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3. The ENETS guidelines: the new TNM classification system

Guido Rindi

Institute of Anatomic Pathology, Università Cattolica del Sacro Cuore Policlinico A. Gemelli, Rome, Italy

ABSTRACT

The WHO definition of the 3 classes of well differentiated endocrine tumor, well differentiated endocrine carcinoma and poorly differentiated carcinoma, allows an efficient general classification of GEP NETs fitting European Union (EU) current clinical use. The ENETS grading and TNM staging systems are complementary tools allowing to improve patients' stratification, and their adoption is strongly recommended. However, the prognostic value of these tools is still under investigation and more data are needed to support their usefulness at all gut sites and in perspective series. Similarly, the four classes system adopted by the WHO for the endocrine tumors of the lung allows a prognostically effective stratification of patients. A common grading system is advocated for lung and GEP NETS. Free full text available at www.tumorionline.it

Introduction

Neuroendocrine tumors (NETs) of the digestive tract are known to originate from cells with endocrine differentiation of the diffuse neuroendocrine system (DNES). Since the discovery of the enterochromaffin (EC) cells in late 19th century, 14 endocrine cell types of the gastrointestinal (GI) tract have been identified, assigned a specific name and characterized by electron microscopy and immunoistochemistry techniques as producing a wide range of hormones and bioactive molecules and displaying a type-specific regional distribution. While some of these cells are present in all portions of the GI tract, others are confined to specific regions, for example cells producing serotonin are distributed from pancreas to large intestine, while those secreting histamine can be found only in the *corpus fundus* of the stomach, and this distribution is strongly related to the specific function of these cells in different parts of the digestive tract[1]. Since endocrine tumor cells are largely similar in phenotype and distribution to their normal counterpart, this complexity is reflected on gastroenteropancreatic endocrine tumors (GEP NETs). Traditional classification of these tumor entities identified 9 tumor counterparts for the 14 normal DES cells, which were referred to as 'carcinoids' because of their less malignant behaviour as compared to the very aggressive small cell carcinoma which present a unique, poorly differentiated cell type and is quite ubiquitous[2]. The World Health Organization (WHO) Classification of 2000[3] abandoned this nomenclature recognizing the site-dependent cell heterogeneity of GEP-NETs and the necessity to regard them as different neoplasms. Today the term "carcinoid" is usually referred to the serotonin-producing GEP-NETs of the ileum or appendix leading to carcinoid syndrome, while the other tumor types are termed "neuroendocrine tumors" followed by their primary location, e.g. neuroendocrine lung, gastric, duodenal, pancreatic, colonic or rectal tumor. The classification of GEP-NETs proposed by the WHO in 2000 integrated and extended previously proposed categorizations by taking into account the morphological and clinicopathological heterogeneity according to tumor anatomical location. Specifically, the WHO identified 5 general categories: well differentiated endocrine tumor; well differentiated endocrine carcinoma; poorly differentiated endocrine (small cell) carcinoma; mixed exocrine-endocrine tumor; tumor-like lesion. Conceptually, this classifi-

cation comprises two major categories according to histology and tumor cell differentiation: well differentiated endocrine neoplasms, including tumor (carcinoid) and carcinoma (malignant/atypical carcinoid), and poorly differentiated endocrine carcinoma, including small and intermediate cell carcinoma. Well differentiated neoplasms typically show an organoid architecture, mild cellular atypia and an indolent behaviour, express the whole set of general markers of endocrine differentiation, first of all chromogranin A, and display abundant large dense core vesicles with variable hormone content. Poorly differentiated carcinomas generally present as large solid structures with marked cellular atypia and extensive necrotic areas, and as a rule display few large dense core vesicles and related markers but widely express, synaptophisin, neuron-specific enolase (NSE) and protein gene product 9.5 (PGP9.5). Several publications have recently focused on the application of the WHO classification system and proved its effectiveness, supporting the concept that the different endocrine tumor types also differ in their clinical behavior. In a population-based study conducted in England and Wales, Lepage *et al.*[4] evaluated survival data relative to 4104 patients diagnosed with malignant digestive endocrine tumors (MDETs) in the period from 1986 to 1999. Interestingly, the authors found that the 5-year survival rate was 56.8% for well-differentiated tumors, compared to only 5.2% for small cell tumors ($P<0.0001$). This result points out to the significant difference between well-differentiated and poorly-differentiated tumors in terms of clinical behaviour, with this differing behaviour which corresponds to a net separation of survival curves for the two classes of tumors[4].

