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The pathological diagnosis of neuroendocrine tumors: common questions and tentative answers

Volante M., Righi L., Berruti A., Rindi G. and Papotti M.
Virchows Arch. 458:393-402, 2011

FAQ 1: terminology of NETs (GEP system)

“can terms such as CARCINOID and NET be used interchangeably?”
FAQ 2: criteria to define malignant potential in NETs

can the malignant potential be defined in NETs (G1 and G2)?

FAQ 3: nomenclature for functioning versus non-functioning tumors

is any immunohistochemically detected hormone production sufficient to label a GEP NET with a suffix “oma” following the hormone?
FAQ 4: grading and staging of NETs

Are grading and staging necessary in all tumors?

FAQ 5: pathological diagnosis of NETs in biopsy/cytology material

Is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?
FAQ 6: the role of Ki-67 in NETs

*Is Ki67 to be determined in all NETs?*

FAQ 7: prognostic factors in NETs

*Are prognostic factors (markers) other than Ki67 to be assessed in all NETs?*
FAQ 8: predictive factors in NETs

Factors (markers) predictive of response to therapy to be assessed in all NETs?

WHO 2000 classification of GEP endocrine tumors

Combined clinico-pathological parameters...

- location, diameter, angioinvasion, presence of metastases

... and functional data (clinico-pathological correlates)

- type of hormonal secretion and clinical syndrome eventually present
**WHO 2000 classification of GEP endocrine tumors**

- **Well-differentiated endocrine tumor**
  - benign/uncertain behavior

- **Well-differentiated endocrine carcinoma**
  - Low grade malignant

- **Poorly differentiated endocrine carcinoma**
  - High grade malignant

- **Mixed Exocrine-Endocrine carcinoma / MEEC**

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**Neuroendocrine Neoplasms**

**WHO Classification 2010 of the Digestive System**

- **Working principles**
  - “Neuroendocrine” defines the peptide hormone-producing tumours and share neural-endocrine markers
  - “Neuroendocrine neoplasm” includes well- and poorly differentiated tumours

- **Premise: All neuroendocrine neoplasms (NENs) have a malignant potential**

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Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach

0) NEN vs non NEN → morphology & NE markers
Morphological patterns in NENs

- Insular (nodular solid nests with peripheral invading cords)
- Trabecular (anastomosing trabeculae or ribbons)
- Glandular (tubules, acini or rosettes)
- Poorly differentiated with no well-organized growth pattern

Immunohistochemical markers in NENs: definition of NE phenotype

Pan-endocrine markers

- Cytosolic (NSE, PGP 9.5)
- Related to secretory granules (chromogranin A)
- Related to synaptic vescicles (synaptophisin, VMAT)
- Intermediate filaments (NF, CK HMW)
- Adhesion molecules (N-CAM)
Glucagon IHC in non-functioning pNET

Islet 1 (Isl1) expression is a reliable marker for pancreatic neuroendocrine tumors and their metastases\(^1\)

Liver mts

CDX2 as a marker of intestinal EC-cells and related well-differentiated neuroendocrine tumors\(^2\)

Primary ileal  LN mts  Liver mts

Definition of NE phenotype

✓ Compromise between sensitivity and specificity
✓ Do not rely on a single marker to establish or disprove the diagnosis of NEN
✓ Immunohistochemical findings must be interpreted in the context of the microscopy (and, if necessary, the clinical and biochemical picture)

Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach

1) NET vs NEC →  
structure + grade

0) NEN vs non NEN → morphology & NE markers
Neuroendocrine Neoplasms
WHO Classification 2010 of the Digestive System

- Neuroendocrine tumor/NET (Carcinoid)
- Neuroendocrine carcinoma / NEC

<table>
<thead>
<tr>
<th>WHO 2000</th>
<th>WHO 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-differentiated endocrine tumour (WDET)</td>
<td>Neuroendocrine tumour</td>
</tr>
<tr>
<td>Well-differentiated endocrine carcinoma (WDEC)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated endocrine carcinoma/small-cell carcinoma (PDEC)</td>
<td>Neuroendocrine carcinoma</td>
</tr>
</tbody>
</table>
Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach

1) NET vs NEC → structure + grade

2) Grade 1-2-3 → mitoses & Ki67

0) NEN vs non NEN → morphology & NE markers

Grading of GEP-NENs According to ENETS/WHO/AJCC

<table>
<thead>
<tr>
<th>Grade</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67 index</td>
<td>≤2</td>
<td>3–20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>MI</td>
<td>&lt;2</td>
<td>2-20</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Ki67 Counting

- **Options to quantify Ki67**
  - Systematically counting a defined number of tumors cells (500-2000) and calculating the positive percentage
  - Using a computerized digital image analysis system to measure the positive percentage
  - A general “eyeballed” estimate of the percentage of positive cells

- The result should be reported as a single percentage reflecting the average of the regions counted, rather than a range of value

