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Cyto-histology in NET: what is necessary today and what is the future?

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Abstract The carcinoid as originally described is part of the relatively large family of neuroendocrine neoplasia found in almost every organ. Historical reasons back their current definitions. Neuroendocrine cancer is most frequently observed in the lung and the digestive tract. In the lung is defined as carcinoid (typical and atypical) for well differentiated, low to intermediate grade, and small cell and large cell neuroendocrine carcinoma for poorly differentiated, high grade. In the digestive system are respectively defined as neuroendocrine tumor (NET) and neuroendocrine carcinoma (NEC) of small and large cell types. Grading and staging are developed for their clinical classification by the World Health Organization (WHO) and the American Joint Committee on Cancer (AJCC). In both anatomical sites the morphological features are overlapping, with bland histology for carcinoid and NET, and aggressive features with extensive necrosis, severe atypia and abundant, atypical mitoses for high grade cancer types. Such features are also essential diagnostic clues in cytological preparations. The confirmation of the neuroendocrine signature by immunohistochemistry is mandatory for the diagnosis; a minimum panel comprising chromogranin A and synaptophysin is recommended in the digestive system. In addition, the application of grading requires the mitotic count and or spotty necrosis assessment for lung, or the mitotic count and the Ki67 assessment in the digestive system.

Keywords Cytology · Histology · Grading · Staging · Classification · Personalized medicine · Tailored therapy

1 Brief historical notes

Neuroendocrine neoplasia is a large family of epithelial tumors that express several antigens shared by tumors of the endocrine and the central nervous systems [1–3]. Such antigens associate with the built-in machinery of the neuroendocrine cell to control, produce and secrete hormones, small neuroendocrine mediators and other factors [4]. This phenotype definition reflects shared gene programs but does not imply any embryological significance. Indeed, the possible origin from the neural crest still stands correctly for certain neuroendocrine cell types only (e.g. the thyroid calcitonin-producing C cell), while it is not valid for the neuroendocrine cell at most other sites (e.g. lung and gastrointestinal tract and pancreas) [5]. By converse and incidentally, the amine uptake and decarboxylation (APUD) feature is recognized for most neuroendocrine cells independently form their embryological origin [6].

As such neuroendocrine neoplasia is called/classified with different and organ-specific definitions [7–13]. The neuroendocrine cancer is recognized within the central (anterior pituitary adenoma) and peripheral (paraganglioma) nervous systems, the endocrine (medullary thyroid carcinoma; adrenal pheochromocytoma), the upper airway (carcinoid, small cell carcinoma), the skin (Merkel cell carcinoma), the lower airway (carcinoid, small/large cell carcinoma), the digestive (neuroendocrine tumor/carcinoma) and the urogenital (neuroendocrine tumor/carcinoma) systems. Such non-uniform (and

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potentially confusing) nomenclature has historical reasons since it was independently generated in different times for different organs to describe organ-specific clinical features. Nonetheless some degree of similarity is generally recognized since at most anatomical sites the neuroendocrine cancer may display low, intermediate and high grade morphological features and clinical malignancy. As a rule, all cancer types share the family-brand antigen signature no matter their grade.

This paper deals with the cyto/histological diagnosis of neuroendocrine neoplasms of the lung and the digestive tract which are recognized as the most commonly occurring neuroendocrine cancer types [14, 15].

2 Current classification tools

2.1 WHO/AJCC classifications

As major health agencies, the World Health Organization (WHO) and the American Joint Committee on Cancer (AJCC) have established well-structured classification tools for both lung and the digestive tract (Table 1) [10, 12, 16, 17]. The nomenclature and the categories are apparently different, bearing however a high degree of similarity.

In the lung, two tumor categories are recognized: the category of carcinoid, either of low grade and defined as "typical carcinoid" (TC), or of intermediate grade as "atypical carcinoid" (AC), and the category of small/large cell neuroendocrine carcinoma (Table 1). The two categories are identified based on histological differentiation features, the carcinoid being well-differentiated, the small/large cell carcinoma being poorly differentiated. The differentiation is established at histology based on classical morphological criteria. The subcategory definition for carcinoid is based instead on the proliferation activity by mitotic count (<2 mitoses per 10 high power field for TC; ≥ 2–10 for AC) and/or by the presence of spotted necrosis (defined as micro-foci of necrosis). For small/large cell carcinoma the separation vs AC is based only on mitotic count/presence of spotted necrosis, and upon subtle morphological features for the separation between small vs large cell carcinoma.

In the digestive tract, the simple, original three tiers system devised in 2010 [10], has now been improved to a two tiers system similar to the one adopted for the lung [16, 17]. The system still utilizes the specific proliferation grading based on Ki67 and mitosis determination (Table 1). Two categories are recognized, the neuroendocrine tumor (NET) and the neuroendocrine carcinoma (NEC). The NET is equalized to a well differentiated neuroendocrine cancer graded as NET G1, G2 and G3, and directly associating with increasing clinical malignancy. The NEC is by default a G3, poorly differentiated, highly malignant cancer and subdivided in small and large cell type. Classical morphological criteria are utilized to define differentiation. The separation between NET G2 and NET G3 is based only on Ki67/mitotic count definition, with overlapping morphology and often clinical behavior. For NET G3/NEC the separation may in some instances be difficult since mostly based on subtle morphological features. Similarly, the separation between small vs large cell NEC may sometime be problematic.

