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Controversy

Heterogeneity of grade 3 gastroenteropancreatic neuroendocrine carcinomas: New insights and treatment implications

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ABSTRACT

Gastroenteropancreatic (GEP) neuroendocrine neoplasms (NENs) are currently classified as grade (G) 1, G2 and G3, in accordance with the 2010 WHO classification. G1 and G2 are named neuroendocrine tumors (NETs) whereas G3 neuroendocrine carcinomas (NECs). While advanced G1 and G2 are usually treated with several different therapies, including somatostatin analogs, chemotherapy, interferon, molecular targeted agents, peptide receptor radionuclide therapy (PRRT) and liver-directed treatments, advanced G3 NECs are usually treated with a platinum-etoposide chemotherapy, trusting their clinical homogeneity is similar to that of small cell lung cancer.

However, over the last years a number of reports suggested that 2010 WHO G3 GEP NECs are more heterogeneous than expected. Therefore, we critically reviewed the literature about this topic and reported pathological and clinical considerations on 2010 WHO G3 GEP NEC category proposing new sub-categories. Over the last five years, six studies specifically investigating large series of G3 GEP NECs have been published, including around 800 patients. Tumor morphology and Ki-67 Labeling Index (that will be mentioned as Ki-67 in this manuscript) combination has been reported as a tool to define two or even three subgroups of this category with different prognosis and potentially different therapeutic approach. Prospective trials are warranted to investigate if several types of therapy other than the platinum/etoposide chemotherapy can be effective in well differentiated GEP NEN with 21–55% Ki-67 and alkylating-based chemotherapy in poorly differentiated GEP NEN with 21–55% Ki-67.

Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP NENs) comprise two main categories, well differentiated (WD) and poorly differentiated (PD), also called G1/G2 and G3, respectively [1]. Poorly differentiated or G3 NENs represent less than one third of all GEP NENs [2–4] and are usually considered a quite homogeneous category to be treated with a platinum-based chemotherapy [5].

Over the last years an increasing number of publications reported that the G3 GEP NENs category is less homogeneous than expected. Tumor morphology and Ki-67 were defined as prognostic factors that can separate this category in two or even three subgroups with significantly different survival.

Therefore a better characterization of the G3 GEP NENs could have practical therapeutic implications. For instance WD GEP NENs with Ki-67 >20% could be treated differently from PD GEP NENs with Ki-67 >70%.

This manuscript reports the current state of the art about this particular topic and personal view by a medical oncologist and a pathologist specifically dedicated to GEP NENs.

Selection of evidence

Articles for consideration in the present paper were retrieved by a keyword-based MEDLINE search using pertinent keywords (gastrointestinal OR gastric OR intestinal OR pancreatic OR pancreas OR gastroenteropancreatic) AND (neuroendocrine AND (neoplasm OR carcinoma) AND (classification OR "predictive factor" OR "prognostic factor" OR chemotherapy OR grading OR Ki-67 OR proliferative index OR heterogeneity OR morphology). Only papers published in English and for which an abstract was available were considered. The search was last updated on May 16th 2016.

In total, 2342 papers were identified (601 with "gastric neuroendocrine tumors", 540 with "intestinal neuroendocrine tumors"

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and 1201 with "pancreatic neuroendocrine tumors"). Retrieved papers were then filtered selecting observational studies, such as case-control and large cohort studies, prospective clinical trials (any phase), systematic reviews and meta-analyses.

Abstracts from the last five years of major scientific congresses were also browsed. Guidelines from NENs international scientific societies and main oncological societies were also considered.

Duplicate papers, case reports, case series were removed. At the end of this process, 573 papers were selected (107 on gastric NENs, 238 on intestinal NENs and 228 on pancreatic NENs).

Finally, articles specifically addressing GEP NENs with G3 or PD or NEC and heterogeneity were identified. Particularly, retrospective large series with re-classification and correlation with overall survival (OS) were definitely selected.

General part: GEP NECs category

Definition and terminology

Neuroendocrine or endocrine

These terms may be considered synonymous [6]. Endocrine cells origin from the embryonic neural crests. Neuroendocrine neoplasms (NENs) are epithelial neoplasms prevalently composed of cells of endodermal origin [1,7,8]. The term "neuroendocrine" will be preferentially used here.

Neoplasm, tumor and carcinoma

The last 2010 WHO classification recommended the more popular, but not fully-biologically adequate term "Tumor" instead of the biologically-correct "Neoplasm". On the other hand, the European Neuroendocrine Tumor Society (ENETS) recommended using "neoplasm" [9–16] to indicate all neuro-endocrine neoplastic proliferations regardless of their differentiation grade, reserving "tumor" only to WD lesions. Accordingly, the term "neuroendocrine neoplasm" will be used here.

