APPENDIX 5

PATHOLOGY

1. Handling and gross examination of gastrointestinal and pancreatic NETs

Specimen handling and gross examination should be performed according to the Royal College of Pathologists (RCPath) guidelines for carcinoma of these organs[1-3] and the ENETS guidance.[4]

1.1 Specimen fixation

The resection specimen should, when possible, be placed on ice immediately after removal and brought as soon as possible, fresh and unopened, to the pathology laboratory, where it should be placed in a large volume of formalin-based fixative.

1.2 Specimen dissection

As outlined in the RCPath dataset for the reporting of gastroenteropancreatic NETs,[5] specimen dissection should be performed according to the RCPath guidelines for carcinomas of the respective organs.[1-3] In general, dissection of specimens from the tubular gastrointestinal tract is based on serial slicing of the intact, tumour-bearing segment of the specimen. Dissection of pancreatoduodenectomy specimens is based on axial slicing of the intact specimen. Non-peritonealised resection margins in colorectal surgical specimens or the circumferential ('dissected') margins of pancreatic specimens are painted with suitable markers to enable subsequent identification of margin involvement.

1.3 Macroscopic assessment

The core macroscopic data to be included in the pathology report are the specimen type; the site and three-dimensional size of the tumour; extension of the tumour within the primary organ and into neighbouring tissues; relationship to other key anatomical structures and the specimen resection margins; and the number and site of lymph nodes retrieved from the main specimen and/or from separately submitted samples.[4, 5]

1.4 Tissue sampling

Representative blocks should be taken from the tumour to demonstrate the deepest point of invasion and/or involvement of adjacent tissues or anatomical structures relevant to WHO classification[6, 7] and TNM staging schemes.[8-10] The closest transection and/or circumferential ('dissected') margin(s) should be sampled. Samples of other tumours or lesions should be processed. One or two random blocks should be sampled from apparently normal background pancreatic parenchyma or gastrointestinal mucosa. In the case of gastric NETs, mucosa from both the corpus and antrum are to be sampled, to provide information on the type of (ECL-cell) tumour. All lymph nodes, whether retrieved from the main specimen or submitted separately, should be embedded in their entirety.

2. Microscopic assessment

2.1 Immunohistochemistry

2.1.1 General neuroendocrine markers

All tumours should be immunostained with a panel of antibodies to general neuroendocrine markers. These include synaptophysin and chromogranin A. Neuron-specific enolase (NSE), PGP9.5 or CD56 are not recommended as these antibodies or markers have poor specificity.[4, 11] Chromogranin staining may be sparse or negative in poorly granulated (i.e. less well-differentiated) tumours, or in L-cell tumours of the appendix and large bowel.[11] If the amount of tissue is limited (e.g. in liver biopsies of tumour metastases), synaptophysin is the best single marker to use. Histochemical stains, such as the Grimelius silver stain, are non-specific and therefore not recommended.

2.1.2 Hormones

The hormones produced will vary with the primary tumour site:

Pancreas: insulin, glucagon, pancreatic polypeptide, somatostatin, gastrin, vasoactive intestinal peptide (VIP), adrenocorticotrophic hormone (ACTH), prolactin.

Stomach and duodenum: gastrin, serotonin, somatostatin, gastrin-releasing peptide (GRP).

Ileum and caecum: serotonin, tachykinins, substance P.

Colon and rectum: serotonin, somatostatin, serotonin, peptide YY.

Appendix: serotonin, somatostatin, enteroglucagon.

If there is a clinical syndrome related to a particular (site-specific or ectopic) hormone, immunostaining may be performed to confirm the source of hormone production. However, occasionally, immunohistochemical hormone detection may not correlate with biochemical or clinical evidence of hormone production by the tumour.

