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
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 |
| IA    | T2 | N0 | M0 | IIA  | T2 | N0 | M0 |
| IA    | T3 | N0 | M0 | IIIB | T3 | N0 | M0 |
| IA    | T4 | N0-1 | M0 | IIIA | T4 | N0 | 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         | 282       |

CBD = common bile duct; LN = lymph node; SMA = superior mesenteric artery.
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, (pro)insulin, 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 ≥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 gastric production. Chronic atrophic gastritis may also cause a secondary elevation of 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.

REFERENCES

1. Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008;26(18):3063-72.
2. Garcia-Carbonero R, Capdevila J, Crespo-Herrero G, Diaz-Perez JA, Martinez Del Prado MP, Alonso Orduña V, et al. Incidence, patterns of care, and prognostic factors for outcome of gastroenteropancreatic neuroendocrine tumors (GEP-NETs): results from the National Cancer Registry of Spain (RCETNE). Ann Oncol. 2010;21(9):1794-803, http://dx.doi.org/10.1093/annonc/mdq222.
3. Pape UF, Berndt U, Muller-Nordhorn J, Bohmig M, Roll S, Koch M, et al. Prognostic factors of long-term outcome in gastroenteropancreatic neuroendocrine tumours. Endocr Relat Cancer. 2008;15(4):1083-97, http://dx.doi.org/10.1677/ERC-08-0017.
4. Strosberg J, Gardner N, Kvol L. Survival and prognostic factor analysis of 147 metastatic neuroendocrine tumors of the mid-gut. Neuroendocrinology. 2009;89(4):471-6, http://dx.doi.org/10.1159/000197899.
5. Ahmed A, Turner G, King B, Jones L, Culliford D, McCance D, et al. Midgut neuroendocrine tumours with liver metastases: results of the UKNETS study. Endocr Relat Cancer. 2009;16(3):805-9, http://dx.doi.org/10.1677/ERC-09-0042.
6. Ekeblad S, Skogseid B, Dunder K, Oberg K, Eriksson B. Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution. Clin Cancer Res. 2008;14(23):7986-903, http://dx.doi.org/10.1158/1078-0432.CCR-08-0734.
7. Durante C, Boukheris H, Dromain C, Vuillard P, Leboulleux S, Elias D, et al. Prognostic factors influencing survival from metastatic (stage IV) gastroenteropancreatic well-differentiated endocrine carcinoma. Endocr Relat Cancer. 2009;16(2):585-97, http://dx.doi.org/10.1677/ERC-08-0301.
8. Janson ET, Holmberg L, Stridsberg M, Eriksson B, Theodorsson E, Wilander E, et al. Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. Ann Oncol. 1997;8(7):865-90, http://dx.doi.org/10.1093/annonc/3a1021.
9. Modlin IM, Gustafsson BI, Moss SF, Pavel M, Tsoiakis AV, Kidd M. Chromogranin A—biological function and clinical utility in neuroendocrine tumor disease. Ann Surg Oncol. 2010;17(9):2427-34, http://dx.doi.org/10.1245/s10434-010-1006-3.
10. Jain R, Fischer S, Serra S, Chetty R. The use of Cytokeratin 19 (CK19) immunohistochemistry in lesions of the pancreas, gastrointestinal tract, and liver. Appl Immunohistochem Mol Morphol. 2010;18(1):9-15.
11. Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, et al. Prognostic factors influencing survival from metastatic (stage IV) gastroenteropancreatic neuroendocrine tumours of the mid-gut. Endocr Relat Cancer. 2008;15(4):1083-97, http://dx.doi.org/10.1677/ERC-08-0301.
12. Rindi G, Villanacci V, Ubaldi A, Scarpa A. Endocrine tumors of the digestive tract and pancreas: histogenesis, diagnosis and molecular basis. Expert Rev Mol Diagn. 2001;1(3):323-33, http://dx.doi.org/10.1586/14737591.1.3.323.
13. Solcia E, Rindi G, Capella C. Histochemistry in pathology. In: Felipe MI, Lake BD, editors. Edinburgh, London, New York: Churchill-Livingstone; 1990:p.397-409.

