ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Pathology: Diagnosis and Prognostic Stratification

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RUNNING HEAD: Diagnosis and Prognostic Stratification

Abstract

The European Neuroendocrine Tumor Society (ENETS) proposed standard of care guidelines for Pathology in 2009. Since then, profound changes in the classification have been made, with the separation of neuroendocrine neoplasia (NEN) into well differentiated neuroendocrine tumours (NET) and poorly differentiated neuroendocrine carcinomas (NEC) in the 2010 WHO classification. The 7th edition of the TNM classification (2009) included NEN for the first time, adapting widely ENETS proposals but with some differences for NEC and for NET of the pancreas and the appendix. Therapy guidelines for gastro-entero-pancreatic NET have been updated 2016. The need for update of the standards of care prompted ENETS to organize a consensus conference which was held in Antibes in 2015; a working group was designated to propose pathological standards of care.

Introduction

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(NEN) into well differentiated neuroendocrine tumours (NET) and poorly differentiated neuroendocrine carcinomas (NEC) in the 2010 WHO classification [2]. The 7th edition of the TNM classification (2009) included NEN for the first time, adapting widely ENETS proposals but with some differences for NEC and for NET of the pancreas and the appendix [3]. Therapy guidelines for gastro-entero-pancreatic NET have recently been updated as well [4]. The need for update of the standards of care prompted ENETS to organize a consensus conference which was held in Antibes in 2015; a working group was designated to propose pathological standards of care.

Gross analysis and processing of tissues

Histopathological analysis of tissue specimens is the gold standard for the diagnosis of NEN. Conventional morphological analysis is completed by immunohistochemistry, required to demonstrate the neuroendocrine phenotype and to evaluate Ki67 index. Samples can be obtained by endoscopy, but mini-biopsy is preferred to the classical fine needle aspiration with smears only [5, 6]. Tissue specimens are gained by biopsy of a primary or secondary tumor, by surgical resection or by endoscopic resection. Tissues are fixed in formalin and embedded in paraffin. Resection specimens require a detailed gross examination to select the proper regions for histological analysis. Gross examination is also crucial to provide data for T- and N-staging and to select the regions to analyze for establishing resection status.

Diagnostic Standards:

Neuroendocrine phenotype:

Table 1 summarizes the mandated and optional immunohistochemical requirements for a histopathological analysis of a NET biopsy. If by hematoxylin/eosin staining, a

neuroendocrine phenotype is suspected, immunohistochemical stainings for synaptophysin and chromogranin-A are required to definitely confirm this hypothesis [7]. Cytokeratin staining might be useful to confirm the epithelial nature of the tumor and to rule out paraganglioma. In well differentiated NET, all tumor cells stain diffusely for synaptophysin because of the diffuse presence of small clear vesicles. The expression of chromogranin A is usually more heterogeneous in the cytoplasm of tumor cells, since it depends on the presence of large neurosecretory granules. Rectal NET may frequently stain negative for chromogranin A with most monoclonal antibodies of current use. Otherwise, care must be taken in diagnosing well differentiated NET without any chromogranin-A expression; other entities, such as solid pseudopapillary neoplasia of the pancreas, acinar cell carcinoma or adrenocortical neoplasms must be ruled out. In poorly differentiated NEC, however, chromogranin-A may be lacking: moreover, in some small cell NEC, synaptophysin also may be focal or absent: in such tumors, the diagnosis of "small cell neuroendocrine carcinoma" is a diagnosis of exclusion. The use of other so-called neuroendocrine markers such as neuron-specific enolase (NSE) or N-CAM (CD56) is discouraged due to their low specificity [8].

Differentiation

According to the WHO classification, NEN are separated into well differentiated NET and poorly differentiated NEC. Initially, the assumption was that all G1-G2 tumors were well-differentiated and all G3 tumors were poorly differentiated. However well differentiated NET can rarely have proliferation indexes >20% especially in the pancreas. These patients survive longer than patients with poorly differentiated NEC [9], but shorter than patients with well differentiated NET. This new entity has by some been classified as well differentiated NET G3 [10]. These well differentiated NET with high proliferation index seem to be characterized by regular network of fine

vessels, organoid growth pattern without expansile growth and absence of geographic necrosis or desmoplastic stroma. Well differentiated morphology correlates with Ki67 index range of 20% to 50% [9-12]. Therefore, the exact Ki67 index as well as differentiation needs to be included into pathology reports. For NEC, small cell and large cell morphology should be described.

