Gastroenteropancreatic (neuro)endocrine neoplasms: The histology report

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Abstract

Based on the year 2000 World Health Organization (WHO) classification and the European Neuroendocrine Tumor Society (ENETS) grading and staging proposals, we here define the minimal guidelines for pathology reporting of (neuro)endocrine neoplasms. The macroscopical description is recommended according to standard procedures and the microscopical description according to recognized architectural and cytological features for endocrine lesions. Minimal diagnostic immunohistochemistry entails the use of chromogranin A, synaptophysin and Ki67. Other potentially useful tests are those for CD56 N-CAM, PGP 9.5 and hormones for diagnosis, the somatostatin receptor subtype 2 for potential radiodiagnoses and therapy, and transcription factors like TTF1 and CDX2, for site of origin. Grading definition is always mandatory as well as TNM staging for surgical specimens.

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1. Introduction

The term (neuro)endocrine tumor of the gastroenteropancreatic (GEP) tract refers to the neoplasm defined as “carcinoid” more than one century ago and actually known as neuroendocrine tumor (NET). The increasing advances in our knowledge of the disease and the recognition of its wide spectrum of differentiation patterns has generated multiple definitions with the passing of time with related confounding clinical and terminological implications (carcinoid; adenocarcinoid; tubular carcinoid; apudoma; neuroendocrine neoplasia; islet-cell tumors; pancreatic endocrine tumors; pancreatic endocrine neoplasia; malignant carcinoid; atypical carcinoid; well differentiated endocrine carcinoma; poorly differentiated endocrine carcinoma; neuroendocrine carcinoma; large cell neuroendocrine carcinoma; small cell neuroendocrine carcinoma; high grade neuroendocrine carcinoma, etc.). For the sake of simplicity and clarity the terms adopted in this article are those currently used in the clinical practice: neuroendocrine tumor (NET) for well to moderately differentiated neoplasms (gastroenteric carcinoids, pancreatic and duodenal endocrine tumors), and neuroendocrine carcinoma (NEC) for high grade, poorly differentiated neoplasms.

The diagnosis of GEP neoplasms is currently a much more common event than in the past. This diagnosis may be difficult especially for the assessment of the malignant potential of well to moderately differentiated lesions. Finally, it has to be...
remarked that a proper diagnosis, adequately supported by specific relevant findings, is crucial for the definition of the relevant therapy and management of the patients according to standardized and efficacious clinical guidelines [1–3].

The sources for the criteria of classification and diagnosis here recommended include the histopathologic classification proposed by the World Health Organization (WHO) [4,5] and the recent proposals of grading and staging of the European Society of Neuroendocrine Tumors (ENETS) [6,7]. The aim of this article is to provide simple guidelines for the application of these tools leading to a reasonable diagnostic accuracy and to useful responses to the clinician’s requests.

2. Epidemiology [8]

The neuroendocrine GEP neoplasms have been reported to present increased incidence and documented high prevalence [8]. Such finding likely reflects better knowledge and recognition of these types of tumors, as well as the wide diffusion of endoscopy and of appropriate and useful diagnostic tools, including chromogranin A determinations on serum and tissues and search for the type 2A subtype of somatostatin receptors (SST2A) either in vivo and in tissues [9,10].

3. Clinical aspects

At variance with the past, the large majority of GEP-NETs identified today belong to the non-functioning group, i.e. not associated with symptoms of hormone hypersecretion. In these cases most clinical information refers to organ-specific symptoms related to the tumor mass or to incidental findings obtained during endoscopic or imaging investigations.

In the case of NETs associated with recognized hormonal hypersecretion the essential clinical features are all useful for the definition of the associated endocrine syndromes (for example, hypergastrinemia in patients with Zollinger-Ellison syndrome). In view of the spectrum of different clinical syndromes potentially associated to GEP-NETs, the reader is referred to the current literature for a more accurate definition [4,5].

