Current Practices and Novel Techniques in the Diagnosis and Management of Neuroendocrine Tumors of Unknown Primary

Andrew E. Hendifar, MD,* Robert A. Ramirez, DO, FACP,† Lowell B. Anthony, MD, FACP,‡ and Eric Liu, MD, FACS§

Abstract: Neuroendocrine tumors (NETs) comprise a heterogeneous group of neoplasms in which tumor staging/prognosis and response to treatments depend heavily on accurate and timely identification of the anatomic primary site or NET subtype. Despite recent technological advancements and use of multiple diagnostic modalities, 10% to 14% of newly diagnosed NETs are not fully characterized based on subtype or anatomic primary site. Inability to fully characterize NETs of unknown primary may cause delays in surgical intervention and limit potential treatment options. To address this unmet need, clinical validity and utility are being demonstrated for novel approaches that improve NET subtype or anatomic primary site identification. Functional imaging using 68Ga-radiolabeled DOTATATE positron emission tomography/computed tomography/magnetic resonance imaging. Using a genomic approach, molecular tumor classification based on differential gene expression has demonstrated high diagnostic accuracy in blinded validation studies of different NET types and subtypes. Given the widespread availability of these technologies, we propose an algorithm for the workup of NETs of unknown primary that integrates these approaches. Including these technologies in the standard workup will lead to better NET subtype identification and improved treatment optimization for patients.

Key Words: neuroendocrine tumors, gene expression profiling, 68Ga-DOTATATE, neoplasms, unknown primary

Neuroendocrine neoplasms comprise a broad, heterogeneous group of tumors that develop from hormone- or neuropeptide-producing cells that function to regulate various physiologic and homeostatic processes. These tumors are diagnosed in approximately 5.25/100,000 people in the United States and may occur at slightly higher frequencies among African Americans (6.50/100,000) than among whites (4.44/100,000). Neuroendocrine neoplasms can vary widely in their secretion of vasoactive substances, histological appearance, malignancy, and prognosis. Well-differentiated (grade 1 and some grade 2) neuroendocrine tumors (NETs) often progress slowly, can secrete hormones that manifest as a set of nonspecific clinical symptoms, and are frequently diagnosed after metastasis to the liver. In contrast, high-grade neuroendocrine carcinomas (NECs) are often more aggressive and associated with a worse prognosis. Partly because of their lack of differentiation, NECs are less likely to secrete vasoactive substances and patients are often diagnosed as having widespread metastatic disease without first presenting with hormone-related symptoms.

Neuroendocrine cells are an important cellular component of multiple organ systems, such as the pulmonary, gastrointestinal, and pancreaticobiliary tracts; therefore, tumors that initiate from neuroendocrine cells can be found in a wide variety of organ systems. Anatomic sites of primary neuroendocrine neoplasms include, but are not limited to, the lung, stomach, jejunum/ileum, pancreas, skin, and at least 7 other anatomic sites (Fig. 1). However, for 10% to 14% of cancers with a confirmed histological diagnosis of neuroendocrine origin, the anatomic primary site remains unknown after standard-of-care diagnostic workup. These diagnostically challenging tumors, which may be referred to as NET of unknown primary, or NET-UPs, typically present as advanced, nonfunctional, or poorly differentiated neoplasms, and treatment options may be limited for patients whose NET-UP cannot be further subtyped based on the anatomic primary site.

An accurate identification of the NET-UP subtype based on the anatomic primary site can have an immediate impact on patient management by directing further diagnostic imaging of potential metastatic sites, informing the optimal surgical procedure, and the appropriateness of targeted drug therapy. Moreover, because most targeted therapies are approved only for specific NET subtypes, insurance coverage for these targeted medicines can be hampered without knowledge of the subtype. This review focuses on the unmet need for accurate NET-UP subtype identification based on the anatomic primary site. A critical review of the current standard workup for NET-UP diagnosis is provided, followed by a discussion of novel imaging and molecular technologies that provide additional information to better characterize the neuroendocrine subtype.
Well-differentiated NETs often secrete hormones or vasoactive substances that the patient may have a functional neuroendocrine neoplasm. Nocturnal diarrhea, and cardiac effects is frequently the first indication though not all NET subtypes have been clinically validated. Diagnostic accuracy of NET subtype classification by gene expression profiling is reported to be 95% or greater, although not all NET subtypes have been clinically validated.