Mitotic index evaluation

- A total of **50 fields** should be counted
- The mitotic rate should be expressed based on the number in **10 high power fields (2 mm²)**
Grading of GEP-NENs according to ENETS/WHO/AJCC

Cumulative Survival

G1 vs. G2 \( P=0.040 \)
G1 vs. G3 \( P<0.001 \)
G2 vs. G3 \( P<0.001 \)

Time (months)


Neuroendocrine Neoplasms (NENs):
a stepwise diagnostic approach

3) TNM Stage I-II-III-IV → size & invasion

2) Grade 1-2-3 → mitoses & Ki67

1) NET vs NEC → structure + grade

0) NEN vs non NEN → morphology & NE markers
TNM Classification of GEP-NENs

✓ SITE-specific

✓ Based on depth of invasion and size

ENETS: 2006/2007

UICC/AJCC: 2009

Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

✓ Similar TNM classifications:
  • Stomach
  • Duodenum
  • Jejunum/ileum
  • Colon/rectum
Staging of upper digestive NENs according to ENETS/WHO/AJCC

Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

- Similar TNM classifications:
  - Stomach
  - Duodenum
  - Jejunum/ileum
  - Colon/rectum

- Different TNM classification
  - Appendix
  - Pancreas

T Category Criteria for Appendiceal NENs is Different Between ENETS and UICC/AJCC

<table>
<thead>
<tr>
<th>ENETS TNM</th>
<th>UICC/AJCC TNM</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T1a: ≤1 cm; T1b: &gt;1 – 2 cm</td>
</tr>
<tr>
<td>≤1 cm; invasion of muscularis propria</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>&gt;2 – 4 cm; or invasion of cecum</td>
</tr>
<tr>
<td>≤2 cm; and &lt;3 mm invasion of subserosa/mesoappendix</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>&gt;4 cm; or invasion of ileum</td>
</tr>
<tr>
<td>&gt;2 cm; or &gt;3 mm invasion of subserosa/mesoappendix</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>invasion of peritoneum/other organs</td>
</tr>
<tr>
<td>invasion of peritoneum/other organs</td>
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</tbody>
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Pathology report of NENs

- Define location and tumor type based on WHO classification
- Define tumor grade (including Ki-67 proliferative index)
- Describe the presence of additional histologic features (multicentric disease, non-ischemic tumour necrosis, vascular or perineural invasion)
- Assess the TNM stage
- Define the resection margins
- Define the hormonal production, if any

Upon request, assess prognostic or predictive factors useful for target therapy (e.g. somatostatin receptors, mTor pathway molecules, other target enzymes, …)

WHO 2010 applicability in the “preoperative” setting?

✓ Definition of a NE neoplasm possible (suspect + markers)

✓ Definition of NET vs NEC usually possible (cell size, atypia, necrosis, growth pattern)

✓ Grading applicable, although with some limitations

WHO 2004 Criteria and CK19 are Reliable Prognostic Markers in Pancreatic Endocrine Tumors

Anja M. Schmitt, MD, Martin Anlauf, MD, Valentin Rousson, PhD, Sonja Schmid,* Andreas Koffer, MD, Florian Riniker,* Juliane Baumgart, MD, Andre Borghorn, MD, Nicole M. Probst-Hensch, MD, Holger Moch, MD, Philipp U. Heitz, MD, Guenter Kloeppel, MD, Paul Komminoth, MD, and Aurel Perron, MD


<table>
<thead>
<tr>
<th>TABLE 1. Multivariate Analysis Including WHO 2004 Classification</th>
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<tr>
<td></td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>WHO classification</td>
</tr>
<tr>
<td>CK19 (positive)</td>
</tr>
<tr>
<td>Necrosis</td>
</tr>
<tr>
<td>Nodular fibrosis</td>
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10 HPF indicates 10 high-power fields, corresponding to an area of 2 mm².
PREDICTIVE MARKER

LINKED TO CURRENT THERAPY

+ METHODOLOGY OF ANALYSIS

AVAILABLE =

CLINICAL RELEVANCE

Expression of Somatostatin receptor types 1-5 in 81 cases of gastrointestinal and pancreatic endocrine tumors. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis.


Virchows Arch. 2002 May;440(5):461-75
Correlation with Scintigraphy: 77%
(107 cases)

Tx response: 75%
(28 patients)

Integration of SSTR IHC and Somatostatin Analog-based Imaging (SRS):

**SSTR IHC**
- Cost effective
- Detection of protein
- Identification of SSTR subtype
- Identification of cell type expressing SSTR
- Applicable retrospectively

**SRS**
- Identification of “functional” receptors
- Detection of SSTR expression in the whole tumor mass
Current therapy modalities in NETs

✓ Biotherapy (somatostatin analogs and interferon)
✓ Peptide receptor radionuclide therapy
✓ Cytotoxic treatment
✓ Targeted therapies

FAQ 1: terminology of NETs (GEP system)

“can terms such as CARCINOID and NET be used interchangeably?”