The two classification systems are well-understood on both the pathology and the end-user clinical side, proved successful in predicting patient survival and useful for patient management and therapy establishment [18–26]. The two systems have a similar structure, however with a different definition for the well differentiated, low grade cancer types, respectively carcinoid in the lung and NET in the digestive system. This difference finds some justification by the known different biology observed for the neuroendocrine cancer of the lung as compared to the gut and pancreas. It is worth recalling that the term "carcinoid" was coined for a neoplastic lesion of the small intestine by Sigfried Oberndorfer in 1907 [1], soon recognized as composed by transformed serotonin-producing, enterochromaffin cells (EC) [27–29]. The carcinoid was subsequently associated with a well-defined hormonal syndrome (the carcinoid syndrome) due to unregulated release of

Table 1 Histology, definition and classification/grading parameters for lung and gut neuroendocrine cancer

Histology	Lung			Gut & pancreas			
	type	mitoses	necrosis	type	grade	mitoses	Ki67
Well-differentiated/low grade	TC	<2	no	NET	G1	<2	<3
Well- differentiated/intermediate grade	AC	2–10	no/yes	NET	G2	2–20	3–20
Well-differentiated/high grade	-	-	-	NET	G3	>20	>20
Poorly-differentiated/high grade	LCNEC	>10	yes	NEC LC	G3	>20	>20
	SCLC	>10	yes	NEC SC	G3	>20	>20

TC Typical carcinoid; *AC* Atipycal carcinoid; *LCNEC* Large cell neuroedocrine carcinoma; *SCLC* Small cell lung cancer; *NET* Neuroendocrine tumor; *NEC* Neuroendocrine carcinoma; *LC* Large cell type; *SC* Small cll type

serotonin and other mediators [30]. In the digestive system, however, neuroendocrine tumors may well be composed by different cell types (e.g. by insulin-producing B cells in the pancreas or by gastrin-producing G cells in the duodenum, etc.), in a fraction of cases associating with well-defined hormonal syndromes [4, 31]. This is the main reason why in the gut the term carcinoid was discarded as uniform label for the neuroendocrine cancer with well-differentiated morphology. On the contrary, in the lung only one type of transformed neuroendocrine cell (the P/D1 type, mainly producing CGRP, tachykinins and serotonin) may compose the carcinoid and in very rare instances may associate with a carcinoid syndrome [32, 33]. As such this definition is still accepted for the low-intermediate grade neuroendocrine cancer of the lung.

Besides grading tools, both WHO and the AJCC offer site-specific staging systems [10, 12, 16, 17]. In the lung, the staging system devised for the more common exocrine cancer, also fits the neuroendocrine cancer family including the small cell cancer [16]. In the digestive system, the original proposals by the European Neuroendocrine Tumor Society (ENETS) [34, 35] proved their efficacy [22–26] and are now endorsed by WHO [10, 17] and AJCC [16]. Notably, while for the WHO the staging systems are foreseen for the all the neuroendocrine cancer types for all sites except pancreas, the AJCC always excludes the NEC.

3 Practice of pathological diagnosis

3.1 Cytology

The following cytological tips apply for both the lung and the digestive neuroendocrine cancer (Table 2). The original, relatively easy and light fine-needle aspiration cytology (FNAC) technique implies the use of 22 (and higher) gauge needles that may return cytological samples with a high degree of diagnostic efficiency [36]. Expert hands and eyes are however mandatory, with variable diagnostic sensitivity and specificity (up to 90%) reported in the literature for a diagnosis of neuroendocrine cancer [37]. The original limit of FNAC which made unique the sample obtained is now overcome by liquid-based cytology for thin layer techniques, allowing repeatable preparations for multiple tests. In addition, in recent years the sampling technique rapidly evolved with the development of more and more efficient needle devices. Larger needles (e.g. 16G and shark-type) are now available making efficient obtaining abundant samples for micro-histology [38]. The cytological preparation for a low/intermediate grade neuroendocrine cancer displays a clean background with relatively monomorphic cells, often in groups and sometimes distributed around gland-like lumens, with mild to moderate atypia, variable amount of cytoplasm from scarce to abundant eosinophilic or granular

Table 2 Major cyto-histological features of neuroendocrine cancer

Cancer Type	Structure	Stroma	Necrosis	Smear	Cell Size/Shape	Nucleus	Cytoplasm
Well differentiated (TC-AC-NET)	Solid islet Trabecular Glandular Mixed	Fine (richly vascular)	no (focal)	Clean/bloody	Medium Round to spindle	Salt & pepper	Abundant (eosinophil/clear)
Poorly differentiated SC-NEC LC-NEC	Solid/organoid	Thick (desmoplastic)	yes (single cell/abundant)	Dirty/bloody	Small (oat) to medium Large	Salt & pepper Prominent nucleoli	Thin rim Abundant

