSG, duodenal primary, and duration of ZES are independently associated with the ECL-cell index; FSG and duration of omeprazole treatment with the α -hCG intensity; and FSG and localized disease with the presence of ECL-cell tumors.

portant was the association of carcinoids with high FSG values ($P = 0.0004$), long ZES duration ($P = 0.0036$), or various clinical/tumor factors (duodenal primary, localized disease, and duration of medical treatment) (Tables 2 and 3). Numerous other variables listed in the footnote of Table 3 did not influence the ECL-cell index, the α -hCG-IR, or the incidence of carcinoids. Multivariate analysis demonstrated only the FSG (odds ratio = 20; $P = 0.0011$) and the presence of localized disease (odds

ratio = 0.051; $P = 0.018$) are independently associated with the presence of carcinoids.

To analyze whether patients with carcinoids have more advanced ECL-cell changes in the biopsies not derived from carcinoids, we calculated a modified ECL-cell index that did not assign any score to carcinoids. Patients with carcinoids had a significantly higher modified ECL-cell index than patients without carcinoids (4.94 ± 0.41 vs. 2.01 ± 0.16 , $P < 0.0001$; data not shown).

A number of histological (any biopsy with at least LH severe), endoscopic (nodules present), clinical (MAO > 70 mEq/h), and laboratory (FSG > 1400 pg/ml) findings showed a relatively high sensitivity (80–100%) and specificity (70–100%) (Table 3) for the presence of carcinoids and therefore could be clinically helpful in identifying a subset of MEN1/ZES patients at high risk of having a carcinoid.

TABLE 3. Sensitivity and specificity of different clinical, tumor, biochemical, and mucosal variables in the detection of patients with ECL-cell tumors

Variable	Detection of ECL-cell tumor (per patient)	
	Sensitivity (%)	Specificity (%)
First biopsy α -hCG intensity = 3+	70	86
Any biopsy α -hCG intensity = 3+	92	50
Any biopsy \geq LH severe	92	89
Dysplasia in any biopsy	53	92
Modified ECL index > 3.51	77	91
No gastritis at biopsy	78	21
Nodules present at biopsy	100	73
More than two nodule biopsies	61	91
FSG > 1400 pg/ml	85	93
Duration ZES diagnosis to biopsy > 15 yr	54	79
Total duration omeprazole > 7 yr	46	93
Duration medical treatment > 9 yr	85	67
Duodenal primary tumor	92	64
BAO > 50 mEq/h	56	80
Age MEN onset < 30 yr	69	59
Duration HPT > 15 yr	69	63
Duration MEN1 > 15 yr	77	64
Family history for MEN1	85	37
No <i>H. pylori</i> at biopsy	100	11

The following variables did not show any significant differences: male gender, age at biopsy more than 46 yr, age at onset ZES more than 36 yr, age at diagnosis of ZES more than 38 yr, BAO more than 32 mEq/h, pain present, diarrhea present, esophageal symptoms present, PPIs at biopsy, sustained hypochlorhydria at biopsy, cured at biopsy, gastric surgery before biopsy, primary tumor greater than 1 cm, primary tumor greater than 2.9 cm, death due to MEN1/ZES, family history of MEN1 present, and gastritis present.

Discussion

The aim of this study was to assess ECL-cell changes in MEN1/ZES patients, including the presence of ECL-cell tumors, and to identify risk factors that could be clinically useful. This project was undertaken because a number of studies suggest ECL-cell tumors may not be uncommon in MEN1/ZES patients and can be clinically important (4, 11, 13, 16, 17, 35). However, a review of the literature supports the conclusion that their frequency is unclear, risk factors for their development are not available, and their relationship to other MEN1/ZES features is unclear, as is their relationship to the gastric ECL-cell changes that may precede their development. First, ECL-cell tumors are reported in 0–33% of MEN1/ZES (mean, $6 \pm 2\%$, 11 small series) (4, 9, 17–20, 36, 37). Second, ECL-cell tumors occurring in ZES are almost all in the 20–25% with MEN1/ZES (16, 18–20). Third, 10–30% of ECL-cell tumors are malignant and can cause symptoms, including carcinoid syndrome (11). Fourth, studies in animals and in humans led to the proposal that chronic hypergastrinemia causes the development of ECL-cell tumors by inducing increasing proliferative changes in gastric ECL cells (hyperplasia

