REVIEW

Diagnostic work-up of gastroenteropancreatic neuroendocrine tumors

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Neuroendocrine tumors are a heterogeneous group of malignancies that present a diagnostic challenge. The majority of patients (more than 60%) present with metastatic disease at diagnosis. The diagnosis is based on histopathology, imaging, and circulating biomarkers. The histopathology should contain specific neuroendocrine markers such as chromogranin A, synaptophysin, and neuron-specific enolase and also an estimate of the proliferation by Ki-67 (MIB-1). Standard imaging procedures consist of computed tomography or magnetic resonance imaging together with somatostatin receptor scintigraphy. 68Ga-DOTA-octreotate scans will in the future replace somatostatin receptor scintigraphy because they have higher specificity and sensitivity. Other positron imaging tomographic scanning tracers that will come into clinical use are 18F-DOPA and 11C-5HTP. Neuroendocrine tumors secrete many different peptides and amines that can be used as circulating biomarkers. The most useful general marker is chromogranin A, which is both a diagnostic and prognostic marker in most neuroendocrine tumors. However, there is still a need for improved biomarkers for early detection and follow-up of patients during treatment. In addition, molecular imaging can be further developed for both detection and evaluation of treatment.

KEYWORDS: Chromogranin A; 68Ga-DOTATOC; Somatostatin Receptor Scintigraphy; Tumor Node Metastasis Staging; Grading.

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INTRODUCTION

Neuroendocrine tumors (NETs) comprise a heterogeneous group of neoplasms that are frequently metastatic at the time of diagnosis, and distance of metastatic disease is, next to grading, one of the most important prognostic factors (1–3). The availability of modern imaging methods for diagnosis and staging of NETs has improved at the same time as the spectrum of therapeutic options in the management of metastatic disease has increased during recent years. The frequency of metastatic disease varies depending on the type of tumor, but in specialized centres, 80–90% of patients who present with small intestinal NETs (carcinoids) and 60–70% of patients with pancreatic NETs have liver metastases. Histology is the strongest predictor of survival. In the most recent SEER (Surveillance Epidemiology and End Results) database analyses, median survival in distant metastatic disease was 33 months in patients with G1/G2 NETs and only 5 months in patients with poorly differentiated tumors (neuroendocrine carcinoma (NEC) G3) (1). In specialized centres for the treatment of NETs, overall 5-year survival rates for patients with stage IV, pancreatic and small intestinal NETs are much higher than those reported in the SEER database (3). In such centres, 5-year survival rates in patients with metastatic midgut NETs exceed 50% (4,5). In a multivariate analysis of patients with well-differentiated tumors and moderately differentiated NETs from the SEER database, disease stage, primary tumor site, histologic grade, sex, race, age and year at diagnosis were predictors of outcome ($p<0.001$). In my centre, in a multivariate analysis of 354 patients with pancreatic NETs, the prognostic factors were TNM stage, World Health Organization (WHO) classification, Ki67, and radical surgery (6). Liver tumor burden, or number of metastases, tumor slope, extrahepatic disease, co-morbidities and performance status represent additional prognostic parameters (3,7). Retrospective data indicate that circulating chromogranin A (CgA) is of prognostic value; highly elevated levels were associated with limited survival (8,9). Other prognostic markers that are available (e.g., CK19, PTEN, TSC-2 expression in tumor tissue) require further validation (10,11).

DIAGNOSTIC WORK-UP

The initial diagnostic approach in patients with NETs includes histological examination, which is always required before therapeutic decisions are made. Clinicians should also consider performing repetitive biopsies to reassess the prognosis if the disease course changes significantly. The following investigations are also required: (a) immunohistochemical markers and detailed histological analysis; (b) assessment of the primary tumor and the extent of extrahepatic spread by imaging, including patterns of

No potential conflict of interest was reported.

hepatic metastases; and (c) biochemical assessment of functionality and general tumor markers.

