YALE JOURNAL OF BIOLOGY AND MEDICINE 71 (1998), pp. 285-290.
Copyright © 1998. All rights reserved.

Natural History, Clinicopathologic Classification and Prognosis of Gastric ECL Cell Tumors

E. Solcia[a], G. Rindi, D. Paolotti, O. Luinetti, C. Klersy[b], A. Zangrandi[c], S. La Rosa[d] and C. Capella[d]

[a]*Department of Human Pathology, University of Pavia and IRCCS Policlinico San Matteo;*
[b]*Laboratory of Statistics and Biometrics, IRCCS Policlinico San Matteo, Pavia;*
[c]*Pathologic Anatomy Service, General Hospital of Piacenza;*
[d]*Department of Clinical and Biological Sciences, University of Pavia at Varese*

A series of 50 gastric endocrine tumors classified according to Rindi et al. [1] comprised 12 small cell neuroendocrine carcinomas (NEC) and 38 ECL cell carcinoids, of which 22 associated with type A chronic atrophic gastritis (A-CAG), eight with hypertrophic gastropathy due to combined Multiple Endocrine Neoplasia and Zollinger/Ellison syndrome (MEN/ZES), and eight sporadic. Variables found to predict tumor malignancy were: size >2 cm, >2 mitoses and >130 Ki67 positive cells/ 10 high power fields (HPF), grade 2 or 3 histology, angioinvasion, p53 protein nuclear accumulation, and the presence of a single tumor. None of these factors increased significantly the predicting ability of tumor classification itself, although grade 2+3 shows 100 percent negative predictive value and Ki67 and angioinvasion 100 percent positive predictive value. When the mostly non-malignant A-CAG and MEN-ZES tumors were analysed against the mostly malignant sporadic and NEC tumors, a positive predictive value of 90 percent and a negative predictive value of 93 percent was obtained. Investigation of a larger tumor series is under way with the aim to develop an optimal model for prognostic evaluation of gastric endocrine tumors.

INTRODUCTION

A few years ago, we developed a clinico-pathologic classification of gastric endocrine tumors which had pathogenetic, histogenetic as well as prognostic implications [1, 2]. Two types of gastrin-dependent, well differentiated ECL cell tumors (carcinoids), arising in a background of type A chronic atrophic gastritis (A-CAG)[e] or of combined Multiple Endocrine Neoplasia/Zollinger-Ellison Syndrome (MEN-ZES), respectively, were separated from a third type of ECL cell tumor, arising independently from known predisposing disease (sporadic ECL cell tumor) and from poorly differentiated, small cell "neuroendocrine" carcinoma (NEC). Most gastrin-dependent tumors proved benign, the sporadic tumors had a substantial, low grade, malignant potential, while NECs showed high grade malignancy. The prognostic value of such classification has been confirmed in subsequent studies of larger tumor series [3-5]. This is an important conclusion considering the fact that until recently the classic histopathologic criteria for prediction of tumor behavior have been found of scarce help to separate benign from malignant low-grade endocrine tumors of the gut and pancreas [6].

[a]*To whom all correspondence should be addressed:* Prof. E. Solcia, Dipartimento di Patologia Umana, Sezione di Anatomia Patologica, via Forlanini, 16, I-27100 Pavia, Italy. Tel.: (39)-382-528474; FAX (39)-382-525866.
[e]*Abbreviations:* A-CAG, type A chronic atrophic gastritis; MEN-ZES, Multiple Endocrine Neoplasia/Zollinger-Ellison Syndrome; NEC, neuroendocrine carcinoma; PP, pancreatic polypeptide.

Previous analysis of pathologic factors influencing tumor behavior suggested, in addition to metastases, tumor size, level of gastric wall invasion, mitotic rate, cellular atypia, and association with carcinoid syndrome [1, 5, 7, 8]. In 1995, all such factors were considered in a classification of gut, pancreas and lung endocrine tumors proposed by a group of european pathologists taking part in a meeting on endocrine tumors held in Monaco [9]. Comparison of the "Monaco" classification with our previous clinicopathologic approach showed broad, though not perfect, overlapping of the two systems when applied to a large serie of gastric endocrine tumors [4].