CURRENT APPROACHES TO NET SUBTYPE IDENTIFICATION

Multimodal diagnostic methods are often used to identify and further characterize neuroendocrine neoplasms, with the specific sequence of techniques depending on the clinical presentation. The standard-of-care diagnostic workup typically includes a detailed history and physical examination, laboratory assays for serum biomarkers, diagnostic imaging, and pathology examination of the tumor tissue. Once a diagnosis of a neuroendocrine neoplasm is confirmed through this multidisciplinary process, additional resolution may be necessary to confidently identify the neuroendocrine subtype. Gene expression profiling provides a molecular approach based on tumor biology for NET subtype classification. Diagnostic accuracy of NET subtype classification by gene expression profiling is reported to be 95% or greater, although not all NET subtypes have been clinically validated.

Biochemical Testing

The onset of hormone-related symptoms such as flushing, nocturnal diarrhea, and cardiac effects is frequently the first indication that the patient may have a functional neuroendocrine neoplasm. Well-differentiated NETs often secrete hormones or vasoactive substances that can be measured by serum or urinary analysis, with the elevated hormone pattern suggestive of a particular NET subtype. Testing for 5-hydroxyindoleacetic acid is usually recommended regardless of the suspected subtype, with additional tests done according to clinical presentation. Plasma chromogranin A and serotonin and urinary or plasma 5-hydroxyindoleacetic acid are often elevated in several NET subtypes (eg, well-differentiated NET, pancreas, and thyroid medullary carcinoma). Other secreted hormones, such as glucagon, gastrin, pancreatic polypeptide, or insulin, may point toward specific NET subtypes. The presence of secreted hormones alone, however, is not considered diagnostic of any particular subtype.

Pathology Evaluation

A complete pathological workup, including histomorphological examination of tumor tissue obtained by surgical resection or less invasive means (eg, needle-guided biopsy or fine needle aspiration), is important for establishing the NET diagnosis. In the context of well-differentiated tumors, histomorphology often provides sufficient resolution to distinguish neuroendocrine neoplasms from other tumors and to further characterize the histology as either small cell or large cell, if applicable. Proliferative markers, such as mitotic count and Ki-67, are also used for grading and may inform prognosis and treatment decisions. However, nonstandard methodology for determining mitotic count and Ki-67 and incomplete characterization of the anatomic primary site may introduce uncertainty that affects grading and staging.

With the exception of the neuroendocrine markers chromogranin A or synaptophysin, immunohistochemical may have limited diagnostic utility based on the tumor's differentiation state and expression of lineage-specific with ranges of specificity that may stress the management of tissue for additional biomarker studies that may follow. Examples of lineage-specific protein markers include TTF-1 that is expressed in 30% to 70% of well-differentiated lung NETs but is also expressed in >40% of metastatic small cell NECs that are not of lung origin. Lack of marker specificity may limit the utility of TTF-1 in a high-grade NET-UP at a distant metastatic site. Other immunohistochemical markers such as CDX2, ISL1, and PDX1 also lack the necessary specificity for accurate subtype identification, with CDX2 positivity indicating a possible intestinal, pancreatic, lung, or ovarian origin. Positive staining for ISL1, PDX1, PAX8, and PAX6 is observed in 45% to 70% of pancreatic neuroendocrine neoplasms, but immune-reactive cells may also point toward a rectal, appendiceal, lung, or ileal origin.

Attempts to identify the subtype by immunohistochemistry should be made using a rational number of immunohistochemical stains. No evidence-based immunohistochemical panel is highly specific to a neuroendocrine subtype, and pathology testing should always be integrated with the clinical presentation and supported by other diagnostic information. Given the movement toward smaller biopsies, tissue management strategies should be used when evaluating NET-UPs. Attempts should be made to find alternative methods to identify the neuroendocrine subtype, such as gene expression profiling that can identify NET subtype in small biopsies and cytology specimens before attempting numerous immunohistochemical panels that may exhaust the biospecimen and are unlikely to increase the diagnostic accuracy.