TC Typical carcinoid; AC Atipycal carcinoid; NET Neuroendocrine tumor; NEC Neuroendocrine carcinoma; LC Large cell type; SC Small cell type

cytoplasm and regularly round nuclei with salt and pepper chromatin (Fig. 1a-e). Ancillary immunohistochemistry (IHC) for neuroendocrine markers available for thin layer cytology may confirm the diagnosis, even providing directions for the grade definition by the use of proliferative markers like Ki67 (Fig. 1b–e). The presence of a dirty background with necrosis, severe cell atypia often with a thin rim of cytoplasm that sometimes may still be abundant, and cell nuclei with salt and pepper chromatin and often with crushing artifacts, all are features indicating a high-grade cancer and supporting a neuroendocrine phenotype to be confirmed by IHC (Fig. 1f-k).

3.2 Histology

The current histological tools have been established long ago by the seminal paper published by Soga and Tazawa in 1971 [39] (Table 2). Such descriptive tools apply for both lung and all the digestive sites. When of low grade and well differentiated, the neuroendocrine cancer displays a similar structure, usually defined as organoid (roughly resembling the organ of origin when present, e.g. the islets of Langerhans in the pancreas), it is characteristically void of necrosis and displays irregularly expansive margins with a delicate stroma
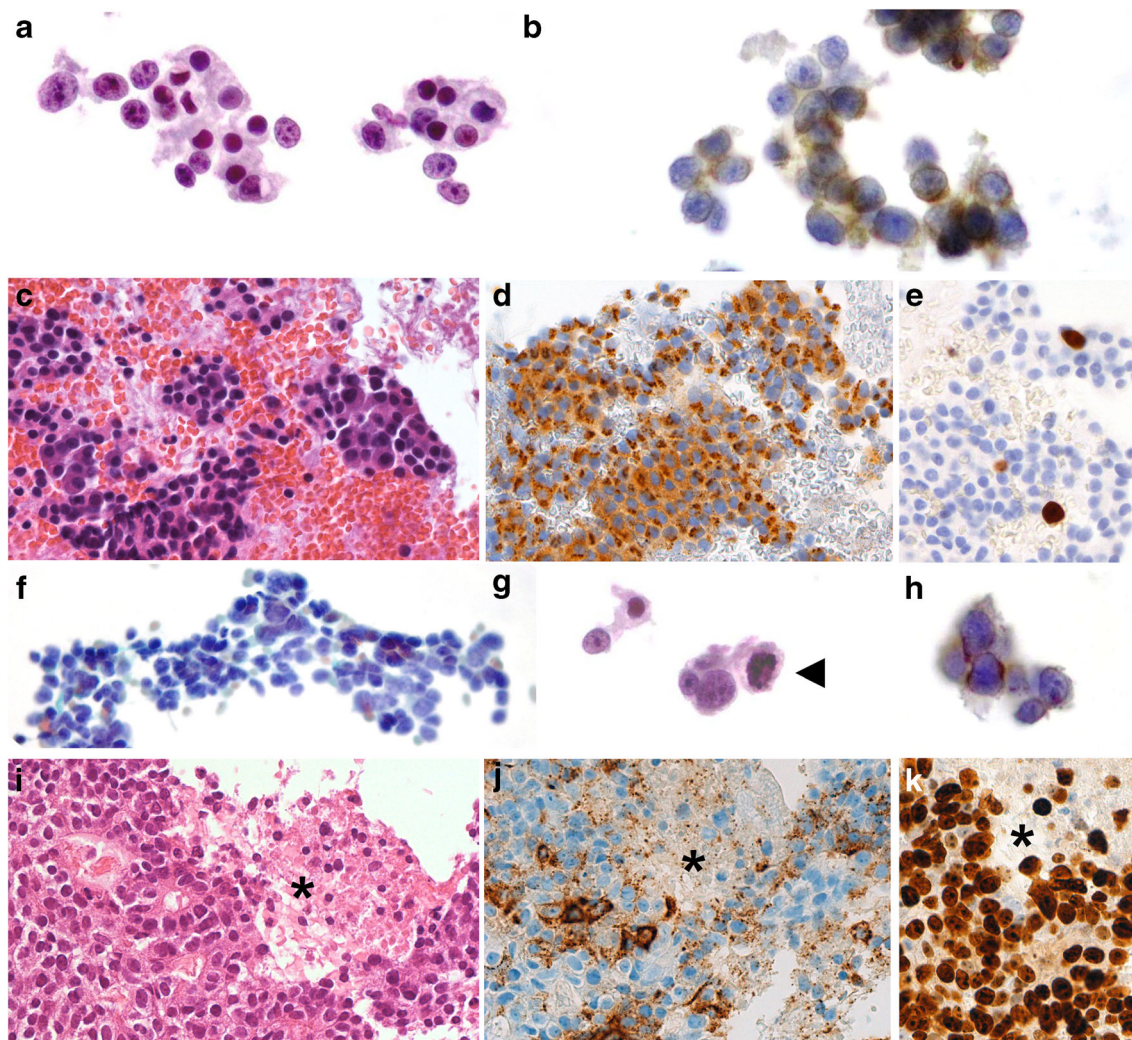