HISTOPATHOLOGY

The neuroendocrine signature of a cell is defined by the expression of general and specific neuroendocrine markers. General neuroendocrine markers are observed in all cell types, and include the cytosol antigens neuron-specific enolase (NSE) and protein gene product 9.5 (PGP 9.5), as well as the secretory vesicle antigens of chromogranin family A for large dense core vesicle (LDCV) and synaptophysin for small synaptic vesicles (SSV). Other neuroendocrine tissue markers are the ATP-dependent vesicular monoamine transporter isoforms (VMAT1 and VMAT2), neuroendocrine secretory protein 55 (NESP55), synaptic vesicle protein 2 (SV2) in both LDCVs and SSV, and neural cell adhesion molecule (N-CAM) (12,13).

Immunohistochemical determination of CgA and synaptophysin as well as proliferation marker Ki-67 (MIB-1) is mandatory. In patients with multiple endocrine neoplasia type 1, specific markers such as gastrin, insulin, and pancreatic polypeptide (PP) should be determined. For patients with unknown primary tumors, TTF1 (bronchial/lung), CDX2 (intestinal serotonin-midgut), and PP/Islet-1/Glucagon (pancreatic) can be used to guide the search for the primary tumor (14,15).

CLASSIFICATION

The current WHO 2010 classification introduces the definition ''neoplasm'' to encompass low- to high-grade neuroendocrine tumors (16). At variance with the WHO 2000 classification, the neuroendocrine connotation is enforced, in recognition of the expression of antigens shared with nerve elements. The classification itself uses a common definition frame that is based on grading and specific staging tools. The definitions are NET for the previous ''carcinoid''/well-differentiated endocrine tumor/carcinoma, and neuroendocrine carcinoma (NEC) for the previous small cell/poorly differentiated carcinoma.

TUMOR STAGING AND GRADING

Staging is performed with the familiar tumor node metastasis (TNM) approach according to the anatomical location of the tumors, and this approach is recommended by the WHO, the American Joint Committee on Cancer and the International Union Against Cancer. However, at variance with the European Neuroendocrine Tumor Society proposal, the WHO-approved TNM staging system is conceived for ''carcinoid'' only, and some parameters for the appendix and the pancreas are different (see Table 1) (17–20).

Grading is performed by definition of proliferation using both the mitotic count and the Ki-67 index, as proposed by the European Neuroendocrine Tumor Society. Notably, both the WHO and the American Joint Committee on Cancer endorse such a grading system.

IMAGING

A standard computed tomographic (CT) scan of the chest, abdomen, and pelvis or magnetic resonance image is mandatory, and should be complemented by somatostatin receptor scintigraphy including single photon emission computer tomography (SPECT)-SRS and triphasic CT (21,22). Positron emission tomographic (PET) scanning using 68Ga-somatostatin analogue (68Ga-DOTATOC-PET/CT) is an alternative if available, as it has higher resolution than additional somatostatin receptor scintigraphy.

Table 1 - The American Joint Committee on Cancer (AJCC) and European Neuroendocrine Tumors Society (ENETS) staging classifications for pancreatic neuroendocrine tumors with cross-tabulation of stage distributions.

AJCC Staging Classification		ENETS Staging Classification	
T1	Tumor limited to the pancreas, <2 cm	T1	Tumor limited to the pancreas, <2 cm
T2	Tumor limited to the pancreas, >2 cm	T2	Tumor limited to the pancreas, 2-4 cm
T3	Tumor extends beyond the pancreas, but not involving the celiac axis or SMA	T3	Tumor limited to the pancreas, >4 cm, or invading duodenum or CBD
T4	Tumor involves the celiac axis or SMA	T4	Tumor invades adjacent structures
N0	No regional LN metastasis	N0	No regional LN metastasis
N1	Regional LN metastasis	N1	Regional LN metastasis
M0	No distant metastasis	M0	No distant metastasis
M1	Distant metastasis	M1	Distant metastasis

Stage	T	N	M	Stage	T	N	M
IA	T1	N0	M0	I	T1	N0	M0
IB	T2	N0	M0	IIA	T2	N0	M0
IIA	T3	N0	M0	IIB	T3	N0	M0
IIB	T1-3	N1	M0	IIIA	T4	N0	M0
III	T4	N0-1	M0	IIIB	Any T	N1	M0
IV	Any T	Any N	M1	IV	Any T	Any N	M1

	ENETS I	ENETS II	ENETS III	ENETS IV
AJCC I	25	59	0	0
AJCC II	0	4	37	0
AJCC III	0	0	18	0
AJCC IV	0	0	0	282

CBD = common bile duct; LN = lymph node; SMA = superior mesenteric artery.