Imaging Techniques

Diagnostic imaging plays multiple roles in the management of NETs, including the initial diagnosis of malignancy, subtype characterization by identifying the anatomic primary site, and assessing the extent of disease. Often occurring in parallel with pathology evaluation, diagnostic imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, endoscopy, somatostatin receptor (SR) scintigraphy (SRS; eg, OctreoScan, Mallinckrodt Nuclear Medicine LLC, Maryland Heights, Mo), and 68Ga–positron emission tomography (PET)/CT are chosen according to clinical presentation and the known or suspected neuroendocrine subtype. The sensitivity of each method varies based on the anatomic site under interrogation, disease stage, and sites of other potential metastases. Frequently, multiple techniques are used in the workup to identify the
neuroendocrine subtype by locating the anatomic primary site, a practice that may increase overall costs and delay the initiation of targeted therapy until the neuroendocrine subtype is fully characterized. A diagnostic imaging plan to identify the neuroendocrine subtype often starts with CT, followed by MRI if liver metastasis is suspected, and then functional imaging in tumors with SR expression. Endoscopy and endoscopic ultrasound can be effective at identifying NETs originating from the gastrointestinal tract. For localized and SR-positive tumors, first-generation imaging methods such as scintigraphy may be sufficient to determine the subtype based on the anatomic primary site to guide subsequent treatment. However, the dependence on a functional SR restricts these scans to well-differentiated tumors that express SR. Furthermore, the spatial resolution of scintigraphy limits the utility of this imaging approach to tumors larger than 1 cm.

\[68\text{Ga-PET/CT}\] is a relatively new imaging technology that has rapidly become standard of care for NET diagnosis. Similar to SRS, \[68\text{Ga-PET/CT}\] also depends on SR expression; however, the increased resolution of PET/CT enables the detection of tumors <1 cm. \[68\text{Ga-PET/CT}\] has demonstrated greater sensitivity in detecting anatomic primary site compared with SRS (100% vs 85%, respectively) and in detecting positive lesions (including in the pancreas, liver, bowel, lung, abdomen, and bone) compared with CT/MRI (95% vs 45%) and single-photon emission CT/CT (31%). Based on a number of studies, \[68\text{Ga-PET/CT}\] seems to have an overall sensitivity of ~60% for identifying the anatomic primary site.

Even in cases where the anatomic primary site was not identified, \[68\text{Ga-PET/CT}\] provided additional resolution to locate previously undetected metastases.

Although \[68\text{Ga-PET/CT}\] is an improvement over SRS, the dependence on functional SR expression limits the ability to detect poorly differentiated tumors and tumors of midgut origin (including in the pancreas, liver, bowel, lung, abdomen, and bone) compared with CT/MRI (95% vs 45%) and single-photon emission CT/CT (31%). Based on a number of studies, \[68\text{Ga-PET/CT}\] seems to have an overall sensitivity of ~60% for identifying the anatomic primary site.

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**CLINICAL IMPACT OF A NET-UP DIAGNOSIS**

Because multidisciplinary approaches are common for the management of neuroendocrine neoplasms, a diagnosis of NET-UP can negatively impact patients throughout the continuum of care. In the absence of timely and accurate characterization of the neuroendocrine subtype, patients with NET-UPs may undergo diagnostic odysseys that subject them to multiple radiologic imaging tests, the possibility of more invasive surgery, and treatment plans that may not include new targeted therapies that are standard of care based on the subtype or anatomic primary site.