The 2010 WHO classification has restricted the use of the term "carcinoma" to PD NENs, termed neuroendocrine carcinomas (NECs), whereas the term "tumor" is limited to WD NENs, termed neuroendocrine tumors (NETs). In 2010 WHO classification the decision to limit the term carcinoma to G3 neoplasms reflects the idea that grading is the only determinant of tumor prognosis [15,17].

Grade of malignancy

Tumor grading and differentiation are different but complementary concepts. Differentiation is a morphological definition and refers to the degree of resemblance of tumor cells with the normal cell counterpart. Tumor grading is defined by both morphology and proliferative index, [Mitotic Index (MI) and Ki-67, see below].

Based on grading, GEP NENs can be classified into two main categories: WD-NETs, and PD NECs [8,21–23].

Well differentiated NETs comprise neoplastic cells uniform for size and features organized in organoid, trabecular, ribbon or gyriform architecture. They present abundant content of secretory granules responsible for intense and diffuse staining for general neuroendocrine markers (Synaptophysin and Chromogranins). Nuclear chromatin is regular with inconspicuous nucleoli, with no atypia. Mitoses are rare or uncommon.

Poorly differentiated NEC comprising large cell (LC) and small cell (SC) (see Table 2), are neoplasms with pleomorphic and highly atypical nuclei, solid growth pattern and abundant non-ischemic necrosis, arranged to form either "map" or "spot" necrosis. Mitoses are plentiful and often atypical. Criteria to distinguish LC from SC and in general for differential diagnosis between PD-NECs and WD-NETs are listed in Table 2 [7,8,24]. Despite these rules, in clinical practice the morphology-based classification of different categories is sometimes challenging.

Although it has been seldom reported that a WD-NET may progress to PD-NEC [25–27] it should be considered that the intratumor heterogeneity could false that. Collecting specimens from the entire neoplasm to compare PD clones with the WD ones indicated that Ki-67 value is likely different between the two areas [28].

The definition of the tumor grade by means of a biopsy is currently under debate, since the analyses of biopsies provide uniform results only in case of PD-NECs with Ki-67 in 2/3 of cells [29]. The concordance rate increases with the number of Ki-67 positive nuclei (Fig. S1). In neoplasms with Ki-67 below the aforementioned threshold, reproducibility is unpredictable [30,31] in particular in liver metastases [32].

Proliferative index cut-off

Ki-67 is a non-histonic nuclear protein expressed during the S phase of cell replication. In 1996, La Rosa et al. showed that patients with a NET expressing the MIB-1 epitope of Ki-67 in >2% of cells have poorer prognosis compared with NET patients with MIB-1/Ki-67% $\leqslant$2% [33]. This finding was confirmed in other studies [20,34]. The 2008 ENETS guidelines suggested that Ki-67 $\geqslant$30% is associated with PD morphology (MI >20 HPF or Ki-67 >20%) [35]. A 20% threshold was established during the Frascati consensus to define NEC [36,37], and this figure was validated during the validation clinical studies of the 2010 WHO classification [17,38,39].

GEP NEN classification: critical comparison between WHO 2000 and 2010 editions

The 2000 classification [7,8] was based on the identification of the following criteria as major predictors of tumor outcome: tumor differentiation, functionality, size, extent of local invasion, lymphovascular invasion giving three classes as listed in Table 3:

(1) Well Differentiated Endocrine Tumor (WDET).
(2) Well Differentiated Endocrine Carcinoma (WDEC)
(3) Poorly Differentiated Endocrine Carcinoma (PDEC).

The two main clinical implications of this classification are based on the distinction of benign from malignant, mainly from a surgical standpoint, and WD from PD, mostly from a medical perspective. The 2000 WHO Classification considered as uncertain and controversial the prognostic relevance of both mitotic count and cell proliferation (as measured by Ki-67).

The 2010 WHO GEP NEN classification [1] is divided into three groups: NETs G1, NETs G2 and NECs G3. "NET G3" is not advised, worthy in Table 1.03 of the blue book [1] it is clearly reported that NETs are by definition WD tumors. Therefore, according to this note a hypothetical entity characterized by WD morphology with >20% Ki-67 should not be included in the G3 category.

This classification validated an easy score system based on proliferative index as defined by Ki-67 antigen immunoreactivity in the "hot spot" areas presenting the highest number of stained nuclei. It is expressed as the Ki-67 percentage of stained tumor cells after scanning of low-power representative tumor sections Ki-67 and/or MI in 50 high power fields (HPF) [1]. When MI and Ki-67 give divergent results, the highest should be reported [1]. Regarding the "high-grade" category, that was named PDEC in the 2000 and NEC G3 in the 2010 Classifications, small differences between the two classifications exist. Basically, based on the same morphological tools, both classifications would include SC, LC and