In a recent investigation of nonfunctioning pancreatic tumors, 17 clinico-pathologic and histochemical variables were considered. Eight (size, vascular and perineural invasion, mitotic and Ki67 proliferative rates, nuclear atypia, capsular penetration and presence of progesterone receptor) were found to be predictive of tumor behavior at univariate analysis [10]. Of these factors, at least two, angioinvasion and >2 percent Ki67 proliferative rate, proved to be strong independent predictors at multivariate analysis. In the present paper five of the above factors (size, vascular invasion, mitotic and proliferative rates, nuclear atypia), in addition to the level of gastric wall invasion and local or distant metastases, have been tested on a series of 50 gastric endocrine tumors, most of which in a previous study [1] formed the basis for the development of the clinico-pathologic classification.

MATERIAL AND METHODS

Fifty gastric endocrine tumors were selected for study from our pathology files in Pavia and Varese, including 37 from the series previously published [1]. Tumors were diagnosed as well differentiated or poorly differentiated based on conventional histopathologic criteria. Clinicopathologic analysis followed the previously assessed guidelines [1] and comprised, in addition to atrophic gastritis or MEN-ZES status, age, sex, presence of local and/or distant metastasis, follow-up and cause of death. Tumor analysis included: size, site, number of growths, level of infiltration, angioinvasion and number of mitoses x10 high power field (HPF x 400). The endocrine nature of the 50 tumors was assessed by an extensive reactivity of tumor cells for general endocrine markers like Grimelius' or Sevier-Munger's silver, chromogranin A, synaptophysin, neuron specific enolase or protein gene product (PGP) 9.5. In addition, paraffin sections were immunostained for serotonin, gastrin, somatostatin, enteroglucagon, pancreatic polypeptide (PP), p53 protein and the proliferative marker Ki67 [1, 10, 11, 12].

Statistical investigation of numerical data comprised descriptive statistics (mean ± standard deviation, frequencies and logistic modelling to measure the association between each of the considered parameters and the malignancy of the tumor (defined as presence of metastasis and/or deep wall invasion). Logistic odds ratios together with the 95 percent confidence interval have been computed by the model. Negative and positive predictive values have been also calculated.

RESULTS

Among the 50 tumors, 12 poorly differentiated, small cell neuroendocrine carcinomas were separated from 38 well differentiated endocrine tumors. Gastrin (seven positive cases out of 38 tested), somatostatin (5/35), serotonin (15/34) and PP (5/33) immunoreactivities were restricted to a small minority (<10 percent) of tumor cells. This finding, coupled with extensive reactivity for markers of neuroendocrine granules or vesicles like silver stains, chromogranin A and synaptophysin [5, 11] as well as with electron micoscopy of 18 cases allowed to diagnose all the 38 well differentiated tumors as more

or less pure ECL cell carcinoids. Focal exocrine differentiation was found in several NECs and occasional carcinoids.

The main clinicopathologic findings of the 50 tumors classified according to Rindi et al. [1] are reported in Table 1. All but one of the 17 metastatic tumors investigated for resulted to be deeply invasive, with invasion of muscularis propria or beyond. On the other hand 17 out of 18 with deep wall invasion had metastases (Spearman rank test = 0.90, p = .0000; 95.1 percent concordance in defining malignancy). Thus, deep wall invasion was assumed as evidence of tumor malignancy in addition to metastases.

In Table 2 the behavior of the tumors classified according to Rindi et al. [1] in respect to five histopathologically assessed variables found to predict behavior in an univariate logistic regression model (Table 3) is given. It appears that, by assuming type 1 and 2 tumors as non-malignant and type 3 and 4 tumors as malignant, Rindi's classification correctly predicts tumors in 93 percent of cases and as malignant in 90 percent of cases. In an attempt to increase the predictive power of the classification, the histopathologic parameters of Tables 2 and 3 were added separately in a model using classification type only as dependent variable. In no case the predictive ability of the multivariate models increased compared to that of tumor type alone. However, Ki67 proliferative index significantly increased the amount of explained variation (log likelihood ratio test: p = 0.03). In addition, all the 12 cases showing blood vessels invasion in the primary tumor were found to be metastatic. Interestingly, nuclear p53 protein accumulation was detected in eight out of 12 NECs, two out of six sporadic (one of which metastatic) and none of 18 CAG or MEN/ZES associated carcinoids.