**NET-UP Impact on Surgical Care**

Specialized surgery optimized for individual NET subtypes is becoming standard of care, with the precise surgical plan and procedure dictated by the known or suspected anatomic primary site. For example, the surgical approach for pancreatic neuroendocrine neoplasms depends on the tumor's differentiation state, with well-differentiated, functional pancreatic NETs requiring stabilization of hormone levels before surgery, whereas nonfunctional pancreatic NETs that are small (<1 cm) may be better suited for monitoring without immediate surgery. Even within different types of functional pancreatic NETs, a splenectomy should be performed in patients with gastrinomas, whereas an insulinoma is likely to undergo enucleation. For some tumors, such as pheochromocytoma or medullary thyroid cancer, radiation therapy in addition to (or in lieu of) surgery may be warranted. In patients with small bowel NETs, a right hemicolectomy with node dissection is appropriate for patients with a primary tumor in the cecum, whereas an ileal tumor may require resection with node dissection and full bowel examination.

Whereas the neuroendocrine subtype provides the anatomic primary site that may inform the surgical plan and location, tumor size and nodal status can help determine the extent of surgery necessary to achieve clinical benefit. For example, surgery for an appendiceal tumor >2 cm may involve right hemicolectomy with node dissection, whereas a smaller tumor on the appendix is likely to require excision only. In the case of a small, nonfunctional pancreatic NET, diligent monitoring rather than excision leads to good outcomes, but a high-grade pancreatic NET with nodal involvement may require aggressive surgery with negative margins.

Poorly differentiated NECs, nonfunctional tumors, or tumors below a certain size (<5 mm) that cannot be further characterized at the subtype level by histology or imaging pose a challenge to surgical management. Before surgery, careful palpation or ultrasound may be necessary to localize the tumor. In addition, correct identification of the anatomic primary site can be complicated in cases where the areas of metastases are much larger than the suspected primary tumor type. Based on the suspected subtype and anatomic site of any metastasis, the surgeon may attempt multiple incisions, with the goal of characterizing all aspects of the malignancy in one operation. For example, in a patient with a lung lesion and liver metastasis, reliable evidence for a lung primary will preclude the need to search for a gastrointestinal primary. However, if evidence points toward a pancreatic or gastrointestinal primary, the surgeon may need to explore several anatomic locations and, if possible, depending on the risk of postsurgical complications, work to remove the primary tumor.

Studies have shown that aggressive surgery to remove the primary tumor and cytoreduce metastases can lower hormone load, manage morbidity due to mechanical obstruction, and slow disease progression. In more advanced disease, diffuse metastases or spread of the tumor to the mesenteric blood vessels can complicate surgery; this underscores the need to plan the surgical intervention using all of the available clinical and diagnostic information on neuroendocrine subtype. The benefit of aggressive cytoreduction is supported by a recent retrospective analysis of 834 patients who were surgically and/or clinically managed at a single center of excellence. In this large study, removal of the primary tumor and cytoreduction of as much of the metastasis as possible was associated with decreased morbidity/mortality and a significant increase in overall survival compared with patients who had a smaller percentage of their tumor burden reduced. When cytoreduction is indicated, the surgery is best performed by a surgeon experienced in NETs.

**Medical Treatment**

As with surgery, optimal medical treatment is based on knowledge of the neuroendocrine subtype. Octreotide and lanreotide are 2 somatostatin analogs approved to treat SR-positive metastatic gastrointestinal NETs (octreotide) and locally advanced or metastatic gastrointestinal or pancreatic NETs (lanreotide). Neither drug is approved for lung NETs, although treatment guidelines list both as options for systemic therapy in SR-positive lung and thymus NETs (the role for somatostatin analogs in treating other lung NETs is unclear). Lung, thymus, and midgut tumors from the gastrointestinal tract may respond to peptide receptor radionuclide therapy with \[\text{177Lu-DOTATATE}\]. Somatostatin analogs can be used in pancreatic NETs to control
symptoms related to hormone overproduction, but they do not seem to reduce tumor size, and patient response may lessen over time. Radiotherapy using $^{131}$I-metaiodobenzylguanidine has been shown to stabilize disease and prolong survival in adrenal and thyroid NETs due to drug uptake by the specialized catecholamine plasma membrane and vesicular transporter system.56