Irrespective of the association with hormone related syndromes, information on abnormal circulating markers, either endemic or not, may be useful. Useful but not essential clinical data includes scintigraphic scans for somatostatin receptors (Octreoscan, G16-DOTA-NOC, C11-5HTP-PET, etc.), radio-metabolic activity (FDG-PET) and, in general, “imaging” by radiologic or ultrasonographic procedures. When dealing with endoscopic biopsies, the definition of the sampled gastrointestinal region and the description of endoscopic findings with particular relevance to the tumor size are mandatory. Imaging of the lesion, especially if provided by endoscopy-associated ultrasonography, is useful but not essential or specific for GEP-NETs. In view of the unexpected diagnosis of GEP-NETs in most cases, however, the most relevant and confirmatory clinical information is often driven by and obtained after the pathological diagnosis of NET.

Occasionally the first diagnosis of NET is made by the pathologist on biopsies mostly taken from the liver and lymph node. In this condition the clinician’s main request is concerned with the identification of the primary tumor location (see the section on immunohistochemistry).

The pathologist should be alerted that the identification of the proper NET category has crucial impact on the treatment of the patients, which differs radically from one category to another.

4. Pathology


Endoscopic samples, small biopsies: detailed examination is not mandatory. A generic description of the number of biotptic specimens or of the size of the largest specimen in the case of needle core biopsies is adequate.

Surgical specimens: adherence to the standard guidelines for tumors of the involved organ is recommended for both the description and the sampling procedure, including for regional and distant lymph nodes collected and the status of the margins [11].

4.2. Basic microscopic features

4.2.1. Descriptive criteria [4–7]

Well differentiated endocrine tumor (NET) (WHO 2000 Categories 1 and 2, Table 1). The architecture of a NET may include: insular solid type (Type A according Soga and Tazawa) [12], with nests usually of small to medium size; trabecular (type B) with cell ribbons in single or, more rarely, double or multiple layers; glandular type (type C) presenting either glandular formations with true glandular lumen or pseudoglands showing a cellular rim surrounding a vascular space or a fibrovascular axis. Tumors with complex structure resulting from various combinations of two or all patterns described above are commonly found. In addition, apparently solid areas may result from the more or less pronounced packing of trabeculae and ribbons.

Different histological patterns may be associated with the region of tumor origin: the glandular type C is frequently found in ampullary tumors, the insular solid pattern A in tumors of the small intestine and appendix, and the trabecular pattern B in tumors of the rectum or the sigmoid colon. These patterns reflect the cell type composing the tumor: in the small intestine the type A structure is typical of enterochromaffin cell (EC) tumors producing serotonin, in the colon-rectum the type B structure usually corresponds to L cell tumors producing enteroglucagon and PYY, whereas in the ampullary region the type C structure more frequently corresponds to D cell tumors producing somatostatin.

The histological features reflecting tumor aggressiveness such as invasion of blood vessels and perineural spaces, of
Table 1
Summary of diagnostic findings

<table>
<thead>
<tr>
<th>WHO category*</th>
<th>Histological pattern</th>
<th>Cytological features</th>
<th>Necrosis</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 1</td>
<td>Organoid</td>
<td>Mild atypia</td>
<td>Absent</td>
<td>CgA+</td>
</tr>
<tr>
<td></td>
<td>– A solid/insular</td>
<td>– monomorphism</td>
<td></td>
<td>Syn+</td>
</tr>
<tr>
<td></td>
<td>– B trabecular</td>
<td>– medium size</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>– C glandular</td>
<td>– abundant cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>– D mixed</td>
<td>– regular nuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– dispersed chromatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– inconspicuous nuclei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO 2</td>
<td>As above</td>
<td>Possible focal</td>
<td>As above</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– more compact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO 3</td>
<td>– solid</td>
<td>Moderate/severe atypia</td>
<td>Extensive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– large, medium, or small size</td>
<td>CgA – (+)</td>
<td>Syn+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– scanty cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– irregular nuclei</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>– nucleoli often</td>
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<td></td>
</tr>
</tbody>
</table>

Legend: WHO 1, well differentiated endocrine tumor, subdivided into subgroups 1A (benign behavior) and 1B (uncertain malignant potential); WHO 2, well differentiated endocrine carcinoma; WHO 3, poorly differentiated endocrine carcinoma (WHO 2000 [4]).