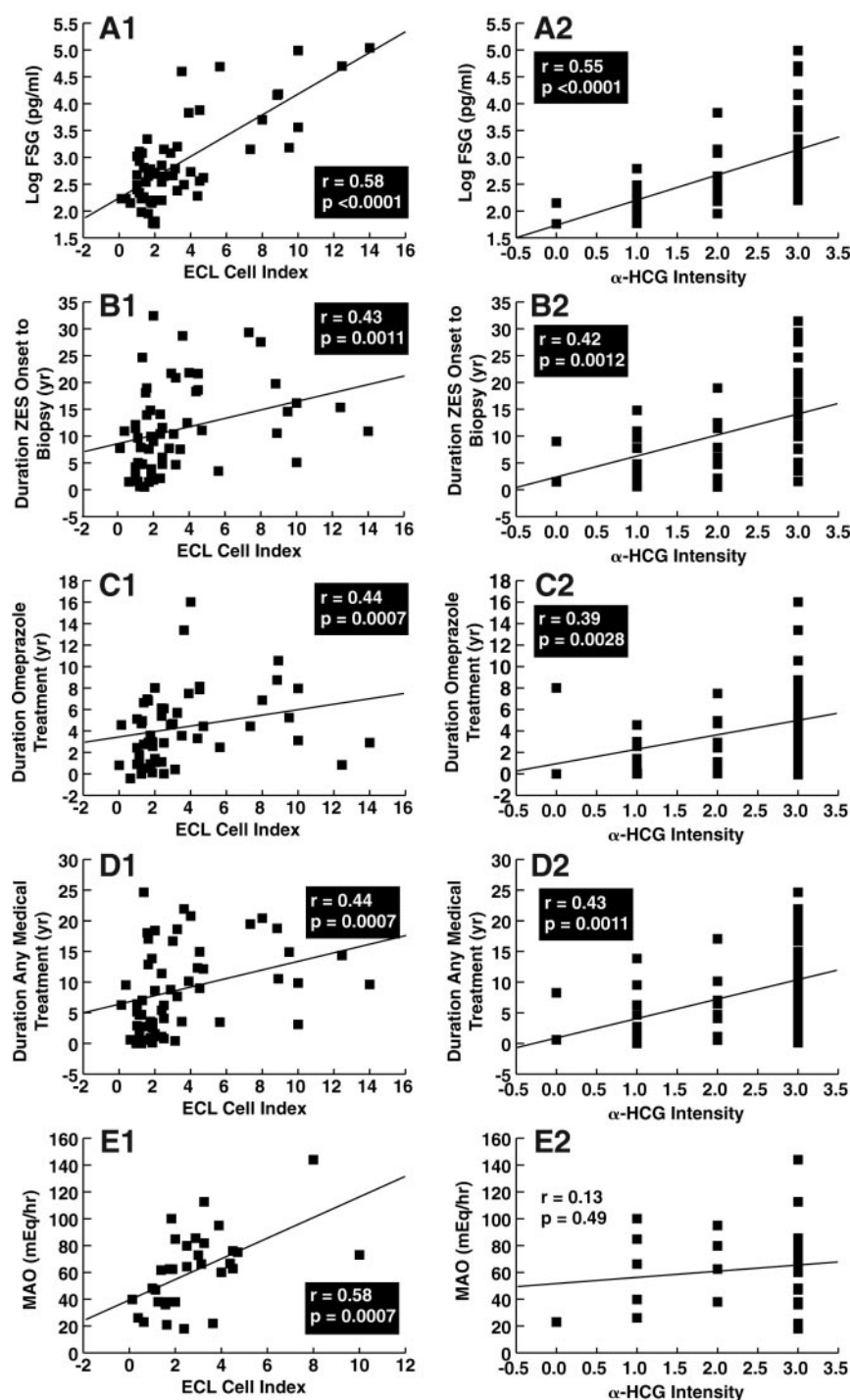


FIG. 4. Correlation between ECL-cell index (*left panels*) or highest α -hCG staining intensity per patient (*right panels*) and various laboratory or clinical ZES features. The mean fasting serum gastrin expressed as a \log_{10} (A1 and A2), duration from onset of ZES to the biopsy (B1 and B2), duration of omeprazole treatment before biopsy (C1 and C2), total duration of any medical acid-reducing treatment (D1 and D2), and the MAO (E1 and E2) are correlated with the ECL-cell index or highest α -hCG staining intensity of a gastric body biopsy. Data are from 57 patients with MEN1/ZES. The correlation coefficient and regression line were calculated by a least-square analysis. Results considered significant are *highlighted*. Each dot represents data from one patient.