DISCUSSION

From the above and previous findings it seems clear that the classification of gastric endocrine tumors based on simple clinico-pathologic criteria developed by Rindi et al. [1] remains the easiest and more informative approach to the study of such tumors. As a strong correlation was found between deep wall invasion (which can be assessed *in vivo* by endoscopic ultrasonography) and metastases, tumors invading the muscularis propria or beyond were included among malignant cases.

In principle, addition of size, mitotic rate, Ki67 proliferative marker, histologic grade, p53 protein accumulation, and angioinvasion should be of help in predicting metastases and more or less malignant behavior. From our findings, it seems that the first four criteria, which can be easily assessed by combined endoscopic and histologic examination, are likely to provide useful information for the identification of tumors requiring surgery, including the rare malignant cases among gastrin-dependent A-CAG or MEN-ZES associated tumors and the higher risk cases among sporadic tumors. Both 53 protein and angioinvasion were mostly confined to small cell neuroendocrine carcinomas, an expected finding in the case of p53, but not of angioinvasion. In the case of well differentiated gastric endocrine tumors angioinvasion seems less informative than it could be anticipated from previous findings on pancreatic tumors [10]. On the other hand a size above 2 cm seems more predictive of malignancy in the case of gastric than of pancreatic endocrine tumors, while a Ki67 proliferative index of more than three percent or with more than 150 positive cells per 10HPF seems to be equally indicative of malignancy for both pancreatic and gastric tumors.

As already suggested for pancreatic tumors [10], an optimal model for prognostic evaluation of gastric endocrine tumors should allow to identify four tumor categories: 1) benign or low risk, 2) increased risk, 3) malignant low grade and 4) malignant high grade. To reach this goal, investigation of a larger tumor series allowing careful multivariate analysis of independent predictive factors is warranted.

Table 1. Clinopathologic profile of 50 gastric endocrine tumors classified according to Rindi et al. [1].

	No.	Age[1] (yrs)	Sex F	Sex M	Size[1] (cm)	Invasion Superficial	Invasion Deep	Metastases	Follow-up A	Follow-up D	Follow-up TD	Follow-up (mo)
ECL cell tumor:												
Type 1 (A-CAG)	22	58 ± 11	12	10	0.7 ± 0.5	21	1	1	17	4	0	60
Type 2 (HG)	8	47 ± 11	5	3	0.8 ± 0.7	8	0	1	4	3	0	135
Type 3 (Sporadic)	8	49 ± 13.8	2	6	2.2 ± 1.1	2	6	5	5	0	3	70
NEC (Type 4)	12	62 ± 9	2	10	3.9 ± 1.5	0	11*	12	1	0	10	7

F, female; M, male; D, dead; TD, tumor-related death; mo, median of months of observation; 1, mean value ± standard deviation; superficial, mucosal + submucosal; Deep, muscularis propria or beyond; A-CAG, type A chronic atrophic gastritis; HG, hypertrophic gastropathy due to MEN-ZES; NEC, "neuroendocrine" small cell carcinoma; *, one case not assessed.

Table 2. Malignancy-predicting variables in 50 gastric endocrine tumors.

	No. of tumors >1	Size ≥2 cm	Grade ≥2	Mitoses ≥2 (10HPF)	Ki67 >130 (10HPF)	Angiovasion	Malignant*
Type 1	16/22	2/21	8/22	3/22	0/19	0/22	1/22
Type 2	8/8	0/7	5/8	3/8	1/5	1/8	1/8
Type 3	1/8	5/8	8/8	4/8	3/6	1/7	6/8
NEC	2/12	11/12	12/12	12/12	11/11	10/11	12/12

*Malignant, 19/20 cases showed metastases, 7 distant and 12, including the type 1 and type 2 cases, to local lymph nodes only; one case, a grade 2 sporadic carcinoid with 3 mitoses/10HPF, had deep wall invasion only.

Table 3. Analysis of behavior predicting variables of gastric endocrine tumors in an univariate logistic regression model.