Context:
Carcinoid vs. (neuro) endocrine tumor/carcinoma terms

Relevance:
Terminology reflects classification and prognosis

Criticism:
Not accepted worldwide
FAQ 1: terminology of NETs (GEP system)

“can terms such as CARCINOID and NET be used interchangeably?”

Answer:

NO
(the term “carcinoid” fails to convey informations related to clinical behaviour)

FAQ 2: criteria to define malignant potential in NETs

can the malignant potential be defined in NETs (G1 and G2)?

Context:
Combination of clinical (presence of metastases) and morphological parameters

Relevance:
Determine the classification (and prognosis) of a NET

Criticism:
Change according to the tumor site; some equivocal/difficult to assess
FAQ 2: criteria to define malignant potential in NETs

*can the malignant potential be defined in NETs (G1 and G2)?*

**Answer:**

YES, applying a site-specific TNM and Grading

FAQ 3: nomenclature for functioning versus non-functioning tumors

*is any immunohistochemically detected hormone production sufficient to label a GEP NET with a suffix “oma” following the hormone?*

**Context:**

Terms such as insulinoma, gastrinoma, etc. and the concept of functioning tumors

**Relevance:**

Functioning tumors are defined by the presence of clinical symptoms related to hormone production

**Criticism:**

Terminology reflects clinical picture rather than pathological features; no link with malignancy
FAQ 3: nomenclature for functioning versus non-functioning tumors

*is any immunohistochemically detected hormone production sufficient to label a GEP NET with a suffix “oma” following the hormone?*

Answer:

**NO**

(unless specific information was provided)

FAQ 4: grading and staging of NETs

*are grading and staging necessary in all tumors?*

**Context:**
Necessity to apply novel grading and staging systems

**Relevance:**
NET stage (ENETS and AJCC) and grade (ENETS) are of prognostic value

**Criticism:**
Recent, to be validated prospectively, sometimes confusing and discordant
FAQ 4: grading and staging of NETs

*are grading and staging necessary in all tumors?*

**Answer:**

**YES**

(applying the criteria of the 2010 WHO classification of digestive system tumors)

FAQ 5: pathological diagnosis of NETs in biopsy/cytology material

*is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?*

**Context:**
The type of information that may be determined in preoperative material from NETs

**Relevance:**
In inoperable cases, biopsy/cytology material may be the only tissue sample available

**Criticism:**
Classification criteria not applicable; immunoprofile (included Ki-67) affected by sampling error
FAQ 5: pathological diagnosis of NETs in biopsy/cytology material

*is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?*

**Answer:**

**YES**
(although classification limited to some cases)

FAQ 6: the role of Ki-67 in NETs

*is Ki67 to be determined in all NETs?*

**Context:**
Diagnostic vs. prognostic vs. predictive role of Ki-67 determination in NETs

**Relevance:**
Most relevant marker in NETs (upon NE differentiation is established)

**Criticism:**
Still poorly reproducible; diagnostic vs. prognostic vs. predictive uses often erroneously interpreted
FAQ 6: the role of Ki-67 in NETs

*is Ki67 to be determined in all NETs?*

**Answer:**

**YES**

(it is presently the best prognostic/predictive parameter for NETs we have. An accurate and reproducible procedure to assess Ki67 index is mandatory)

FAQ 7: prognostic factors in NETs

*are prognostic factors (markers) other than Ki67 to be assessed in all NETs?*

**Context:**
Molecular and immunohistochemical markers of prognostic value

**Relevance:**
Correct classification and proliferative index are the only prognostic features of relevance, to date

**Criticism:**
Several molecules proposed, none turned to be useful in clinical application
FAQ 7: prognostic factors in NETs

are prognostic factors (markers) other than Ki67 to be assessed in all NETs?

Answer:

NO
( not yet. Work in progress.)

FAQ 8: predictive factors in NETs

are factors (markers) predictive of response to therapy to be assessed in all NETs?

Context:
Molecular and immunohistochemical markers of predictive value

Relevance:
Predictive interest of detection of Somatostatin Receptors (5 types)

Criticism:
Several molecules proposed, none turned to be useful in clinical application
FAQ 8: predictive factors in NETs

are factors (markers) predictive of response to therapy to be assessed in all NETs?

Answer

NO

(only upon request by the clinician. Most requested: Somatostatin Receptors, but data are questionable)

FAQ 9: what next?
open issues...

- Applicability of new WHO classification to be tested
- Grading to be implemented (G2, location)
- Optimal staging system to be validated

- Prognostic/predictive markers to be validated
- Quantitative and reproducible evaluation of immunohistochemical markers

- Gene expression profiling

Gene expression profiling by RT-qPCR on Formalin-fixed Paraffin embedded tissues.
Sample 1: 977/94 NET
Sample 2: 8943/96 Malignant Insulinoma
Sample 1: 977/94 NET
Sample 2: 8943/96 Malignant Insulinoma