and dysplasia) (10, 14). Although a number of small studies reported such findings in MEN1/ZES, the relationship of the extent of the ECL-cell changes and development of carcinoids has not been systematically studied. Studies on sporadic ZES

patients identified a number of factors influencing ECL-cell changes in sporadic ZES patients (10, 38). However, it is currently unclear whether these conclusions apply to MEN1/ZES patients (10, 16, 36, 38). Moreover, there are minimal data on the clinical, laboratory, or tumor variables influencing the incidence of carcinoids in MEN1/ZES patients. This lack of information has occurred for a number of reasons. Almost all series contain small numbers of MEN1/ZES patients (mean 5 ± 1 ; $n = 12$ series) (4, 17–20, 36, 37). Moreover, sufficient numbers of gastric biopsies were not systematically taken in most studies. Recent studies in sporadic ZES show that to adequately assess ECL-cell changes and occurrence of ECL-cell tumors, numerous systematic biopsies (six to eight per patient) from different areas of the stomach (lesser curvature and greater curvature) are needed (10, 30).

The present prospective study has none of these limitations. A large number of biopsies (*i.e.* six to eight) were systematically taken from the greater/lesser curvature, any mucosal abnormality biopsied, and α -hCG expression systematically assessed in a large number of patients. Furthermore, assessments were performed in MEN1/ZES patients that were part of a prospective study (4, 21), so findings could be related to other features of MEN1 and ZES.

Our results demonstrate ECL-cell changes are universal in MEN1/ZES patients with no patient having a normal ECL-cell pattern in all biopsies. This result differs from a number of small series (all fewer than 10 patients) that report 14–65% of MEN1/ZES patients have normal ECL-cell patterns (17, 18). However, it is in agreement with other series (16, 19, 20, 38) reporting ECL-cell hyperplasia in 92–100%. These results are also similar to findings in sporadic ZES in which 80–100% show some ECL-cell change (10, 16, 17, 35). In the majority of MEN1/ZES patients, the ECL-cell changes are advanced with 53% having advanced hyperplasia (at least LH mild). This percentage is higher than in most small series of MEN1/ZES patients where 25–47% (mean $35 \pm 4\%$, six series) had this extent of changes

(16–20). It is also a higher percentage than occurs in sporadic ZES, where 7–53% have an ECL change of at least LH (18–20). Furthermore, 23% of our patients had ECL-cell tumors, which is higher than the 0–14% (mean $2 \pm 1.5\%$, nine series) reported

TABLE 4. Guidelines and summary of clinically important findings of ECL-cell changes in patients with MEN1/ZES

Guidelines and summary	
1	All MEN1/ZES patients have some degree of ECL-cell changes; in 53%, these changes are advanced (at least LH mild), and 23% have ECL-cell tumors (gastric carcinoids).
2	Particularly important risk factors are high FSG levels, long disease duration (<i>i.e.</i> >7 yr), and high α -hCG intensity on biopsy (<i>i.e.</i> 3+).
3	In addition to routine gastric biopsies, all gastric nodules, which are frequent in these patients, should be biopsied, because they are frequently associated with an ECL-cell tumor.
4	An ECL-cell change of at least LH severe in any biopsy, the presence of mucosal nodules, or a FSG of more than 1400 pg/ml have the highest sensitivity (92, 100, and 85%) and specificity (89, 73, and 93%) for detecting patients with ECL-cell tumors.
5	Because MEN1/ZES patients are at risk for developing ECL-cell tumors, they require regular gastroscopy with multiple biopsies of all parts of the stomach and systematic biopsies of all mucosal lesions to detect these carcinoids. Both the clinical/laboratory factors and the biopsy results can be used to identify a subgroup of patients meeting the criteria mentioned above (no. 4) that need to be followed more closely because they have a higher risk for developing carcinoids.

by most, but not all (25–33% incidence) (37), small series of MEN1/ZES patients (4, 17–20, 36). This percentage is higher than reported in patients with sporadic ZES, where no ECL-cell tumors are reported in most series (16, 18–20), even though at least one study (10) used a large number of biopsies similar to the present study. Data from this study combined with studies from the literature support the conclusion that ECL-cell tumors are more than 70 times more common in MEN1/ZES than in sporadic ZES patients. Because duration of hypergastrinemia and FSG levels in our MEN1/ZES patients are comparable to those of 106 sporadic ZES patients investigated using a similar protocol (10), the predilection of MEN1/ZES patients for developing carcinoids cannot be explained by more severe hypergastrinemia or more prolonged exposure to the trophic effect of gastrin in our patients. These results support the proposal that the genetic changes in MEN1 patients render ECL cells more sensitive to the proliferative effect of gastrin (39).