Table 2
Grading according to ENETS [6,7]

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic index (10 HPF)*</th>
<th>Ki67 index (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;2</td>
<td>≤2</td>
</tr>
<tr>
<td>G2</td>
<td>2–20</td>
<td>3–20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

*HPF: High Power Field, 2 mm²; based on at least 50 fields (at 40× magnification) in tumor areas with higher mitotic density ("hot spots"); **MIB1 antibody; % on 500–2000 cells in tumor areas with higher density.

the minimal immunohistochemical panel (see the following section).

The differential diagnoses mostly include epithelial exocrine neoplasms such as in the pancreas the acinar cell tumor and the solid pseudopapillary tumor, in the ampullary region the adenocarcinoma and, in the whole GI tract, the epithelioid variants of the gastrointestinal stromal tumor (GIST). Specific immunohistochemical analyses are mandatory for the potential differential diagnosis [4,5].

Poorly differentiated neuroendocrine carcinoma (NEC) (WHO 2000 Category 3, Table 1). The histological arrangement of NECs is characterized by irregular, solid nests or sheets with frequent occurrence of necrotic changes commonly located in the center of the neoplastic clusters. In several regions including the esophagus, stomach, pancreas, duodenum and colon, in addition to the solid undifferentiated pattern, the tumors may present areas with organoid structure, though less defined than in the well differentiated counterparts and often with coarse trabeculae.

The tumor cytology corresponds to that of high-grade epithelial neoplasms with cells of either large or medium-small size, less frequently with the features of the small cell carcinoma of the lung. The cytoplasm, sometimes evident and eosinophilic, is more often restricted to a narrow perinuclear rim. The nuclei show severe atypia with frequent and often atypical mitoses (G3 according to ENETS). The NECs may commonly be associated with a non-endocrine component, usually represented by adenocarcinoma, adenoma or squamous carcinoma [14].

The endocrine nature of the tumors has to be confirmed by the minimal immunohistochemical panel (see the following section).

The differential diagnoses mostly include non-endocrine epithelial neoplasms or, less frequently, the exceedingly rare poorly differentiated tumors of different origin (PNET, Ewing sarcoma, desmoplastic small round cell tumor, leukemic localizations, etc.). Specific immunohistochemical analyses are mandatory to discriminate between differential diagnoses [4,5].

4.2.2. Microscopic description [15]

1) Surgical specimens: the morphologic description together with the immunohistochemical findings supporting the diagnosis of NET must be reported. The tumor grading including the mitotic count and the number of high power fields
used for the determination (recommended 50) as well as the Ki67 proliferation index and the number of counted cells (recommended 500 to 2000) have to be mentioned. Also to be reported are all parameters necessary for definition of TNM and staging according to ENETS. Since the TNM parameters differ according to the anatomic location of origin of the tumor, referral to published parameters [6,7] is recommended. At the end of the description the observed pTNM data should be reported.

(2) Small biopsies: as for surgical specimens, the histological and immunohistochemical features supporting the diagnosis and the data leading to the definition of the grading have to be described. If available (as for example in liver biopsies with known primary tumor), or in the presence of clinical information provided by the clinician (and in particular imaging data), the definition of TNM and stage according to ENETS is strongly recommended.