Furthermore, several more recent studies conducted at single institutions demonstrated the clinical relevance of the WHO classification into the three classes of well-differentiated endocrine tumors (WDETs), well-differentiated endocrine carcinomas (WDECs) and poorly-differentiated carcinomas (PDECs). Among these, Pape *et al.*[5] evaluated retrospectively 202 patients with gastric (n = 48), duodenal (n = 23) and pancreatic (n = 131) NETs; Fisher *et al.*[6] assessed 166 cases of pancreatic NETs and Ekeblad *et al.*[7] evaluated 241 cases of pancreatic NETs. In all these studies, statistical significant differences in survival were maintained between WDETs, WDECs and PDECs, with both WDECs and PDECs which showed significantly shorter survival compared with WDETs.

ENETS grading and TNM staging working proposals for GEP NETs

The WHO classification of 2000 has largely demonstrated its prognostic efficacy and reproducibility in clinical practice and is currently at the basis of all current therapeutic algorithms for the management of NETs. However, while for PDECs a poor prognosis is warranted, predicting the behaviour of well-differentiated neoplasms is often problematic. In well-differentiated neoplasms, three categories can be defined according to the WHO system at the time of diagnosis: benign behaviour tumors or tumors with potentially benign course; uncertain behaviour tumors or tumors with potentially low grade malignant course; and carcinomas with low grade malignant course. Thus, the WHO classification has a major limitation in the incapacity to discriminate true benign behaviour from low-grade malignant behaviour of well-differentiated NETs[8].

In the attempt to integrate the WHO classification and to overcome the difficulties encountered in its practical application, in 2006 and 2007 the European Neuroendocrine Tumor Society (ENETS) proposed a new set of criteria for the grading and staging of GEP NETs[9,10]. The concept underlying these working proposals is that all GEP-NETs probably have a malignant potential, but since their biologic behaviour differs from tumor type to tumor type, their malignancy can be better defined on the basis of the grade and stage of the disease. The proposed grading and staging systems were established at two Consensus Conferences held in Frascati (Rome), organized by the ENETS and based on the ENETS guidelines on the diagnosis and treatment of digestive NETs[11]. Specifically, the grading system is based on mitotic count and Ki67 index and defines three tumor categories as follows: G1, <2 mitoses per 2 mm^2 (10 high-power fields, HPF, 40×magnification) and/or Ki-67 index ≤2%; G2, 2–20 mitoses per 2 mm^2 and/or Ki-67 index between 3% (intended as >2%) and 20%; G3 with 21 or more mitoses per 2 mm^2 and/or Ki-67 index >20% (Table 1).

Table 1 - Grading proposal for midgut, hindgut and foregut (neuro)endocrine tumors

Grade	Mitotic count (10HPF)*	Ki-67 index (%)**
G1	<2	≤2
G2	2-20	3-20
G3	>20	>20

*10HPF (High Power Field) = 2 mm^2, at least 40 fields (at 40x magnification) evaluated in areas of highest mitotic density; **MIB1 antibody; % of 2000 tumor cells in areas of highest nuclear labelling.