Fig. 1 Cytology and micro-histology of neuroendocrine cancer. **a-e** Well differentiated, low grade (G1-G2) neuroendocrine tumor (NET) of the pancreas; **a**) thin layer cytology: tumor cells are round and monomorphic, show abundant, eosinophilic cytoplasm, relatively regular round nuclei with salt and pepper chromatin and inconspicuous nucleoli and **b**) positive staining at immunohistochemistry (IHC) for chromogranin A; **c**) Micro-histology preparation obtained by Fine Needle Tissue Acquisition (FNTA) showing regular cells with no mitosis and necrosis, at IHC with diffuse chromogranin A positive staining (**d**) and only very rare nuclei labelled for Ki67 (**e**). **f-k**) Poorly differentiated, high grade (G3) neuroendocrine carcinoma (NEC) of the pancreas; **f**) conventional cytology preparation (smear) showing a group of cancer cells with irregular shapes, mostly small in size, irregular nuclei and, at thin layer cytology, showing one evident mitosis (**g,** arrowhead) and a thin rim of cytoplasm positive for chromogranin A at IHC (**h**); **i**) micro-histology FNTA preparation with solid structure, evident necrosis (asterisk), highly atypical, small-size cells with focal chromogranin A expression at IHC and diffuse uniform nuclear labelling for Ki67 (**k**). (**a**, **f** and **g**) Papanicolau stain; (**c** and **i**) hematoxylin and eosin; (**b**, **d**, **e**, **h**, **j**, **k**) immunoperoxidase; original magnification ×1000 (**a**, **b**, **g**, **h**); ×400 (**c**, **d**, **e**, **f**, **i**, **j**, **k**)

especially rich in vessels (Fig. 2a, d). Such general structure is then defined as composed by: i) solid islets (A Type, by Soga and Tazawa 1971), typical of the small intestinal NET and well-fitting the original carcinoid description by Oberndorfer in 1907 [1]; ii) cell trabeculae, ribbons and nests often anastomosing (B Type by Soga and Tazawa 1971) typically found in the large intestine, but also observed in the lung, the stomach, and the pancreas; iii) glandular structure, with cell arranged around gland-like spaces (C Type, by Soga and Tazawa 1971), typically observed in the duodenum and the ampullary region; and iv) the mixed structure, a very frequent occurrence in which some or all of the previous morphology models are encountered in the same tumor (Mixed Type by Soga and Tazawa 1971). In addition, a structure of "…lower or atypical differentiation…" was also foreseen though with undefined features (D Type) and underscoring cases not fitting the previous one. A-C Types are invariably composed by medium-size, round to polygonal, monomorphic epithelial cells with abundant eosinophilic cytoplasm, round nuclei with salt and pepper chromatin and inconspicuous nucleoli, overall mild to moderate atypia (Fig. 2a–e). The mitotic count is usually rather low as well. Some degree of higher atypia, proliferative activity and even focal, spotty necrosis can be

observed in intermediate grade cancer in lung AC and NET G2 and G3 in the digestive system (Fig. 2 and 3a-d).

When of high grade and poorly differentiated, the neuroendocrine cancer equally displays a sort-of organoid structure, though much less defined, with infiltrative margins, a thicker stroma up to evident desmoplasia, typically abundant necrosis often defined as "geographical chart necrosis" characterized by ample, irregular areas of necrosis with neutrophil infiltrate and surrounded by a thick rim of residual vital cancer cells (Fig. 3 e, f). Cells are usually severely atypical and either irregularly round or spindle, with an ample, irregular nucleus with salt and pepper chromatin surrounded by a thin rim of cytoplasm (the "oat cell", small cell type), or equally severely atypical but with large irregular nuclei often with evident nucleoli and more abundant cytoplasm (large cell type).