2.1.3 Ki-67

The tumour should be stained with an antibody to Ki-67 protein, preferably MIB-1, to generate a Ki-67 index. As Ki-67 immunolabelling can be influenced by tissue fixation, antigen-retrieval and staining protocols, regular and adequate quality control of the immunostaining process is highly recommended.[12]

2.2 Proliferative activity

Proliferative activity is an integral part of the WHO 2010 and ENETS grading systems. It can be assessed by the mitotic count or Ki-67 index. Assessment should be made in the tumour area with the highest proliferative activity, which may be easier to identify by Ki-67 immunostaining. To allow accurate assessment, screening for mitoses of at least 50 HPF (1 HPF = 2 mm^2) or counting 500-2,000 tumour cells to establish the Ki-67 index is recommended.[4, 6] Accurate counting of a Ki-67 immunostained section may be facilitated by the use of an eye-piece grid or printed microscopic pictures of the selected assessment field. If grade differs between the mitotic count and Ki-67 index, the higher grade should be used.[6] The Ki-67 index has been shown to have diagnostic and prognostic relevance in NETS. However, as

controversy continues to exist over the optimal cut-off points (review in Vilar *et al* 2007[13]), it is important to report on the actual Ki-67 index.

2.3 Grading

The three-tiered WHO 2010 grading classification is based on morphological criteria and the proliferative activity of the tumour. G1 and G2 NETs are composed of uniform cells, showing round nuclei with stippled chromatin and inconspicuous nucleoli. Nuclear atypia is mild to moderate, the number of mitoses is low (<20 mitoses/10 HPF), the tumour cells are arranged in an organoid pattern, and tumour necrosis is absent. In contrast, G3 neuroendocrine tumours (NECs) are characterized by marked nuclear atypia, multifocal necrosis and a high mitotic activity (>20 mitoses/10 HPF). Some of these NECs will show organoid features resembling G1 or G2 NETs. G3 NECs of the GI tract encompass tumours of small cell and large or intermediate cell type. Given the current uncertainty as to whether the large cell variant is as chemosensitive as the small cell type, the tumour cell type of G3 NECs should be stated.[14]

The cut-off values for mitotic count and Ki-67 index of the WHO 2010 grading scheme[6] are identical to those of the ENETS grading system[8, 9] and defined as follows (Table 4) :

- G1: mitotic count <2 mitoses/10 HPF and/or Ki-67 index ≤ 2%
- G2: mitotic count 2-20 mitoses/10 HPF and/or Ki-67 index 3-20%
- G3: mitotic count >20 mitoses/10 HPF and/or Ki-67 index >20%.

2.4 Resection margins

The minimum clearance for NETs has not been established. However, the majority of NETs are relatively well-circumscribed, and therefore it has been suggested that resection can be regarded as complete, even if the margin is very close. Evaluation of resection margins by intra-operative frozen section examination is usually not performed for NETs. While several studies previously suggested that a positive margin after resection of a pancreatic NET does not seem to be critical for long-term survival,[15-19] a recent analysis of non-metastatic well-differentiated pancreatic NETs revealed a strong correlation between microscopic margin involvement and shortened disease-free survival.[20]

2.5 NETs of unknown primary

Biopsies from metastatic NETs, mainly liver biopsies, require immunohistochemical confirmation of the neuroendocrine nature of the cancer (see 2.1.1). The grade of tumour differentiation should be assessed according to the WHO and TNM systems,[6, 8, 9] as this has important management implications.[21] Assessment of the proliferative activity in these biopsy samples is obviously limited by intratumoural heterogeneity and differences between primary tumour and metastases.[22] Hormone production by the tumour cells may assist in identification of the primary tumour site (see 2.1.2). In addition, TTF1 is present in 43% of well-differentiated pulmonary tumours, but cannot be used for poorly differentiated NETs, because 50% of these in extra-pulmonary location are positive. While CDX2 is expressed in 86% of appendiceal and colonic NETs, expression of this marker is much lower or absent in gastric and rectal NETs. Cytokeratin staining (CK7, CK20) is not helpful.[23, 24]