4.2.3. Immunohistochemistry [1–3]

The minimal immunohistochemical tests recommended by the ENETS guidelines are: chromogranin A (CgA), synaptophysin (Syn) and Ki67 [1–3]. CgA and Syn can be regarded as diagnostic tests whereas Ki67 has a predictive prognostic relevance. The usefulness of CgA and Syn for defining the neuroendocrine nature of a tumor is widely supported [4,5]. In NETs, CgA and Syn immunoreactivity is diffuse, though with variable intensity. In NECs CgA immunoreactivity is often focal, or weak and diffuse, whereas Syn immunoreactivity is usually intense and diffuse. In the latter tumors the loss of CgA immunoreactivity reflects the reduction or absence of endocrine granules depending on the deficient differentiation of the neoplastic cells. At variance with this statement is the negative CgA immunoreaction in some well differentiated NETs, in particular those of the rectum, even in the presence of abundant intracellular endocrine granules and heavy immunoreactivity for the hormones glicentin and PYY [16]. For the diagnosis of NECs it is recommended to rely on convincing immunoreactivity for at least two markers (CgA and Syn). Owing to the frequent poor CgA immunoreactivity in these tumors, however, the use of other general neuroendocrine markers such as the cytosolic components neuron-specific enolase (NSE) and protein-gene-product 9.5 (PGP 9.5), or the membrane molecule CD56 N-CAM, may be necessary, though depending on the experience of the user pathologist. As a rule, Syn appears to be the most sensitive marker for detecting the endocrine differentiation of the tumors. However, its specificity together with that of other neuroendocrine markers is somewhat reduced by the observation that some non-endocrine neoplasms react intensely and specifically for neuroendocrine markers (for example Syn, NSE, and CD56 in the solid pseudopapillary tumor of the pancreas). This condition provides the basis for the recommendation of at least two positive general neuroendocrine markers to substantiate the neuroendocrine differentiation of the tumors. The minimal number of hormonal markers necessary for the routine diagnostic work includes antibodies against insulin, gastrin and serotonin (the latter replaceable with the argentaffin reaction according to Masson-Fontana) either on clinician’s demand and/or to provide information useful for a better evaluation of the clinical profile and for the patient’s follow-up.

Similarly, on specific request of the oncologist, the assessment of SSTR2A somatostatin receptors in tumor tissue may be necessary. To this purpose the use of the score recently proposed by Volante et al. [10] is recommended.

The use of a minimal immunohistochemical panel including transcription factors CDX2, TTF1 and the hormones serotonin, gastrin and insulin is also recommended for NET or NEC metastases in liver or lymph node biopsies, in which the most common diagnostic requests refer to the primary tumor localization [17]. Immunoreactivity for CDX2 (NET and NEC) or serotonin (NET) suggests an intestinal origin [18], for TTF1 (NET and NEC) a pulmonary origin, for insulin a pancreatic origin, and for gastrin a pancreatic or duodenal origin. The interpretation of such data requires caution because of incomplete specificity. In particular, the expression of TTF1 in metastatic NECs has been observed also in NECs of extrathoracic origin [17,19,20], with the exception of the cutaneous Merkel cell carcinoma [21].

4.2.4. Diagnosis [4–7,15]

The diagnosis for both small biopsies and surgical specimens must include the definition of the endocrine tumor (NET or NEC), the degree according to ENETS and, if relevant to the clinical purposes or expressly requested by the clinician, the prevailing cell type (functioning tumors).

5. Histology report: checklist

Clinical information

- Anatomic location and size of the tumor (mandatory for biopsy samples);
- Clinical features related to hormone hypersecretion, laboratory findings on hormones and NE markers and imaging data (useful but not essential).

Gross description

- Depending on the anatomic location and according to standard scheme for each organ (for surgical specimens).

Microscopic description

- Description of the histological structure and of cell features;
- Mitotic index (per 10 HPF in at least 50 evaluated fields, if possible) and of the Ki67 proliferation index (% on 500–2000 cells);
- Description, if possible, of the histological parameters of malignancy (angio-lympho invasion, perineural invasion, occurrence of necrosis, infiltration of the capsule, if present, and of gastrointestinal wall invasion, detailing the level, or of adjacent organs and tissues);
- Description of the minimal immunohistochemical findings (CgA, Syn and Ki67); optional, and on the clinician’s demand, SSTR2A score according to Volante et al. [10];
• Description of the metastases if present;
• Description of the status of margins;
• Description of other findings which may be present (inflammation, other neoplastic or hyperplastic lesions, etc.).

**Concluding diagnosis**
• Diagnosis according to the current WHO categories, grading according to ENETS, pTNM and staging according to ENETS; definition of the predominant cell type (at least for insulin B, gastrin G and serotonin EC producing cells) and suggestion for the primary in liver and/or node metastases when unknown.

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**Conflict of interest**

No conflict of interest is declared by the authors.

**Note added in proof**

The terminology and diagnostic/report guidelines here presented are in agreement with the recently published WHO 2010 classification [22].

**References**