14. Anlauf M, Garbrecht N, Baurersfeld J, Schmitt A, Henropp T, Komminoth P, et al. Hereditary neuroendocrine tumors of the gastroenteropancreatic system. Virchows Arch. 2007;451(Suppl 1):S29-38, http://dx.doi.org/10.1007/s00428-007-0450-3.

15. Srivastava A, Hornick JL. Immunohistochemical staining for CDX-2, PDX-1, NISP-55, and TTF-1 can help distinguish gastrointestinal carcinoid tumors from pancreatic endocrine and pulmonary carcinoid tumors. Am J Surg Pathol. 2009;33(4):462-32.

16. Rindi G, et al. World Health Organization classification of tumours, pathology and genetics of tumours of the digestive system. In: Bosman FT, Carneiro F, editors. Lyon: IARC Press; 2010.9-2 p.

17. Rindi G, Kloppe1 G, Allman H, Caplin M, Couvelard A, de Herder WW, et al. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch. 2006;449(4):395-401, http://dx.doi.org/10.1007/s00428-006-0250-1.

18. Rindi G, Kloppe1 G, Couvelard A, Komminoth P, Korner M, Lopes JM, et al. TNM staging of midgut and hindgut (neuro)endocrine tumors: a consensus proposal including a grading system. Virchows Arch. 2007;451(1):757-62, http://dx.doi.org/10.1007/s00428-007-0452-1.

19. Sobin LH, Gospodarowicz M, Wittekind C. UICC: TNM classification of malignant tumours. 7th ed. Oxford: Wiley-Blackwell; 2009.

20. Strosberg JR, Cheema A, Webster J, Han G, Coppola D, Kvols LK. Prognostic validity of a novel American Joint Committee on Cancer Staging Classification for pancreatic neuroendocrine tumors. J Clin Oncol. 2011;29(22):3044-9, http://dx.doi.org/10.1200/JCO.2011.35.1817.

21. Sundin A, Vullierme MP, Kaltsas G, Plockinger U. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: a consensus proposal including a grading system. Virchows Arch. 2006;451(4):757-62, http://dx.doi.org/10.1007/s00428-007-0452-1.

22. Perri M, Erba P, Volterrani D, Lazzeri E, Boni G, Grosso M, et al. Octreoscan: a universal imaging technique for neuroendocrine tumors: as a universal imaging technique for neuroendocrine tumors. Acta Oncol. 1989;28(3):325-9, http://dx.doi.org/10.3109/02841868909111201.

23. Arnold R, Wilke A, Rinkas J, Mayer C, Kann PH, Oberg K, et al. Chromogranins—new sensitive markers for neuroendocrine tumors. Acta Oncol. 1989;28(3):325-9, http://dx.doi.org/10.3109/02841868909111201.

24. Welin S, Stridsberg M, Cunningham J, Granberg D, Skogstedt C, et al. Improved staging of patients with carcinoid and islet cell tumors with 18F-dihydroxy-phenyl-alanine and 11C-5-hydroxy-tryptophan positron emission tomography. J Clin Oncol. 2008;26(9):1489-95, http://dx.doi.org/10.1200/JCO.2007.15.1126.

25. Korse CM, Taal BG, de Groot CA, Bakker RH, Bonfrer JM. Chromogranin-A and N-terminal pro-brain natriuretic peptide: an excellent pair of biomarkers for diagnostics in patients with neuroendocrine tumors. Clin Cancer Res. 2008;14(11):3260-7, http://dx.doi.org/10.1158/1078-0432.CCR-07-2476.

26. Wang SC, Parekh JR, Zuraek MB, Bergland EK, Warren RS, et al. Identification of unknown primary tumors in patients with neuroendocrine tumors. Clin Cancer Res. 2010;16(3):978-85, http://dx.doi.org/10.1158/1078-0432.CCR-09-1759.