68Ga-DOTATOC-PET/CT may help to identify the primary tumor, and is a reliable method for early detection of bone metastases in patients with NETs (23–25). For the detection of small pancreatic NETs, endoscopical ultrasonography seems superior to PET/CT (26). In general, PET should be replaced by PET/CT; 18F-DOPA PET/CT and 5-HTP-PET/CT are promising diagnostic tools, and may be considered if they are available and if somatostatin receptor imaging is negative (27,28). However, their use in the standard work-up may not be recommended at this time. Although recent studies indicate a prognostic value of FDG-PET in well-differentiated NETs, it is not recommended as a routine imaging method (29). Further studies are needed to support its role as a prognostic tool. In special situations, for example if liver transplantation is being considered, FDG-PET/CT can be considered for G2 tumors as well as 18F-DOPA-PET/CT or 5HTP-PET/CT if available. Investigation of the large bowel may be useful, either by means of a colonoscopy plus ileoscopy, or by means of a colon CT with a neutral enema. In a retrospective analysis of 123 patients with NET liver metastases of unknown primary, in 47% the primary tumor was localized in the small or large intestine by lower endoscopy (30). If the primary tumor is suspected to be in the small intestine, double balloon enteroscopy or video capsule endoscopy may be performed if available and considered necessary for the therapeutic management. If the CT study of liver metastases is inconclusive, T2-weighted thin-slice dynamic gadolinium-enhanced magnetic resonance imaging, or, if available, contrast-enhanced ultrasonography, should be performed. Magnetic resonance imaging is considered superior to CT in the detection and follow-up of liver metastases, and is a preferable choice in clinical trials (21).

The imaging report should include segmental information on the distribution of liver metastases. Although there are no standardized imaging techniques to reliably measure liver tumor burden, an experienced radiologist can use such reports to estimate the percentage of liver tumor involved.

CIRCULATING MARKERS IN CLINICAL PRACTICE

The minimal biochemical work-up for NETs includes circulating chromogranin A and assessment of a specific marker to assess functionality, such as urinary 5-HIAA evaluation in carcinoid syndrome. Additional assessment of insulin, C-peptide, (proinsulin), gastrin, pancreatic polypeptide, vasoactive intestinal peptide, glucagon, and calcitonin should be useful, depending on the functional status of the tumor, clinical symptoms, and histological features.

Chromogranins are a family of glycoproteins found in many hormone-producing organs, and early on they were discovered to be elevated in the plasma of patients with endocrine tumors. Plasma chromogranin A (CgA) has been reported to be a prognostic biomarker in GEP-NETs, correlating with hepatic tumor burden and with shorter survival. In the setting of radically operated midgut carcinoids, elevation of CgA has been reported to be both a diagnostic marker and an early marker of recurrent disease. A decrease in CgA levels has been used as a marker of response to treatment in clinical trials, for which biochemical response usually is defined as a $\geq$50% reduction of CgA (31–33). The combination of CgA levels and levels of N-terminal pro-brain natriuretic peptide (Nt-proBNP) correlate significantly with carcinoid heart disease (i.e., right-sided heart failure due to tricuspid regurgitation and/or pulmonic stenosis because of valve fibrosis (probably) caused by elevated circulating serotonin) (34).

There are, however, several limitations to the use of CgA as a biomarker in GEP-NETs. Treatment with proton-pump inhibitors can cause a secondary increase in CgA as a result of the increased gastrin production. Chronic atrophic gastritis may also cause elevated CgA. Impaired renal function may cause accumulation of the peptide, which also results in falsely elevated levels. Many patients with midgut carcinoids are initially misdiagnosed with irritable bowel syndrome, sometimes several years before the correct diagnosis is made (35). There have been reports of elevated CgA in irritable bowel syndrome and in inflammatory bowel disease. CgA is thus not of value as a screening test in the evaluation of unclear diarrhea.

There has been impressive progress in the field of biomarkers as well as molecular imaging. However, we still need more sensitive markers for early detection and follow-up. Furthermore, new markers delineating sensitivity to various therapies are warranted. Molecular imaging is in its early stages and has the potential to be a significant tool in the management of patients with NETs.

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