Grading:

Once the neuroendocrine nature of a tumor is demonstrated, the proliferative activity has to be assessed using Ki67 staining and performing a staining index. The percentage of positive tumor nuclei has to be assessed and reported. Grading is performed as defined in WHO and UICC/AJCC classifications (See Table 2). Ki67 index seems to be more accurate and reproducible than mitotic count [13] [14] and is the only counting possible on biopsy samples. Therefore, Ki67 index is regarded as compulsory and mitotic count optional. Grading can be performed as well on primary tumors as on metastases, but some heterogeneity exists between both and between different metastases [15, 16] [17]; the proliferation index is often higher in metastases. If not enough material for hotspot selection and analysis of 2000 tumor cells is available, undergrading might occur [18], this is occurring in EUS-obtained mini-biopsies [5, 19]. Grading is not recommended on smears from fine needle aspiration, but reliability is increasing in mini-biopsies, also gained by endoscopic procedures [6]. The risk of undergrading decreases between 200 and 2000 cells examined [19, 20] and was minimal when > 2000 cells were counted [20]. Finally, the amount of tissue needed depends on the purpose of the analysis. Only a limited number of cells is enough for discriminating well differentiated NET G1/G2 from poorly differentiated NEC G3 but might be not sufficient for an accurate grading.

Optional diagnostic markers

The use of optional or additional markers including hormones or transcription factors may be employed in the setting of a neuroendocrine tumor metastases of unknown primary site: Serotonin and cdx-2 positivity are in favor of a primary of the small intestine, islet-1 (Isl-1) expression is found in primaries of the pancreas and duodenum and TTF1 in primaries of the lung and in medullary thyroid carcinoma [21], the second together with calcitonin. All these markers are of no use in the setting of poorly differentiated NEC [22].

Immunohistochemical detection of somatostatin receptors (SSTR), especially SSTR2, is feasible and indicated in the absence of in-vivo somatostatin imaging studies [23, 24]. In the case of questionable vascular invasion, immunohistochemistry for endothelial cell markers such as CD34 or special stains for the visualization of vessel walls might be of help.

Pathological report

Table 3 summarizes the minimum requirements for pathological reports of resection specimens or biopsy specimens of NEN.

Needs for research

MGMT expression or methylation may serve as a predictive marker of response to temozolomide based chemotherapy in PanNET: clinical trials are on the way to address this issue. In the same regard, translational studies are needed to define biomarkers predicting response to other therapies such as targeted therapies or other chemotherapeutic strategies. The new category of NET G3 needs to be better defined pathologically, possibly by the inclusion of molecular markers in order to have a more solid basis to define the therapeutic consequences of this tumor type. At last, increasing molecular evidence may suggest a grouping of NET according to

mutational, expression or methylation profiles, but so far no therapeutic strategies are

based on these findings.

Conclusions

The proposed standard procedures for diagnosing NEN should now follow the WHO

and TNM classification systems that are under revision. A standardized diagnosis is

the basis for a standardized treatment as well as for studies to be comparable.

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Table 1. Mandatory and optional elements for assessing a biopsy specimen containing a tumor with features of a GEP-NEN

Mandatory
Morphology and differentiation on HE section

Immunostaining for neuroendocrine markers	
–	Synaptophysin and chromogranin A

Immunostaining for proliferation marker	
–	Ki67/MIB1

Optional

Immunostaining for hormones such as insulin, gastrin, serotonin and others	
–	In the context of hormonal symptoms, liver metastases of an unknown primary or follow-up of a tumor with a hormonal syndrome

Immunostaining for transcription factors (TTF1, CDX2, Isl-1)	
–	In the context of a carcinoma of unknown primary

Immunostaining for somatostatin receptor (i.e. SSTR2)	
–	If not available by in-vivo technique such as SRS imaging

Immunostaining for vessel markers	
–	To determine angioinvasion

Table 2. Grading of GEP-NENs:

Grade	Mitotic count, 10 HPF[1]	Ki67 index, %[2]
G1	≤ 2	<3[3]
G2	2-20	3-20
G3	>20	>20

[1] HPF = high-power field = 2 cm^2, at least 40 fields evaluated in areas at highest mitotic density.
[2] MIB1 antibody; % of 500 to 2,000 cells in areas of highest nuclear labeling. If less cells, the number of assessed cells should be noted.
[3] <3 could replace ≤2 in the 2010 WHO classification in order to include decimal numbers between 2 and 3

Table 3. Minimum requirements of pathology reports, given for the example of pancreatic NET, according to CAP guidelines

Type of specimen:	Excisional biopsy, parial pancreatectomy, Whipple resection, total pancreatectomy
Tumor site:	pancreatic head, body, tail, uncinated process.
Tumor size:	in cm and 3 dimensions
Tumor focality:	unifocal, multifocal
Tumor functionality	Insulinoma, Glucagonoma, Somatostatinoma, Gastrinoma,VIPoma, Serotinin producing, other, nonfunctional
Histologic differentiateion:	well differentiated, poorly differentiated*
Proliferation rate:	Ki-67 index + optionally mitotic count
Tumor necrosis:	present, absent
Microscopic tumor extension	confined to pancreas, invading peripancreatic soft tissue, invading other organs
Margins:	margins uninvolved by tumor, closest margin in cm, margins involved by tumor
Lymphovascular invasion:	present, absent
Perineural invasion:	present, absent
TNM staging (UICC 7th edition):	
Lymphnodes:	number of lymphnodes examined, number of lymphnodes involved
Additional features	

* note that for poorly differentiated NEC the TNM system of adenocarcinomas of the pancreas is applied.