Influence of multiple endocrine neoplasia type 1 on gastric endocrine cells in patients with the Zollinger-Ellison syndrome

T Lehy, G Cadiot, M Mignon, P Ruszniewski, S Bonfils

Abstract

The influences of multiple endocrine neoplasia type 1 (MEN 1), hypergastrinaemia, age, and sex on gastric endocrine cell densities were studied in 48 patients with the Zollinger-Ellison syndrome of either the sporadic type (n=31) or associated with MEN 1 (n=17). The mean fundic argyrophil cell density was higher in women ($p<0.05$). It showed no appreciable difference between young and old women but it declined with age in men. The mean argyrophil cell density, when adjusted for sex, was higher (+48·5%, $p=0.06$) in patients with Zollinger-Ellison syndrome associated with MEN 1 than in those with sporadic type disease. This measurement was not significantly different between the two groups of patients when antisecretory treatments were considered. In patients with sporadic type disease, fundic argyrophil cells showed a normal pattern (16%) or diffuse (71%) or linear (13%) hyperplasia. In patients with MEN 1 diffuse and linear hyperplasia were of the same order (53% and 47%). Furthermore, fundic argyrophil endocrine tumours developed in five of 17 – that is, 29·5% of patients with associated MEN 1 while none was seen in patients with sporadic type disease. These tumours showed an exclusive or prominent enterochromaffin like cell population. Antral gastrin and somatostatin cell densities and fasting serum gastrin concentrations were similar in the two groups of patients with Zollinger-Ellison syndrome. Whatever the underlying mechanism for carcinoidosis, the risk of developing fundic enterochromaffin like cell tumours in Zollinger-Ellison syndrome patients who present with MEN 1 is probably higher than was initially estimated and suggests that regular follow up of these patients is necessary.

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Proliferation of fundic argyrophil endocrine cells, mainly enterochromaffin like (ECL) cells, is mostly under the control of gastrin in animals and in man. Indeed, lifelong hypergastrinaemia, induced by long term administration of inhibitors of gastric acid secretion or partial fundectomy as well as exogenous gastrin administration, have been associated with pronounced hyperplasia of gastric argyrophil cells or development of ECL cell carcinoids in rats.[1-8] In patients with the Zollinger-Ellison syndrome, who have long standing hypergastrinaemia originating from the tumour, proliferation of fundic argyrophil cells, mainly of the ECL type, has also been observed.[9-12] In these patients, we and others have reported scattered cases of fundic argyrophil carcinoids.[12-14]

Factors other than hypergastrinaemia may influence gastric endocrine cell populations, these include age in man[15] and female sex, which, in the rat, seems to promote ECL cell proliferation and carcinoid development.[2] Especially, in man with the Zollinger-Ellison syndrome, fundic argyrophil endrocrine tumours have been shown to be associated with multiple endocrine neoplasia type 1 (MEN 1) syndrome.[12-14]

This study in a large population of patients with Zollinger-Ellison syndrome aimed to search for a differential pattern of gastric endocrine cells between patients with sporadic type Zollinger-Ellison syndrome and those with the syndrome and MEN 1, and to establish whether there is a real predominance of ECL cell tumours in the second group. The influences of age and sex were also analysed. Our histological observations enable us to confirm the strong influence of the above mentioned factors on fundic argyrophil cells and particularly that of MEN 1 since fundic argyrophil cell tumours were found to occur only in patients with MEN 1, with a prevalence of 29·5%.

Methods

PATIENTS

Forty eight patients (31 men, 17 women) with clinically biologically, and/or histologically documented Zollinger-Ellison syndrome[16] and a median age of 53·5 years (range 17–83 years) were studied. The median duration of disease (as defined by duration of symptoms) was 8 years (range 0·2–36 years) and the median time since diagnosis was 4·5 years (range 0–15 years). Thirty one patients had sporadic Zollinger-Ellison syndrome and 17 had the syndrome associated with MEN 1. Sixteen of the 17 patients with MEN 1 had hyperparathyroidism. Other associated endocrine involvements were pituitary adenomas in four (three with prolactin oversecretion), insulinomas in three, glucagonoma in one, and bilateral hyperplasia of the adrenal cortex in one. The characteristics of the two groups of patients with Zollinger-Ellison syndrome are given in Table I.

The study was approved by the Human Studies Committee, Hôpital Bichat, France.