The degree of hypergastrinemia correlates with the extent of ECL-cell changes in animal studies and in patients with atrophic gastritis (10, 20, 40). In ZES patients (primarily sporadic), the results are contradictory. Some studies report a positive correlation between these two variables (10, 17, 20, 35); however, no correlation was found in others (36, 41). Furthermore, there are no studies demonstrating that the level of hypergastrinemia correlates with the occurrence of ECL-cell tumors in ZES patients, as it does in atrophic gastritis (40). A number of results in our study support the conclusion that the magnitude of hypergastrinemia is directly correlated to the level of ECL-cell changes and the presence of carcinoids in MEN1/ZES patients. First, there was a highly significant positive correlation between the ECL-cell index and FSG level (Fig. 4A1). Second, the MAO, which has been shown to reflect the parietal cell mass, which is affected directly by the magnitude of hypergastrinemia in ZES patients (24, 42), was correlated to the ECL-cell index in the present study (Fig. 4E1). Third, patients with low FSG values (<490 pg/ml) or

with localized disease, which have been previously reported to have lower FSG levels (24), had a lower ECL-cell index (Tables 2 and 3).

Age or gender have been reported to influence ECL-cell changes and/or the development of ECL-cell tumors in animal and in some (16, 40), but not all (10, 35) human studies. We did not find such a correlation. These results are similar to findings in a recent study of sporadic ZES patients (10). Our results support the proposal that gender or age might be affecting the extent of hypergastrinemia in these different non-ZES groups of patients, which then affects the severity of ECL-cell changes. In contrast, in the current study, there was no significant difference in FSG levels in male and female or in old and young ZES patients (data not shown).

Although there are no data specifically on MEN1/ZES patients, the duration of hypergastrinemia and treatment with PPIs have been associated with increased ECL-cell changes in some (17, 38), but not all (10, 18, 35–37, 41), studies of ZES patients. In the present study, ZES duration was independently associated with the severity of ECL-cell changes and the presence of carcinoids (Fig. 4B1 and Tables 2 and 3). This result differs from findings in sporadic ZES patients (10). The reason for this difference is currently unclear, although it could be related to the earlier onset of ZES in MEN1 patients (4). Also, hypercalcemia from the hyperparathyroidism can increase serum gastrin levels in MEN1/ZES patients (4, 34, 43), and at the time of the biopsies in the present study, more than half of the MEN1 patients had a prior parathyroidectomy, so the serum gastrin levels were very likely higher in the past. Previous studies demonstrated ectopic expression/overexpression of α -hCG by neuroendocrine tumors (42). In the stomach, α -hCG was associated with hypergastrinemic states, which in some cases correlated with the presence of malignancy (10, 44). We found proliferative ECL cells ectopically express α -hCG in most MEN1/ZES patients, and overexpression correlates with the severity of ECL-cell changes, similar to results in sporadic ZES (10). Both high FSG and long duration of omeprazole treatment independently correlated with high α -hCG-IR. Strong α -hCG-IR was present in virtually all patients with carcinoids, even those with a low ECL-cell index (Fig. 3), supporting the proposal that high α -hCG-IR may be an independent predictor for carcinoids in ZES (10). Whereas in the pancreas in some studies (45), α -hCG-IR is associated with tumor malignancy, in the stomach in our study and others, it is largely restricted to preneoplastic changes (15).

Our study identified important findings/risk factors for the presence of advanced ECL-cell changes and/or ECL-cell tumors, from which can be proposed guidelines summarized in Table 4. Particularly important findings were that 53% of MEN1/ZES patients have advanced ECL-cell changes and 23% ECL-cell tumors and the demonstration of the necessity to biopsy all gastric nodules. Our study shows that gastric nodules are more frequent than previously reported (36), occurring in 44% of patients, and they frequently harbor carcinoids. The use of the risk factors summarized in Tables 2 and 3 should allow identification of a subgroup of MEN1/ZES patients at greater risk for developing ECL-cell tumors and allow stratification for follow-up. Long-term follow-up stud-

ies of these patients will be particularly important to attempt to identify risk factors for malignancy, the best methods to assess growth, and the best treatment methods for patients with multiple ECL-cell tumors.

In conclusion, this study provides important insights into the effect of chronic hypergastrinemia on ECL cells sensitized to the mitogenic effect of gastrin in MEN1/ZES patients. Our data provide risk factors, which can be used to develop guidelines for the management of these patients. Most importantly, our study shows that patients with MEN1/ZES, especially those with high FSG, gastric nodules and severe ECL-cell changes, are at significantly increased risk for developing carcinoids and should be monitored accordingly.

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