2.6 Mixed endocrine-exocrine tumours

These neoplasms are defined as composed of intimately admixed endo- and exocrine tumour cell populations, which each represent at least 30% of the tumour mass.[6, 25] Scattered individual neuroendocrine cells within an otherwise conventional adenocarcinoma are a common finding without clinical significance that should not be reported as a mixed tumour. The NET component should be confirmed immunohistochemically (see 2.1.1), while histochemical detection (alcian blue/PAS staining) of intracytoplasmic mucin droplets may be helpful to ascertain adenocarcinomatous differentiation. PAS positivity can occasionally be seen in the lumina of pure ETs with a glandular growth pattern, however, this does not represent evidence of exocrine differentiation. Immunostaining for CEA (monoclonal antibody) and CA19.9 has been recommended for affirmation of exocrine differentiation, however systematic validation of these markers is currently outstanding. Immunostaining for cytokeratins is not helpful.[24]

Goblet cell carcinoid of the appendix is a mixed tumour characterised by intimate admixture of neuroendocrine and signet ring/goblet cells showing mild to moderate atypia, low proliferative activity (Ki-67 index <20%) and an organoid growth pattern.[6] These tumours should be carefully distinguished from mixed

adenoneuroendocrine carcinoma (MANEC)[6] or adenocarcinoma ex goblet cell carcinoid, which is characterised by marked cytological atypia, a disorderly growth pattern, a much higher proliferative activity and aggressive behaviour.[26-30]

The significance of immunohistochemical detection of neuroendocrine marker expression in <30% of poorly differentiated carcinoma is currently not clear. As it is presently not known whether these tumours represent a separate entity, a descriptive diagnosis with documentation of the extent of both components is recommended.[14, 25]

3. Pathology report

The pathology report should contain the core data as set out in the RCPath guidelines for neuroendocrine digestive tumours.[5] To assist remembering all data items, the use of the RCPath site-specific proformas is advised. In addition, staging and grading should be performed according to the recently published WHO 2010 classification,[6] the UICC TNM 7th edition[10] and the ENETS staging system for NETs of the stomach, pancreas and appendix.[8, 9]

Table 1. WHO 2010 classification of gastroenteropancreatic NETs.[6]

1. Neuroendocrine tumour (NET) G1
2. Neuroendocrine tumour (NET) G2
3. Neuroendocrine carcinoma (NEC; G3; large cell or small cell type)
4. Mixed adenoneuroendocrine carcinoma (MANEC)

Table 2. TNM staging criteria for NETs of the digestive tract and pancreas according to UICC TNM 7th edition.[10]

	T-stage			
Site	**T1**	**T2**	**T3**	**T4**
Stomach	Invasion of (sub)mucosa and size ≤1 cm	Invasion of muscularis propria or size >1 cm	Invasion of subserosa	Perforation of serosa or invasion of adjacent structures
Duodenum, ampulla, upper jejunum	Invasion of (sub)mucosa and size ≤1 cm	Invasion of muscularis propria or size >1 cm	Invasion of pancreas or retroperitoneum	Invasion of peritoneum or other organs
Lower jejunum, ileum	Invasion of (sub)mucosa and size ≤1 cm	Invasion of muscularis propria or size >1 cm	Invasion of subserosa	Invasion of peritoneum or other organs
Colon, rectum	Invasion of (sub)mucosa T1a: size <1 cm T1b: size 1–2 cm	Invasion of muscularis propria or >2 cm	Invasion of subserosa/pericolic /perirectal fat	Invasion of peritoneum or other organs/structures
Appendix	Size ≤2 cm T1a: <1 cm T1b: >1 cm – <2 cm	Size ≥2 – ≤4 cm or extension to caecum	Size >4 cm or extension to ileum	Perforation of peritoneum or invasion of other organs
Pancreas	Limited to pancreas and size <2 cm	Limited to pancreas And size >2 cm	Outside pancreas but no invasion of coeliac axis/SMA any size	Invasion of coeliac axis / SMA