METHODS

Except for eight patients who had never been treated with antisecretory drugs at the time of the study, the remainder took part in a prospective

INSERM Unité 10 and Department of Gastroenterology, Hôpital Bichat, Paris, France
T Lehy
G Cadiot
M Mignon
P Ruszniewski
S Bonfils

Correspondence to:
Dr Th Lehy, INSERM U.10, Unité de Gastroenterologie, Hôpital Bichat, 170 bld Ney 75018 Paris, France.

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follow up protocol with estimation of several parameters at each visit (every 6–12 months). Patients underwent gastric endoscopy with biopsy specimens taken for determination of the histological endocrine cell status. They also had the fasting serum gastrin concentration determined using standard radioimmunoassay for detection of CT-gastrin 17/34, sulfated or not. The evolution of the gastrinoma tumoural process was assessed by endoscopy (for duodenal tumours), ultrasonography, and computed tomography.

Quantitative estimation of gastric endocrine cells

After a 12 hour fast, detailed upper gastrointestinal endoscopy was performed by the same two well trained endoscopists, using an Olympus GIF-XQ endoscope. Particular care was taken to detect any mucosal abnormalities or polypoidal formations, which, if present, were biopsied. In addition, mucosal biopsy specimens (mean, 5) were taken from the greater curvature on well defined areas in the corpus (at 50 cm from dental arches) and antrum (at 2–3 cm above pylorus) as described elsewhere.[12] They were fixed in Bouin's fluid for 24 hours and routinely processed and embedded in paraplast. Tissues were sectioned at a thickness of 4 μm. Instead of biopsy specimens, gastrectomy specimens were investigated for 10 patients: five were untreated and five were being followed up and had finally to undergo total gastrectomy. These specimens were all fixed in Bouin's solution. On oxyntic tissue sections, argyrophil endocrine cells (mainly ECL cells)[10] were stained with the Grimelius silver impregnation technique.[17] On antral tissue sections, gastrin and somatostatin cells were immunostained using antibodies raised in our laboratory[12] diluted to 1:1000 and the peroxidase-antiperoxidase complex (ICN immunobiological IL, USA) or the avidin-biotin peroxidase complex (ABC) (Vectastain ABC kit, Vector Labs Inc, CA, USA) methods.

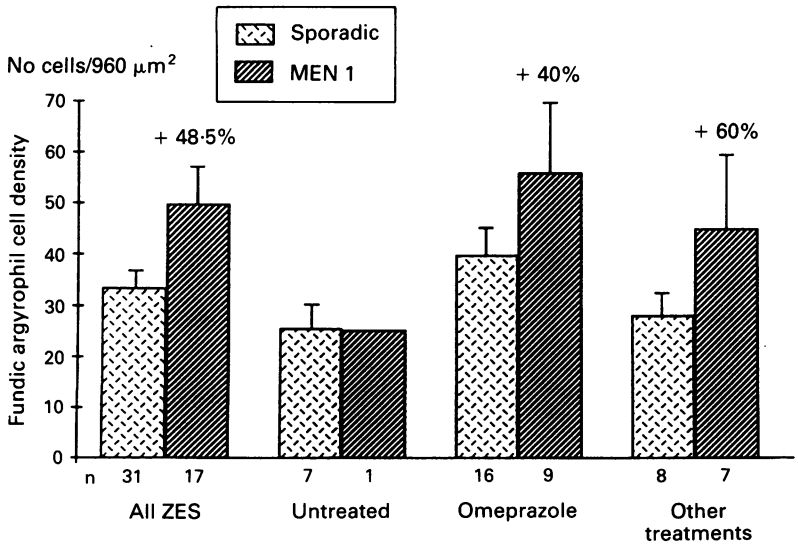

Figure 1: (A) Mean fundic argyrophil cell densities in all patients with sporadic Zollinger-Ellison syndrome and in all those with associated multiple endocrine neoplasia type 1. Difference between values of the two groups almost reached statistical significance (analysis of variance according to Fisher on means adjusted for sex, p=0·06). (B) Mean argyrophil cell densities in the same two kinds of patients, grouped according to the treatment followed (none, omeprazole, other treatments: ranitidine and/or octreotide). Differences were never significant.