27. Orlefors H, Sundin A, Garske U, Juhlin C, Oberg K, Skogseid B, et al. Elevated plasma chromogranin A is the first indication of recurrence in radically operated midgut carcinoid tumors. Neuroendocrinology. 2008;6(7):820-7, http://dx.doi.org/10.1016/j.cgh.2008.02.052.

28. Orlefors H, Sundin A, Garske U, Juhlin C, Oberg K, Skogseid B, et al. Plasma chromogranin A and pro-brain natriuretic peptide as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. J Clin Endocrinol Metab. 2005;90(6):3392-400, http://dx.doi.org/10.1210/jc.2004-1938.

29. Wang SC, Parekh JR, Zuraek MB, Bergland EK, Warren RS, et al. Elevated plasma chromogranin A and pro-brain natriuretic peptide as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. J Clin Endocrinol Metab. 2005;90(6):3392-400, http://dx.doi.org/10.1210/jc.2004-1938.

30. Wang SC, Parekh JR, Zuraek MB, Bergland EK, Warren RS, et al. Elevated plasma chromogranin A and pro-brain natriuretic peptide as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. J Clin Endocrinol Metab. 2005;90(6):3392-400, http://dx.doi.org/10.1210/jc.2004-1938.

31. Wehn S, Stridsberg M, Cunningham J, Granberg D, Skogstedt C, et al. Plasma chromogranin A as marker for survival in patients with metastatic endocrine gastroenteropancreatic tumors. Clin Gastroenterol Hepatol. 2008;6(7):820-7, http://dx.doi.org/10.1016/j.cgh.2008.02.052.

32. Prasad V, Ambrosini V, Hommann M, Hoesch D, Fasti S, Baum RP. Detection of unknown primary neuroendocrine tumours (CUP-NET) using (68)Ga-DOTA-NOC receptor PET/CT. Eur J Nucl Med Mol Imaging. 2010;37(1):67-77, http://dx.doi.org/10.1007/s00259-009-0305-3.

33. Putzer D, Gabriel M, Henninger B, Kendler D, Upritchy C, Dobrozensky G, et al. Bone metastases in patients with neuroendocrine tumor: (68)Ga-DOTA-Tyr3-octreotide PET in comparison to CT and bone scintigraphy. J Nucl Med. 2009;50(8):1214-21, http://dx.doi.org/10.2967/jnumed.108.060236.

34. Gabriel M, Decristofo1 C, Kendler D, Dobrozensky G, Heute D, Upritchy C, et al. (68)Ga-DOTA-Tyr3-octreotide PET in neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and CT. J Nucl Med. 2007;48(4):508-18, http://dx.doi.org/10.2967/jnumed.106.035667.

35. Andrzejewska A, Thorsen BE, Hommerstad H, Tvermoen K, et al. Natural course of small, asymptomatic neuroendocrine pancreatic tumours in multiple endocrine neoplasia type 1: an endoscopic ultrasound imaging study. Endocr Relat Cancer. 2006;13(4):1195-202, http://dx.doi.org/10.1677/erc.1.01220.

36. Orlefors H, Sundin A, Garske U, Juhlin C, Oberg K, Skogseid B, et al. Whole-body (11)C-5-hydroxytryptophan positron emission tomography as a universal imaging technique for neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and computed tomography. J Clin Endocrinol Metab. 2005;90(6):3392-400, http://dx.doi.org/10.1210/jc.2004-1938.

37. Koopmans KA, Neels OC, Kema IP, Elinga PH, Sluiter WJ, Vanghi11lewe K, et al. Improved staging of patients with carcinoid and islet cell tumors using (68)Ga-DOTA-Tyr3-octreotide PET/CT. J Clin Oncol. 2008;26(9):1489-95, http://dx.doi.org/10.1200/JCO.2007.15.1126.