The G1 and G2 well-differentiated NETs usually display diffuse and intense expression of the two general immunohistochemical neuroendocrine markers, chromogranin A and synaptophysin. Punctate necrosis is per se indicative of a more aggressive tumor and points to a G2 or G3 status, which is then determined by the mitotic count and the proliferation fraction. G3 indicates a poorly differentiated neuroendocrine carcinoma with high mitotic counts/Ki-67 index, fields of necrosis,

significantly reduced chromogranin A expression and intense staining for synaptophysin, meeting the current WHO histological criteria. As for mitotic count, mitoses should be counted on hematoxylin and eosin stained slides in at least 40HPF when possible. The mitoses should be assessed in areas where they are most frequent after a general slide survey. For the Ki-67 assessment, the MIB1 antibody is recommended at the conditions that have been established at the laboratory in question. The Ki-67 index should be assessed in 2,000 tumor cells in areas where the highest nuclear labeling is observed (often but not exclusively at the tumor periphery). Of note, recently cut-off changes for the Ki-67 from 2%-20% to 5%-20% have been proposed by Scarpa and coworkers[8], but this proposal is under investigation and awaits validation in further studies. So far, the ENETS grading proposal has been tested in four retrospective studies on a total of about 929 cases and also in a recent prospective study on 297 patients, and has proved to be an effective and independent predictor of survival in the foregut. In the aforementioned study by Pape *et al.*[5] on 202 GEP-NETs, the majority of which pancreatic, the proposed grading system was able to differentiate significantly between cellular proliferation rate according to Ki-67, with Cox regression analysis which confirmed an increased risk of reduced survival for patients with grade 2 or 3 NETs compared to grade 1 NETs.

The ENETS staging system was developed on the basis of the well-known tumor-node-metastasis (TNM) format as working template. For tumor sizes, the limits indicated for T1 and T2 are those defined tumors of "benign behaviour" and "uncertain behaviour", respectively, according to the WHO site-specific clinico-pathological correlations, while deeply invasive and large tumors are included in the T3 and T4 categories, taking into account site-specific features. Lymph-node (N) and distant metastasis (M) were defined as absent (respectively N0 or M0) or present (respectively N1 or M1). Accordingly, a staging system was defined as follows: stage I for T1 NETs with limited growth, stage II for larger or more invasive T2 or T3 tumors in absence of metastases, stage III for tumors invading the surrounding structures (stage IIIA) or with regional node metastases (IIIB) and stage IV implying the presence of distant metastases[9]. Table 2 shows the ENETS TNM/staging proposal for the endocrine tumors of the pancreas, which so far has demonstrated to be a valid and powerful tool for the prognostic stratification of GEP NETs.

The new classification system was tested in five retrospective series of about one thousand cases providing a clear separation of all stages in some of them[12], a separation of stage I and II from stage III and IV only[5,6] or more complex separation in others[7], but always demonstrating its effectiveness as predictor of survival.

Table 2 - Proposal for a TNM classification and disease staging for endocrine tumors of the pancreas

TNM (Tumor - Node - Metastases)	
T-primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor limited to the pancreas and size <2 cm
T2	Tumor limited to the pancreas and size 2-4 cm
T3	Tumor limited to the pancreas and size >4 cm or invading duodenum or bile duct
T4	Tumor invading adjacent organs (stomach, spleen, colon, adrenal gland) or the wall of large vessels (celiac axis or superior mesenteric artery). For any T, add (m) for multiple tumors
N – regional lymph nodes	
NX	Regional lymph node cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M – distant metastaes	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastases

Classification of lung neuroendocrine tumors

With regard to lung NETs, their classification was implemented before the GEP NETs system. On the basis of the WHO general categories of well-differentiated neuroendocrine neoplasm and poorly-differentiated endocrine carcinoma, lung NETs can be subdivided into four classes: tumor (carcinoid), carcinoma (malignant/atypical carcinoid), large cell neuroendocrine carcinoma and small/intermediate cell carcinoma. More specifically, lung NETs represents a spectrum of proliferations that can be classified into a four-step system including typical carcinoid (≥0.5 cm in size, <2 mitosis/2 mm^2), atypical carcinoid (≥0.5 cm, presence of focal necrosis, 2-10 mitosis/2 mm^2), large cell neuroendocrine carcinoma and small cell carcinoma. Also this system, which was developed by Travis *et al.*[13] in 2004 on the basis of clinically relevant data, demonstrated its prognostic effectiveness in providing a clear separation of typical carcinoids from atypical carcinoids, which in fact are two clearly distinct diseases with very different prognosis, and of typical and atypical carcinoids from large cell and small cell carcinoma. These data are robust and well-consolidated, as demonstrated by the study of Righi *et al.* on 218 malignant cases showing a clear difference in survival for all these tumor classes[14].