TABLE I *Characteristics of the two groups of patients with the Zollinger-Ellison syndrome (ZES)*

	Sporadic ZES	*ZES+MEN 1*
No of patients (M/F)	31 (20/11)	17 (11/6)
Sex ratio	1·82	1·83
Age (yr) (median and range)	49 (17–83)	46 (29–64)
Disease duration (yr)	7 (0·2–36)	10 (0·5–28)
Omeprazole treatment:		
No of patients	16	9
Treatment duration (month) (median and range)	24·5 (2–67)	33 (1·5–69)
Dosage (mg/day) (median and range)	80 (20–160)	80 (20–160)
Other treatments (R and/or SMS):		
No of patients	8	7
Treatment duration (month) (median and range)	65 (3–156)	49 (24–144)
Dosage R (mg/day) (range)	600–900	600–900
Dosage SMS (μg/day)	400	400

R=ranitidine; SMS=octreotide.
MEN 1: multiple endocrine neoplasia of type 1.

Counts were always performed by the same two independent observers. Nucleated endocrine cells were counted in adjacent but non-overlapping areas, along the entire length of well oriented mucosal sections using a calibrated ocular grid, 240 μm wide, at magnification ×400. In practice, the mean (SEM) number of counts made per patient was 32 (2) for argyrophil cells and 48 (3) for gastrin and somatostatin cells. The cell count represented the number of endocrine cell nuclei seen in a column of mucosa extending through the muscularis mucosa to the surface. This cell column underlies a unit area of mucosal surface, the dimensions of which correspond to the thickness of the section and to the width of the ocular grid – that is, 960 μm². Density was expressed as the mean number of cells per unit mucosal area (for more detail, see ref 12). When tumours were present in the oxyntic mucosa of the biopsy specimens taken at the standard sites, counts were always done outside these areas. Although gastrectomy specimens were carefully examined for possible endocrine cell abnormalities, quantitative estimation of endocrine cell densities have been performed strictly in areas corresponding to the site of biopsy specimens during gastroscopy.

Fundic argyrophil cell densities in patients with Zollinger-Ellison syndrome were compared with previously published values in 10 healthy control subjects.[12] In these controls, gastric endoscopy, serum gastrin, and gastric acid secretion were normal. Mucosal oxyntic biopsy specimens were taken from the same defined area and tissues were processed in the same way as in the present study.

Qualitative evaluation of fundic argyrophil cell growth

As a complement to the estimation of cell densities, qualitative evaluation of argyrophil cell growth, according to the graduation recently established by Solcia *et al*,[18] was performed independently by the two observers on the fundic specimens of the 48 patients, without knowledge of the source of the samples.

Histopathological examination of tumours

When fundic endocrine tumours were found,

they were stained with the Grimelius argyrophilic method and immunostained with other endocrine markers such as neuron specific enolase antibodies (Immunotech, France) and monoclonal chromogranin A antibodies (Biosoft, Clonatec, France). Masson's argentaffin reaction was applied to detect enterochromaffin (EC) cells. Immunoreactivity with antibodies against gastrin, somatostatin, enteroglucagon (from our laboratory),[19] human pancreatic polypeptide (ICN immunobiological), and peptide YY (Milab, Sweden) was studied using ABC technique (dilution of primary antibodies 1:1000). Ultrastructural examination of tumours was performed under a Jeol JEM-1200 EX electron microscope after routine glutaraldehyde-osmium tetroxide fixation. Granule morphometry was done by measuring the diameter of all granules in at least 4–5 tumoural cells from each carcinoid examined, at a final magnification of 27 000.

Statistical analysis

Gastric endocrine cell densities were expressed as mean (SEM). For patients with iterative studies, only mean gastric cell densities obtained from the last set of biopsy specimens or from the gastrectomy specimen were taken into consideration. Serum gastrin concentrations were given as median and range. Results of the two groups were compared using the non-parametric Mann-Whitney U test. Means of argyrophil cell densities were also compared taking sex into consideration (analysis of variance according to Fisher). The level of significance was set at $p<0.05$.

Results

QUANTITATIVE ESTIMATION OF GASTRIC ENDOCRINE CELL DENSITIES

Marked hyperplasia of fundic argyrophil cells by reference to values found in healthy controls[12] (mean density/unit area: 9·8 (0·9) cells, range 6–15) was noted in the groups of patients with either sporadic type Zollinger-Ellison syndrome or MEN 1 associated Zollinger-Ellison syndrome (33·5 (3·5) cells and 49·8 (8·8) cells, respectively, $p<0.0001$). Comparison of these two groups showed that the mean argyrophil cell density was higher in the patients with MEN 1 than in those with sporadic disease (+48·5%), however, the difference did not reach statistical significance with the Mann-Whitney U test ($p<0.10$, Fig 1).