Several targeted therapies are approved for different neuroendocrine subtypes. The mTOR inhibitor everolimus and tyrosine kinase inhibitor sunitinib are approved first-line treatment of progressive pancreatic NETs.57,58 Because these targeted agents act primarily by inhibiting angiogenesis,59 they may be particularly effective in slowing disease progression of highly angiogenic pancreatic tumors. Everolimus is also approved for unresectable, locally advanced, or metastatic well-differentiated gastrointestinal and lung NETs.58 For medullary thyroid carcinoma, the pan–tyrosine kinase inhibitor vandetanib was shown to have a significant impact on progression-free survival.60 Immunotherapy using checkpoint inhibitors has shown benefit in small cell lung cancer in the refractory setting61 and Merkel cell carcinoma.62

For some clinical presentations such as large tumors or extensive metastases, or in cases of high-grade tumors with a high proliferative rate, cytotoxic chemotherapy specific to the neuroendocrine subtype may be the recommended treatment. For example, whereas there may be some benefit with temozolomide in advanced bronchopulmonary and thymic NETs, there seem to be few, if any, benefits of cytotoxic chemotherapies or platinum-based treatments in patients with advanced gastrointestinal NETs.16,63 Cisplatin and etoposide may be appropriate for extrapulmonary

| TABLE 1. Medical Treatments for Advanced NETs Based on Subtype53 |
|------------------------|------------------------|------------------------|------------------------|
| NET Subtype            | Well-Differentiated GI NET | Pancreatic NET | Well-Differentiated Lung NET | Thyroid Medullary NET | SCLC |
| Therapy                | • Somatostatin analog    | • Somatostatin analog | • Somatostatin analog | • Vandetanib | • Platinum-based chemotherapy + etoposide with/without atezolizumab |
|                        | • Everolimus             | • Everolimus        | • Everolimus             |                  |                  |
|                        | • Lu$^{177}$-DOTATATE    | • Lu$^{177}$-DOTATATE| • Capecitabine + temozolomide |                  |                  |
| GI indicates gastrointestinal; $^{177}$Lu, $^{177}$lutetium; SCLC, small cell lung cancer; SSA, somatostatin analog.

FIGURE 2. Diagnostic algorithm of NET-UPs. The integration of clinical findings, biochemical testing, imaging results, and pathology can identify the NET subtype based on the anatomic primary site in 85% of cases, leading to subtype-specific treatment and better outcomes.66 Ga-radiolabeled DOTATATE PET/CT ($^{68}$Ga-PET/CT) may be considered for patients who have clinical or biochemical evidence of a NET but negative $^{68}$Ga-PET/CT results. Biopsy tissue should be conserved for molecular testing by the 92-gene assay in patients who have clinical evidence of a NET but negative $^{68}$Ga-PET/CT results. Furthermore, in cases of limited biopsy tissue such that complete pathology characterization is not possible, or for poorly differentiated tumors that are not amenable to functional imaging, molecular tumor classification by the 92-gene assay provides a molecular determinant of NET tumor type and subtype that can inform treatment decisions. 5-HIAA, 5-hydroxyindoleacetic acid; FNA, fine needle aspirate; IHC, immunohistochemistry.
TABLE 2. Performance of the 92-Gene Assay in the Identification of NET Subtype

| NET Subtype                        | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value |
|-----------------------------------|-------------|-------------|---------------------------|---------------------------|
| Gastrointestinal carcinoid (n = 12)| 1.00        | 1.00        | 1.00                      | 1.00                      |
| Lung carcinoid (n = 11)           | 0.91        | 1.00        | 0.98                      | 0.98                      |
| Pancreatic islet cell carcinoma (n = 10) | 0.80        | 0.98        | 0.89                      | 0.97                      |
| Merkel cell carcinoma (n = 10)    | 1.00        | 0.97        | 0.83                      | 1.00                      |
| Small/large cell lung carcinoma (n = 11) | 0.91        | 1.00        | 1.00                      | 0.98                      |
| Thyroid medullary (n = 11)        | 1.00        | 1.00        | 1.00                      | 1.00                      |
| Adrenal-pheochromocytoma (n = 10) | 1.00        | 1.00        | 1.00                      | 1.00                      |

EMERGING DIAGNOSTIC TECHNOLOGIES

New methods that accurately identify the neuroendocrine subtype in a timely and cost-effective manner may improve outcomes for the 10% to 14% of patients with NET-UPs. One novel diagnostic technique, molecular tumor classification by gene expression profiling, may fill this unmet need. A new algorithm that integrates this novel approach into the standard diagnostic workup for NET-UPs is shown in Figure 2.