References

1. Rindi G, Villanacci V, Ubiali S: Biological and molecular aspects of gastroenteropancreatic neuroendocrine tumors. Digestion, 62: 19-26, 2000.

2. Williams ED, Sudler M: The classification of carcinoid tumors. Lancet, 1: 238-239, 1963.
3. Solcia E, Klöppel G, Sobin LH: Histological Typing of Endocrine Tumours, ed. 2. WHO International Histological Classification of Tumours. Springer-Verlag Berlin, 2000.
4. Lepage C, Rachet B, Coleman MP: Survival from malignant digestive endocrine tumors in England and Wales: a population-based study. Gastroenterology, 132 :899-904, 2007.
5. Pape UF, Jann H, Müller-Nordhorn J, Bockelbrink A, Berndt U, Willich SN, Koch M, Röcken C, Rindi G, Wiedenmann B: Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. Cancer, 113: 256-265, 2008.
6. Fisher L, Kleeff J, Esposito I, Hinz U, Zimmermann A, Friess H, Büchler MW: Clinical outcome and long-term survival in 118 consecutive patients with neuroendocrine tumours of the pancreas. Br J Surgery, 95: 627-635, 2008.
7. Ekeblad S, Skogseid B, Dunder K, Öberg K, Eriksson B: Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. Clin Cancer Res, 14: 7798-7803, 2008.
8. Scarpa A, Mantovani W, Capelli P, Beghelli S, Boninsegna L, Bettini R, Panzuto F, Pederzoli P, Delle Fave G, Falconi M: Pancreatic endocrine tumors: improved TNM staging and histopatological grading parmit a clinically efficient prognostic stratification of patients. Mod Pathol 2010, *in press.*
9. Rindi G, Klöppel G, Alhman H, Caplin M, Coulevard A, de Herder WW, Eriksson B, Falchetti A, Falconi M, Komminoth P, Körner M, Lopes JM, McNicol A-M, Nilsson O, Perren A, Scarpa A, Scoazec J-Y, Wiedenmann B and all other Frascati Consensus Conference participants: TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch, 449: 395-401, 2006.
10. Rindi G, Klöppel G, Coulevard A, Komminoth P, Körner M, Lopes JM, McNicol A-M, Nilsson O, Perren A, Scarpa A, Scoazec J-Y, Wiedenmann B: TMN staging of midgut and hindgut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch, 451: 757-762, 2007.
11. Plöckinger U, Rindi G, Arnold R, Eriksson B, Krenning EP, de Herder WW, Goede A, Caplin M, Öberg K, Reubi JC, Nilsson O, Delle Fave G, Ruszniewski P, Ahlman H, Wiedenmann B: Guidelines for the diagnosis and treatment of neuroendocrine gastrointestinal tumours. A consensus statement on behalf of the European Neuroendocrine Tumour Society (ENETS). Neuroendocrinology, 80: 394-424, 2004.
12. La Rosa S, Klersy C, Uccella S, Dainese L, Albarello L, Sonzogni A, Doglioni C, Capella C, Solcia E: Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. Human Pathol, 40: 30-40, 2009.
13. Travis WD, Brambilla E, Muller-Mermelink HK, Harris CC: World Health Organisation Classification of Tumors. Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart, 4th edn. WHO: Geneva, p 1, 2004.
14. Righi L, Volante M, Tavaglione V, Billè A, Daniele L, Angusti T, Inzani F, Pelosi G, Rindi G, Papotti M: Somatostatin receptor tissue distribution in lung neuroendocrine tumours: a clinicopathologic and immunohistochemical study of 218 'clinically aggressive' cases. Ann Oncol, 21: 548-555, 2010.