No change was noted between the group of patients with MEN 1 and that with sporadic Zollinger-Ellison syndrome when antral gastrin cell densities (30·8 (6·0) *v* 27·5 (2·6) cells/unit area, respectively), somatostatin cell densities (5·8 (0·8) *v* 6·0 (0·7) cells/unit area, respectively), or fasting serum gastrin concentrations (Table II) were compared.

TABLE II *Fasting serum gastrin concentrations (pg/ml) in patients with the Zollinger-Ellison syndrome (ZES)*

	Sporadic ZES		*ZES+MEN 1*	
	Median (no)	*Range*	*Median (no)*	*Range*
All ZES	391 (28)	61–72 617	436 (16)	97–184 000
Omeprazole	499 (16)	82–72 617	589 (9)	135–184 000
Other treatments	396 (8)	136–1450	368 (7)	97–10 520

Serum gastrin values were obtained in 44 patients but not in 4 untreated patients who were studied when gastrin radioimmunoassay was not available.

TABLE III *Fundic argyrophil cell densities in patients with the Zollinger-Ellison syndrome (values mean (SEM))*

	Women	*Men*
All population	51 (8) (n=17)	33 (4) (n=31)*
Age:		
<40 yr	43 (11) (n=4)	38 (7) (n=5)
40–60 yr	55 (14) (n=8)	37 (7) (n=17)
>60 yr	52 (14) (n=5)	22 (3) (n=9)**

*$p<0.05$ compared with women.
**$p<0.05$ compared with men aged <40 yr (Mann-Whitney U test).

Influence of antisecretory drugs

Further analysis according to the treatment administered indicated that the mean fundic argyrophil cell densities were always higher in MEN 1 than in sporadic type Zollinger-Ellison syndrome patients, whatever the treatment, but the difference was never significant (Fig 1).

Influence of sex and age

For all patients with Zollinger-Ellison syndrome and independently of the groups to which they belonged, the mean fundic argyrophil cell density was significantly higher in women than in men, $p<0.05$ (Table III). Analysis of this parameter in terms of age showed that argyrophil cell densities were roughly stable in young and old women while they declined in old men (Table III). The mean fundic argyrophil cell density was higher in women with MEN 1 than in those with sporadic type Zollinger-Ellison syndrome but the difference was not significant. A similar observation was made for men. The decrease in argyrophil cell density was noted in old men whatever the group of Zollinger-Ellison syndrome patients to which they belonged (data not shown).

When mean fundic argyrophil cell densities in the group of patients with sporadic Zollinger-Ellison syndrome and that associated with MEN 1 were adjusted for sex, the difference almost reached statistical significance (analysis of variance $F=3.965$, $p=0.06$) (Fig 1).

QUALITATIVE OBSERVATION ON FUNDIC ARGYROPHIL CELLS

According to the recently proposed histopathological classification for fundic argyrophil cell growth,[18] 16% of patients with sporadic type Zollinger-Ellison syndrome had normal values, 71% showed diffuse hyperplasia, and 13% linear hyperplasia. Neither dysplasia nor carcinoid growth were seen. In patients with Zollinger-Ellison syndrome associated with MEN 1, all cell densities were above the highest control value; 53% of these patients had diffuse hyperplasia and 47% linear hyperplasia. One patient had a dysplastic (precarcinoid) growth and five had fundic