The above descriptive features accommodate most neuroendocrine cancer cases; however, border-zone cancers may pose some diagnostic problems. For border-zone cancer it is intended a neoplasm that displays a morphology that better fits the lower category but, based on proliferation index assessment (in specific by mitotic count only in the lung and by either mitotic count and Ki67 in the digestive tract), fits the immediately higher category. In the lung, neuroendocrine
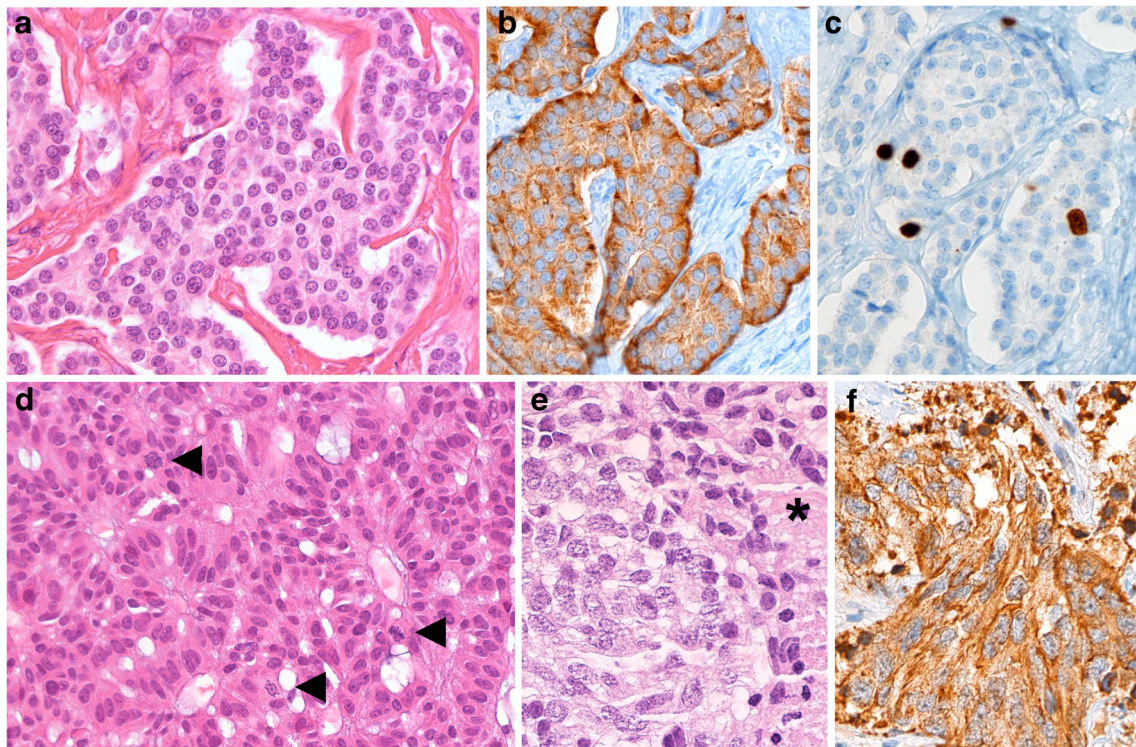