The biology of NETs is likely influenced by the anatomic primary site in which the initial neoplastic events take place, such that a well-differentiated NET of the gastrointestinal tract has a distinct biology from a well-differentiated NET originating from the lung. Therefore, interrogation of tumor biology by gene expression profiling provides a molecular approach to classify NETs into subtypes that segregate with the anatomic primary site. Gene expression-based molecular cancer classifiers predict tumor types and subtypes based on comparison of sample expression profiles to a database of gene expression profiles from known tumors. Currently, there are 2 clinically available molecular tumor classifiers based on differential gene expression. In one assay (Tissue of Origin, Cancer Genetics Inc, Rutherford, NJ), microarray analysis of >2000 genes and an associated algorithm provided a rank order of 15 different tumor types and were shown to have an overall sensitivity of 88% in a validation study including 547 tumors. However, this assay does not report NET tumors or NET subtypes. The 92-gene assay (CancerTYPE ID, Biotheranostics, Inc, San Diego, Calif) is a validated real-time reverse transcriptase polymerase chain reaction–based laboratory-developed test that provides a molecular classification of tumor type for 50 tumor subtypes. Importantly for this patient population, the 92-gene assay further categorizes NETs into 1 of 7 NET subtypes with an overall sensitivity of 95% (95% confidence interval, 87%–98%) for NET subtyping. For this reason, the 92-gene assay is the focus of new technology here.

The 92-gene assay uses real-time reverse transcriptase polymerase chain reaction to measure the collective expression of 87 informative genes (and 5 reference genes to normalize gene expression) from RNA collected from formalin-fixed, paraffin-embedded tissue. The associated algorithm generates a prediction of tumor type and subtype based on the similarity of the unknown tumor sample to a reference database of more than 2000 known tumor types and subtypes. The reference database contains gene expression data from 291 well-characterized NETs of various subtypes determined by histological examination of the tumor tissue and evaluation of available pathology data by a board-certified pathologist. The genes included are primarily derived from transcription factors and signal transduction pathways that provide genomic information related to cell lineage. Other genes within the assay evaluate proliferative and differentiation status. As such, the classification scheme reflects both anatomic primary site and differentiation status: in the classification scheme, well-differentiated NETs are separated into gastrointestinal carcinoid and lung carcinoid; subtypes for NECs include the pancreas (pancreatic islet cell carcinoma), skin (Merkel cell carcinoma), and lung (small/large cell lung carcinoma). In addition, the assay categorizes thyroid (thyroid medullary carcinoma) and adrenal gland (pheochromocytoma) tumors.

Clinical validation demonstrated an overall sensitivity of 87% to identify 28 main tumor types and 82% accuracy for 50 different subtypes with 96% to 100% specificity. In a subgroup analysis, the assay accurately identified 99% of NET carcinomas, with 95% accuracy in identifying NET subtype (Table 2). In terms of clinical utility, one prospective study demonstrated a 37% improvement in overall survival for patients with cancer of unknown primary who received assay-directed therapy compared with historical trials that used carboplatin/cisplatin therapy.

In a recent database analysis that included 24,484 consecutive cases submitted for clinical testing, the 92-gene assay rendered a molecular diagnosis of NET in 6.3% of cases. Small/large cell lung carcinoma was the most frequently identified NET molecular diagnosis (50%), followed by gastrointestinal carcinoid (14%), islet cell (14%), Merkel cell (10%), and lung carcinoid (9%). The assay identified all 7 NET subtypes in liver biopsy tissue, which accounted for 39% of all cases. The findings from this analysis highlight the clinical utility of molecular classification to identify distinct NET tumor types/subtypes to improve diagnostic precision and treatment decision making. This analysis is corroborated by another recent study, in which retrospective analysis identified a primary tumor site with >95% certainty for 35 (92%) of 38 patients with NET-UPs.