Table 3. TNM staging criteria for NETs of the stomach, appendix and pancreas according to the ENETS system.[8, 9]

	T-stage			
Site	**T1**	**T2**	**T3**	**T4**
Stomach	Invasion of (sub)mucosa and size ≤1 cm	Invasion of muscularis propria or subserosa or size >1 cm	Penetration of serosa	Invasion of adjacent structures
Appendix	Size ≤1 cm and invasion of submucosa or muscularis propria	Size ≤2 cm and invasion of submucosa, muscularis propria and/or ≤0.3 cm into subserosa/meso-appendix	Size >2 cm and/or >0.3 cm into subserosa/ mesoappendix	Invasion of peritoneum or other organs
Pancreas	Limited to pancreas and size <2 cm	Limited to pancreas and size 2–4 cm	Limited to pancreas and size >4 cm or invasion of duodenum or bile duct	Invasion of coeliac axis / SMA, stomach, spleen, colon, or adrenal gland

Table 4. Grading criteria for proliferative activity according to WHO 2010[6] and ENETS grading schemes.[8, 9]

Grade	Mitotic count	Ki-67 index
1	<2 mitoses / 10 HPF	≤2%
2	2–20 mitoses / 10 HPF	3–20%
3	>20 mitoses / 10 HPF	>20%

Table 5. WHO 2000 classification of gastroenteropancreatic NETs.[7]

	Classification			
Site	**Well-differentiated NET Benign behaviour**	**Well-differentiated NET Uncertain behaviour**	**Well-differentiated neuroendocrine carcinoma Low-grade malignant**	**Poorly differentiated neuroendocrine carcinoma High-grade malignant**
Pancreas	Confined to pancreas <2 cm size <2 mitoses/10 HPF Ki-67 index ≤2% No vascular invasion No perineural invasion Functioning insulinoma or non-functioning tumour	Confined to pancreas and one or more of the following: ≥2 cm size, 2–10 mitoses/10 HPF, Ki-67 index >2% Vascular invasion, Perineural invasion	Invasion of adjacent organs and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF
Stomach	Non-functioning tumour Confined to mucosa-submucosa ≤1 cm size No vascular invasion	Non-functioning tumour Confined to mucosa-submucosa >1–2 cm size With or without vascular invasion	Functioning tumour of any size Non-functioning tumour >2 cm size Or any size with invasion of muscularis propria or beyond and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF
Duodenum, upper jejunum	Non-functioning tumour Confined to mucosa-submucosa ≤1 cm size No vascular invasion	Non-functioning tumour or functioning gastrinoma Confined to mucosa-submucosa >1 cm size With or without vascular	Functioning or non-functioning tumour of any size with invasion of muscularis propria or beyond and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF

		invasion		
Distal jejunum, ileum	Non-functioning tumour Confined to mucosa-submucosa ≤1 cm size No vascular invasion	Non-functioning tumour Confined to mucosa-submucosa ≤1 cm size Vascular invasion	Functioning tumour of any size Non-functioning tumour >1 cm size Or any size with invasion of muscularis propria or beyond and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF
Colon, rectum	Non-functioning tumour Confined to mucosa-submucosa ≤2 cm size No vascular invasion	Non-functioning tumour Confined to mucosa-submucosa ≤2 cm size Vascular invasion	Functioning tumour of any size Non-functioning tumour >2 cm size Or any size with invasion of muscularis propria or beyond and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF
Appendix	Non-functioning tumour Confined to appendiceal wall ≤2 cm size No vascular invasion	Non-functioning tumour Extension into mesoappendix >2–2.5 cm size Vascular invasion	Functioning tumour of any size Non-functioning tumour >2.5 cm size Or any size with deep invasion into mesoappendix and/or metastases Ki-67 index ≤30%	Large cell or small cell carcinoma >30% Ki-67 index >10 mitoses/10 HPF

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