Fig. 2 Histology of well-differentiated neuroendocrine cancer. **a-c)** Low grade (G1-G2) neuroendocrine tumor (NET) of the stomach, possible clinical-pathological Type 2 (MEN1-associated); (**a**) trabecular-anastomosing structure void of necrosis with evident hyaline stroma composed by monomorphic cells with regular round nuclei and abundant eosinophilic cytoplasm at immunohistochemistry (IHC) strongly and diffusely positive for synaptophysin (**b**) and only few cells labelled for Ki67 (**c**); **d-f)** atypical carcinoid of the lung; (**a**) trabecular structure void of necrosis though with evident multiple mitoses (arrowhead); (**b**) solid structure with focal, spotty necrosis (asterisk) and strong IHC positive staining for chromogranin A in cells mostly with spindle shape (**f**). (**a, d, e**) Hematoxylin and eosin; (**b, c, f**) immunoperoxidase; original magnification ×200 (**d**); ×400 (**a, b, c, e, f**)
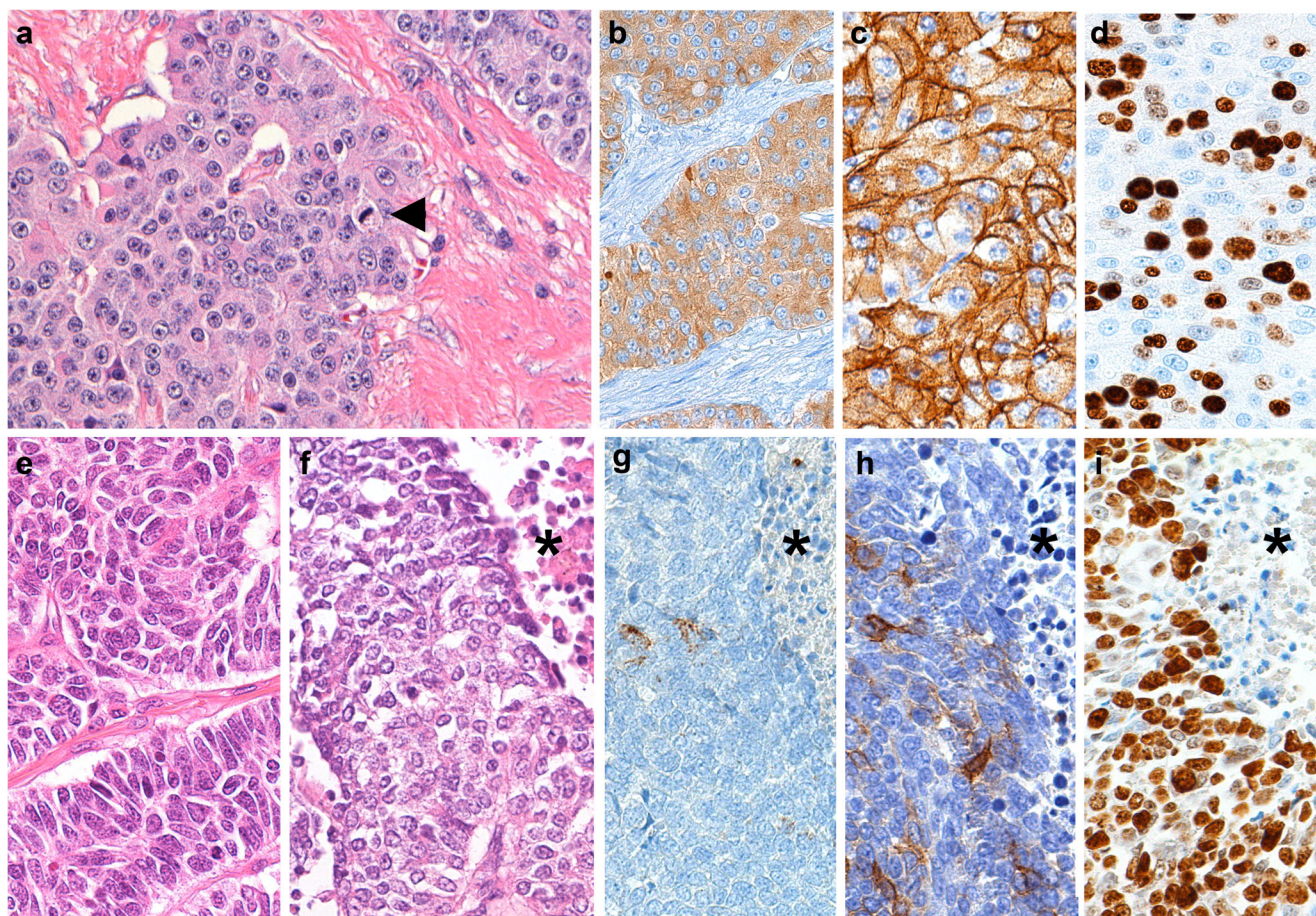
Fig. 3 Histology of well and poorly differentiated aggressive neuroendocrine cancer. **a-d** High grade, G3, well-differentiated neuroendocrine tumor (NET) of the stomach, clinical-pathological Type 3; (**a**) solid-organoid structure void of necrosis, with abundant and thick hyaline stroma, composed by relatively monomorphic though highly atypical cells with evident mitoses (arrowhead), regular nuclei with evident nucleoli and abundant eosinophilic cytoplasm; at immunohistochemistry (IHC) cancer cells are strongly positive for synaptophysin (**b**), for the receptor subtype 2A for somatostatin (SSTR2A) with cell membrane reinforcement (**c**) and diffusely for Ki67 in their nuclei (**d**). **e- i**) High grade, poorly differentiated small cell carcinoma of the lung with organoid (**e**) and solid structure (**f**), evident necrosis (asterisks, **f-i**), few cell positive at IHC for chromogranin A (**g**, center of the micrograph), SSTR2A (**g**) and almost all cells with nuclei positive for Ki67 (**i**). (**a, e, f**) Hematoxylin and eosin; (**b, c, d, g, h, i**) immunoperoxidase; original magnification ×400

cancers that at morphology fit the definition of AC but exceeds the mitotic count of the AC category (above 10 mitoses per 10 HPF), are acknowledged and by rule defined as Large Cell Neuroendocrine Carcinoma (LCNEC), despite lacking the poorly differentiated morphology required for a LCNEC diagnosis [12]. In the digestive tract and pancreas not much was known before the introduction of the WHO 2010 three tiers grading system. The application of such tool revealed that the G3 category is numerically limited and indeed heterogeneous [40], comprising cases with relatively well-differentiated morphology [41, 42], discrepant mitotic count vs Ki67 index [43] and with either more well-differentiated morphology and relatively high Ki67 index (between 20 and 55%) (Fig. 3d), poorly differentiated morphology with relatively low Ki67 index (between 20 and 55%) and poorly differentiated morphology and very high Ki67 index (>55%) [44]. Such cases are indeed rare but may pose significant diagnostic problems being in some instances virtually impossible to assign either to the NET G3 or to the NEC category [45, 46]. The use of ancillary IHC for markers of poor prognosis (e.g. P53, Rb, etc) may be of help (see below).

Overall according to the above rather structured histological features, it would be expected that the rule "same face, same biology" may apply to neuroendocrine cancers no matter their origin. It's however everyday oncology practice to observe that the clinical behavior and the response to conventional therapy or to targeted agents do vary according to the anatomical site of cancer occurrence, likely depending on yet unknown site-specific features.