Variable	OR_{log}	95% CI	p value	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
Type* 3 vs. 1	63	4.8-819	0.002				
3 vs. 2	21	1.5-293	0.0024	93.3	75	75	93.3
2 vs. 1	3	0.16-54.6	0.46				
3+4 vs. 1+2	126	16.3-976.2	0.000	93.3	90	90	93.3
No. of lesions >1	0.12	0.03-0.44	0.001	73.3	75	65.2	81.5
Size > .2 cm	52	8.53-317.11	0.000	92.9	80	88.9	86.7
Grade ≥2	Predicts malignancy perfectly		0.000 (χ^2)	56.6	100	60.0	100
Mitoses ≥2/10HPF	28.3	6.0-1346	0.000	8.3	85.0	77.3	89.3
Ki67 (per unit)**	1.02	1.00-1.04	0.000	100	76.5	100	85.7
≤130/10HPF	Predicts nonmalignancy perfectly		0.000 (χ^2)	100	88.2	100	92.3
Angiovasion absence	Predicts nonmalignancy perfectly		0.000 (χ^2)	100	66.7	100	83.3

OR, odds ratio; CI, confidence interval; PPV, positive predicting value; NPV, negative predicting value; *Type 4 (NEC) predicts malignancy perfectly; ** per unit of Ki67 positivity.

Acknowledgements: This work was supported in part by grants from the Italian Ministry of Health (to IRCCS Policlinico San Matteo) and Ministry of University and Research (to E.S. and C.C.).

REFERENCES

1. Rindi, G., Luinetti, O., Cornaggia, M., Capella, C., and Solcia, E. Three subtypes of gastric argyrophil carcinoids and the gastric neuroendocrine carcinoma: a clinicopathologic study. Gastroenterology 104:994-1006, 1993.
2. Solcia, E., Capella, C., Sessa, F., Rindi, G., Cornaggia, M., and Riva, C. Gastric carcinoids and related endocrine growths. Digestion 35(suppl):3-22, 1986.
3. Rappel, S., Altendorf-Hofmann, A., and Stolte, M. Prognosis of gastric carcinoid tumours. Digestion, 56: 455-462, 1995.
4. Rappel, S., Rindi, G., Bordi, C., Capella, C., Solcia, E., Altendorf-Hofmann, A., and Stolte, M. Gastric carcinoid tumours in atrophic autoimmune gastritis: classification, differential diagnosis and prognosis. Verh. Dtsch. Ges. Path. 80:199-207, 1996.
5. Rindi, G., Bordi, C., Rappel, S., La Rosa, S., Stolte, M., and Solcia, E. Gastric carcinoids and neuroendocrine carcinomas: pathogenesis, pathology and behavior. World J. Surg. 20:168-172, 1996.
6. Kloppel, G. and Heitz, P.U. Pancreatic endocrine tumors. Path. Res. Pract. 183:155-168, 1988.
7. Mizuma, K., Shibuya, H., Totsuka, M., and Hayasaka, H. Carcinoid of the stomach: a case report and review of 100 cases reported in Japan. Ann. Chir. Gynaecol. 72:23-27, 1983.
8. Wilander, E., El-Sahly, M., and Pitkanen, P. Histopathology of gastric carcinoids: a survey of 42 cases. Histopathology 8:182-193, 1984.
9. Capella, C., Heitz, P.U., Hofler, H., Kloppel, G., and Solcia, E. Classification of neuroendocrine tumors of the lung, pancreas and gut. Virchows Arch. 425:547-560, 1995.
10. La Rosa, S., Sessa, F., Capella, C., Riva, C., Leone, B.E., Klersy, C., Rindi, G., and Solcia, E. Prognostic criteria in nonfunctioning pancreatic endocrine tumours. Virchows Arch. 429:323-333, 1996.
11. Rindi, G., Buffa, R., Sessa, F., Tortora, O., and Solcia, E. Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. Histochemistry 85:19-28, 1986.
12. Buffa, R., Rindi, G., Sessa, F., Gini, A., Capella, C., Jahn, R., Navone, F., De Camilli, P., and Solcia, E. Synaptophysin immunoreactivity and small clear vesicles in neuroendocrine cells and related tumours. Molec. Cell. Probes 2:367-381, 1988.