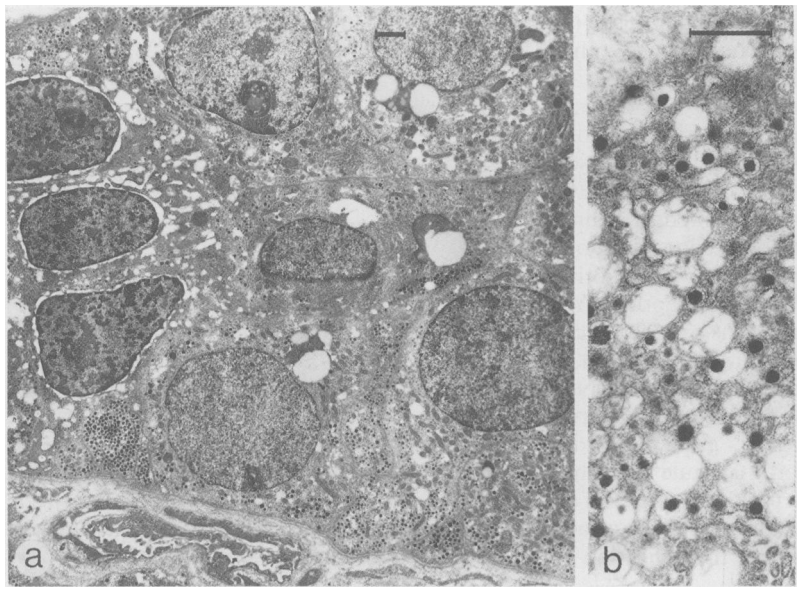

Figure 2: (A) Electron micrograph of fundic argyrophil tumour in case 5. It was composed of endocrine cells containing round, electron dense granules, most of which were non- vesicular. The granule diameter averaged 220 nm in some cells (atypical enterochromaffin like (ECL) cells) and 110 nm in other cells, which could correspond to P cells (original magnification ×5750; bar=1 μm). (B) Electron micrograph of fundic argyrophil tumour in case 4. Detail of typical vesicular ECL granules (original magnification ×18 000; bar=1 μm).

argyrophil carcinoids associated with linear hyperplasia. The latter developed within two of six women and three of 11 men – that is 29·5% of the MEN 1 patients. Characteristics of patients with carcinoids are indicated in Table IV. All these patients exhibited biological stigmata of primary hyperparathyroidism. Progression of the gastrinoma was found in only three of the five patients.

Macroscopically, upper gastrointestinal endoscopy showed sessile polypoid formations, covered with normal or erythematous mucosa, sometimes eroded on the surface and difficult to visualise because of enlarged gastric folds. At the time of first detection, they were usually located in the transitional zone between the corpus and antrum. When they developed, they were distributed throughout the oxyntic mucosa. Their diameter varied from 0·5–1·3 mm (cases no 3 and 5), 5–8 mm (case no 4), to several cm (3–4 cm on the gastrectomy specimen, case no 2). In all patients tumours were intramucosal, infiltrating the muscularis mucosa (n=5) and the submucosa (n=4). They were stained with the Grimelius silver method, reacted rather weakly with neuron specific enolase antibodies, and in general strongly with chromogranin A antibodies, while they did not express argentaffinity or immunoreactivity for gastrin, somatostatin, enteroglucagon, human pancreatic polypeptide, and peptide YY. Electron microscopy provided a conclusive diagnosis of ECL cell tumours in two cases. A mixed population of ECL cells and cells resembling P cells by the granule size was observed in patient no 5 while very heterogeneous cellular proliferation with a prominent ECL cell component was found in a fourth case (case no 1). In case no 3, microcarcinoids were discovered by chance on gastrectomy specimens, thus electron microscopy was not performed and cellular type(s) could not be determined. Tumoural ECL cells were either typical, containing vesicular electron dense granules or atypical with non-vesicular granules displaying a coarsely granular structure. Both aspects were seen in case no 2. The mean diameter of ECL cell granules was 208 (16) nm (case no 4), 220 (10) nm (case no 5), and 246 (17) nm (case no 2) (Fig 2A and B). The ultrastructural aspect of case no 1 has already been described.[13]

TABLE IV *Characteristics of patients with the Zollinger-Ellison syndrome at the time of appearance/detection of fundic carcinoid tumours*

No, sex (age) (yr)	*Disease duration (yr)*	*Antisecretory treatment (duration)*	*Serum gastrin (pg/ml)*	*Lesions**	*Cell type*
1 F (55)	12	Anti-H_2 (5 yr)	14 000	Multiple polyps†	EC, ECL P/D_1, A-like
2 M (36)	15	Anti-H_2 (5·5 yr) then OM (3·5 yr)	4 193	Isolated polyps‡	ECL
3 M (36)	2	Anti-H_2 (2 yr)	2 390	Microcarcinoids§	–
4 F (49)	28	Anti-H_2 (7·5 yr) then [OM+SMS) (4 yr)	4 718	≈12 Polyps¶	ECL
5 M (52)	8	Anti-H_2 +P (6·5 yr) then OM (7·5 mo)	229	1 Polyp**	ECL/P

P=pirenzepine, OM=omeprazole, SMS=octreotide.
*All proved to be argyrophil (macro or micro) carcinoid tumours.
†Total gastrectomy 3 months later; about 100 visible polyps.[14]
‡Total gastrectomy 4 years later (serum gastrin concentration reaching 13 883 pg/ml). Abnormal fundic mucosa almost quite invaded by carcinoid tumours.
§Discovered fortuitously on the gastrectomy specimen (unsatisfactory observance of medical treatment in an African patient).
¶One fundic polyp was removed at the end of anti-H_2 treatment (serum gastrin: 1063 pg/ml) but the diagnosis of carcinoid was not definitely proved at that time.
**One year later: 3–4 polyps noted on the greater curvature in the antro-fundic transitional zone (serum gastrin: 500 pg/ml).