3.3 Ancillary techniques

Immunohistochemistry is the tool used to make the diagnosis of neuroendocrine cancer, to define its aggressiveness and to provide solid clues for its origin in the metastatic setting.

A small panel of antibodies is required to uncover the neuroendocrine family signature, in spite of the vast number of neuroendocrine antigens (either general to all or specific for a single cell type) tested and available [4]. The minimum panel comprises chromogranin A and synaptophysin. Chromogranin A is likely the most specific though not the most sensitive marker available, given the fact that its efficacy depends on the amount of large dense core vesicles (LDCV, the subcellular structure where chromogranin A is located) available in the cytoplasm of the neuroendocrine cancer cell [4]. It's therefore diffusely and intensely expressed in cells that are closer to the normal counterpart and thus in the well-differentiated neuroendocrine neoplasm (Figs. 1 and 2), while usually expressed in few cells in poorly differentiated cancers (Fig. 3g). Synaptophysin is a molecule with unknown function found within the small synaptic-like vesicle (SSV) in the cytoplasm of the neuroendocrine cell [4] (Figs. 2b, f and 3b, g). For poorly understood reasons SSVs are abundant in the cytoplasm of the neuroendocrine cancer cell, of both well and poorly differentiated types. In light of this feature it is considered a very sensitive marker of neuroendocrine differentiation. However, it is also relatively poorly specific since found in cancer types other than neuroendocrine (e.g. the solid pseudo-papillary tumor of the pancreas, etc.) [10]. On the same line the cytoplasmic marker Neuron Specific Enolase (NSE) or the membrane marker CD56 may also be of help, though of low specificity for the neuroendocrine nature assessment. Hormone immunohistochemistry can also be considered, but only upon specific clinical request. Indeed since gut hormones are cell-type specific, their demonstration in cancer cells may have some diagnostic and clinical value [4]. This is the case for neuroendocrine tumors determining hormonal hypersecretion (functioning tumors). The confirmation of hormone expression on tumor tissue may prove the source of hormonal production, additionally suggesting the primitive cancer origin. This may also apply for those debated conditions in which hormone levels are increased though in absence of defined clinical features (e.g. PP-producing tumors). Hormones to be considered should mirror the tissue hormonal distribution (e.g. in the stomach somatostatin, gastrin, etc.) [47], the most frequent anatomical site of neuroendocrine tumor occurrence and, most importantly, the hormonal syndrome observed in the patient by clinicians, no matter its unusual setting (Table 3). A caveat is the well-known high plasticity of neuroendocrine cancer that may change its hormone production over time. In some instances, finally, hormone demonstration on tissue specimens may help elucidating the clinical background leading to tumor development. This is the case for neuroendocrine tumors of the stomach that mostly develop in a hypergastrinemic condition. Indeed, the demonstration of hyperplastic gastrin-producing cells of the antrum or, alternatively, of a gastrin-producing tumor in the duodenum (rarely in the pancreas) may define the tumor clinical-pathological subtype (Type 1 vs Type 2) [48]. Commercial antibodies for gut hormones are usually effective for the above requirements, though as a common rule experience is needed for interpretation of immunohistochemistry data.

Ki67 is the most popular if not the most effective tool to define the degree of aggressiveness of the neuroendocrine cancer. Its use is not supported by the WHO classification of the lung, though is outlined its utility for small biopsy [12, 49]. On the contrary it is an intrinsic component of the classification of digestive neuroendocrine neoplasms (Figs. 1, 2 and 3) (Table 1).

Other markers may be of some utility. Some of the most popular IHC markers include transcription factors like the thyroid transcription factor 1 (TTF-1) for the lung, the islet 1 (Isl1) and PAX8 for the pancreas and the caudal type homeobox 2 (CDX29) for the intestine, in the metastatic setting to help elucidating the possible primitive source of the neuroendocrine cancer [10, 17, 50]. Their use however needs caution since their possible wider expression, e.g. TTF1 in high grade neuroendocrine cancer outside the lung [51, 52] or CDX2 can be found in neuroendocrine tumors of the pancreas as well [17]. IHC for the somatostatin receptor subtypes 2A (sst2A) and 5 (sst5) may also be performed (Fig. 3c, h). Their use, though usually not recommended, may be foreseen upon request by the clinician to help the patient therapy tailoring in the absence of nuclear medicine *in vivo* methods [e.g. ^{68}Ga-peptides positron emission tomography (PET)]. Of note, since receptor preservation in formalin-fixed, paraffin-embedded tissue may be problematic, somatostatin receptor immunohistochemistry was traditionally difficult and poorly effective [53]. The relatively recent commercial introduction of reliable sst2A and sst5 monoclonals made these tests efficient and highly reproducible [53–55]. The use of sst5 is so far of value only in specific settings, e.g. to help tumor type definition in duodenal neuroendocrine tumors [56]. Its significance for clinical/therapeutical purposes remains however unclear/undefined.