Discussion

The present results provide new data on the influence that sex and age may have on fundic argyrophil endocrine cell populations in patients with Zollinger-Ellison syndrome and emphasise the role that MEN 1 plays in the ECL cell carcinoidosis development.

Our work indicates that fundic argyrophil cell densities were significantly higher in women than in men. A difference in these cell numbers related to sex has already been pointed out in rats given omeprazole.[2] Furthermore, the percentage of animals who developed tumours was much higher in females than in males.[2] It has also been shown recently that, in patients with untreated duodenal ulcer, argyrophil cells were significantly more numerous in women than in men.[20] However, the influence that sexual hormones *per se* may have on fundic argyrophil cell proliferation is unknown. We also confirm in our patients the previous observations made in normal subjects by Green *et al*,[15] concerning the evolution of endocrine cell densities with age. Indeed, while fundic argyrophil cell densities declined with age in men, no such tendency was evident in women.

Comparing the two groups of patients with Zollinger-Ellison syndrome, there was no difference in gastrin and somatostatin cell densities or in the fasting serum gastrin concentration. However, as reported by others in a few patients,[21] higher numbers of fundic argyrophil cells (+48·5%) were observed in patients with MEN 1 associated than in those with sporadic type Zollinger-Ellison syndrome. Although the difference was not quite significant (p=0·06), this observation is certainly of biological interest

since it seems that the risk of ECL cell carcinoidosis exists essentially in those with MEN 1 associated Zollinger-Ellison syndrome. Moreover, histopathological examination of fundic argyrophil cell growth allowed us to make several observations. In patients with sporadic type Zollinger-Ellison syndrome, in addition to a normal pattern of argyrophil cells (in 16%), cell hyperplasia was mainly diffuse (71%). In patients with MEN 1 associated Zollinger-Ellison syndrome, however, diffuse and linear cell hyperplasia were roughly of the same order (53% and 47%, respectively). No hyperplasia strictly corresponding to the micronodular definition[18] or as described in atrophic gastritis[22] was observed. Nevertheless, argyrophil micronodules were often seen, scattered in the fundic mucosa. In our series, fundic argyrophil cell tumours developed in five of 17 patients with MEN 1 while none was seen in the 31 patients with sporadic Zollinger-Ellison syndrome, even when argyrophil cell hyperplasia was as marked as in some patients with tumours. Furthermore, a precarcinoid growth was also noted in another patient with MEN 1. In an earlier report,[12] we have mentioned two patients with fundic argyrophil carcinoid growths among those studied. One case was previously published[13] and the second was in a patient in whom the first polyp was discovered and biopsied in December 1986. Since that time the second patient's lesion had progressed in such a way that gastrectomy had to be performed in January 1991 and fundic argyrophil cell tumours were discovered in three other patients and were progressing in two of them. Although ECL cell type was predominant in all tumours, other endocrine cell types have been noted in two tumours.

The clinical and biological profiles of Zollinger-Ellison syndrome patients who develop fundic ECL cell tumours cannot be determined from the study of such a small number of cases. Indeed, gastrinoma growth did not appear as an essential factor and, as shown in Table IV, neither sex, level of hypergastrinaemia, duration of disease, nor the type and duration of antisecretory treatment can, independently, account for neoplastic changes.

Solcia *et al*,[14] in a recent review of the published reports, gathered 14 cases of fundic argyrophil carcinoids (including our first two cases), all occurring in patients with Zollinger-Ellison syndrome and MEN 1. Detailed information on nine cases indicated that carcinoids were found in five women and four men: this did not suggest a prominent sex influence. In addition, these authors reported the personal communication of three possible unpublished cases of carcinoids developing in patients with sporadic Zollinger-Ellison syndrome. All patients with MEN 1 who had fundic carcinoid tumours had hyperparathyroidism and one may question the role played by humoral factors such as serum calcium or parathormone, or both. In our patients, values for these two factors varied considerably between individuals as well as within individuals, during the period of follow up. The causal factor in carcinoidosis is perhaps genetic, as discussed by others.[14] Whatever the underlying mechanism, the risk of developing fundic argyrophil endocrine tumours within the patients with Zollinger-Ellison syndrome and MEN 1 is probably higher than initially estimated and suggests regular survey of these patients.

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