Finally, for digestive neuroendocrine cancer, negative prognosis markers like the retinoblastoma gene (Rb) and *p53* (P53) products are often demonstrated in poorly differentiated NEC and only rarely in high grade NET [45, 57]. By converse NECs retain the expression of the death domain associated protein (DAXX) or of the chromatin remodeler gene (ATRX) that are frequently lost in the well differentiated forms [46]. Such markers, together with clinical information, were suggested as helpful to separate NET G3 vs NEC when morphology fails, a situation that may involve up to 61% of such rare cases, as reported for the a series of 33 cases investigated by Tang et al. [46].

4 Guidelines recommendation

International and national scientific societies and agencies like the European Neuroendocrine Tumor Society (ENETS), the

Table 3 Hormones of potential utility for the diagnosis of neuroendocrine cancer according to tumor cell type, most frequently observed primary anatomical site, cancer type and possible clincal setting

Hormone		Cell type*	Primary Anatomical Site	Neuroendocrine cancer type	Possible clinical setting
Gastrin		G	Stomach Duodenum/jejunum Pancreas	Well differentiated (TC-AC-NET)	Type 1, 2, 3 NETs** Zollinger-Ellison syndrome (+/− MEN1) " " " "
Glucagon/peptides		A/L	pancreas/colon-rectum		Glucagonoma syndrome/undefined
Insulin		B	Pancreas		Insulinoma syndrome
PP/PYY peptides		PP/L	pancreas/colon-rectum		Undefined
Serotonin		EC	Small intestine		Carcinoid syndrome
Somatostatin		D	Duodenum Pancreas		Somatostatinoma syndrome " "
Rare	VIP	P/D1 nd	Lung Pancreas		Verner-Morrison syndrome " " "
	ACTH	P/D1 nd	Lung Pancreas	Well differentiated (TC-AC-NET) poorly differentiated (SC/LC-NEC)	Cushing syndrome " "

Glucagon/peptides glucagon and proglucagon-derived peptides including glicentin; PP/PYYpeptides Pancreatic polypeptide and peptide *YY VIP* Vasoactive intestinal polypeptide; *ACTH* Adrenocorticotropic hormone; *see ref. [47]; *G* Gastrin-producing cell, *A/L* Glucagon-producing cell of the pancreas/glicentin/PYY-producing cell of the intestine, mainly colon-rectum; *PP* Pancreatic polypeptide cell; *EC* Enterochromaffin, serotonin-producing cell; *D* somatostatin-producing cell; *nd* Not defined *TC* Typical carcinoid, *AC* Atipycal carcinoid, *NET* Neuroendocrine tumor; *NEC* Neuroendocrine carcinoma; *LC* Large cell type, *SC* Small cell type; **see ref. [48]

North American Neuroendocrine Tumor Society (NANETS), the National Comprehensive Cancer Network (NCCN), the American Joint Cancer Committee (AJCC) and the European Society for Medical Oncology (ESMO) define and regularly update the recommended histopathology approach and report [33, 58–63]. In brief the neuroendocrine cancer must be classified and named according to WHO bluebooks published for gut, lung and pancreas. The minimum IHC recommended panel includes chromogranin A, synaptophysin and Ki67 (digestive cancer only). Other tests are allowed when required. Some other morphological features with prognostic impact such as neural and vascular invasion should be described too. The pathology report should comprise the neuroendocrine cancer definition, the grade (gut and pancreas only) and the stage, when possible. The stage should be defined according to WHO 2010 (gut), 2015 (lung) and 2017 (pancreas) and the AJCC 2017 [10, 12, 17].

5 What's next

The future of the morphological definition of neuroendocrine cancer will reproduce the future knowledge of its biology. Despite commonplace, such statement indeed reflects the classification progression of the last decades. While at the beginning of the last century silver impregnation techniques allowed a correct cyto/histological diagnosis, now immunohistochemistry for neuroendocrine markers is the basis for it [64]. The same is now applied for gene products relevant for therapy (e.g. ssts) or prognosis (Ki67, PCNA, P53, DAXX,

ATRX, Rb, etc.). The recent great improvement for patient personalized therapy relied on informative classification tools, improved knowledge of neuroendocrine cancer biology and new or better agents for key molecular targets [65–69]. Two directions at least can be foreseen: one toward the definition of better and more refined prognostic markers to go beyond Ki67 for a molecular grading, and a second one to define markers to predict therapy response for individual patients. While for a more robust prognosticator the challenge is ongoing and fueled by the multiple, literally thousands, of potential genes emerging by high throughput gene investigations [70–73], relatively little is available for markers of therapy response [74–76]. More is expected to be done in both directions.

6 Concluding remarks

From the original description of carcinoid to the current knowledge of neuroendocrine cancer many aspects changed. A new, relatively large family of cancer is now recognized, classified and diagnosed. What has not changed, though, is the cyto/histological essence of the carcinoid and the high grade neuroendocrine cancer that still are recognized as so in the current pathology practice as they used to, based exclusively on morphology.

Compliance with ethical standards

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