In this population, gastrointestinal NETs were most common (37%), followed by pancreatic (26%), bronchial carcinoid (13%), large cell neuroendocrine carcinoid (8%), Merkel cell (5%), and pheochromocytoma (3%).

In addition to the strong performance to identify the NET subtype, several additional features of the 92-gene assay highlight the potential for enhanced clinical utility in NET-UPs. First, the assay demonstrated high performance from a wide range of primary and metastatic biopsy sites, indicating that this approach has utility for NET patients whose clinical presentation can be heterogeneous. Second, the assay showed strong performance (91% sensitivity) in cytology and limited tissue samples which may be relevant in NET-UPs given the frequency of minimally invasive procedures in potential metastatic sites like the lung and liver. Third, in cases where it may be standard practice to default to large immunohistochemical panels, the assay demonstrated significantly higher accuracy in tumors that required more than 9 stains...
to render a diagnosis. A limitation of the technology is that classification of NETs is limited to the 7 NET subtypes that were included in the algorithm training. In addition, NETs originating from the rectum, which account for 17% of NETs by incidence, are not part of the classification algorithm.

**DISCUSSION**

A primary site diagnosis is essential for patients with NETs because of the heterogeneity of clinical symptoms, disease progression, treatment responsiveness, and prognosis. In approximately 10% to 14% of NETs, the initial diagnostic workup is unable to determine the tumor subtype, which may lead to delayed or suboptimal treatment approaches that have a negative effect on patient outcomes. In contrast, an accurate diagnosis of NET subtype can direct optimal surgical and medical interventions at the beginning of the treatment period.

Advances in genomics provide physicians with new technologies to identify the NET subtype based on the anatomic primary site. One example is the 92-gene assay, which has shown excellent specificity and sensitivity for NET classification. In addition, the clinical utility to identify distinct NET tumor types/subtypes to improve diagnostic precision and treatment decision making has been recently demonstrated. In the proposed diagnostic algorithms to identify the NET subtype based on the anatomic primary site, the 92-gene assay, which has shown excellent specificity and sensitivity for NET classification. In addition, the clinical utility to identify distinct NET tumor types/subtypes to improve diagnostic precision and treatment decision making has been recently demonstrated. In the proposed diagnostic algorithm for NET-UPs (Fig. 2), these emerging technologies are integrated with traditional approaches. The newer imaging technique, 68Ga-PET/CT, may be considered for patients who have clinical or biochemical evidence of NET but negative scans based on first-generation imaging methods. Molecular testing with the 92-gene assay is proposed for patients with clinical evidence of NET but with an unknown primary site or subtype after traditional workup or after inconclusive 68Ga-PET/CT testing. Furthermore, in cases of limited biopsy tissue such that complete pathology characterization is not possible, or for poorly differentiated tumors that are not amenable to functional imaging, biopsy tissue should be conserved for molecular tumor classification by the 92-gene assay.

In summary, accurate identification of NET subtype is critical for developing a targeted treatment plan. Multimodal diagnostic methods are often used to identify subtypes of neuroendocrine neoplasms. Genomic testing has evolved to be able to further characterize NET-UPs that may lead to improved patient care. The 92-gene assay has shown the ability to subtype NET-UPs in select studies; however, only 7 NET subtypes are identified, whereas some common NET subtypes, such as those originating from the rectum, are not part of the classification algorithm. Despite these limitations, the proposed algorithm takes into account that the 92-gene assay is the only genomic classifier to date that provides any NET subtype information for the determination of a patient care program. The algorithm, though clinically meaningful, would benefit from a prospective validating study in the future.

Emerging approaches such as molecular tumor classification may help fill the diagnostic gap that exists for NET subtype identification, particularly for community oncologists who may not have access to a pathology center of excellence with subspecialty practitioners.

**REFERENCES**

1. Zandee WT, Kamp K, van Adrichem RC, et al. Effect of hormone secretory syndromes on neuroendocrine tumor prognosis. *Endocr Relat Cancer*. 2017;24:R261–R274.

2. Hauso O, Gustafsson BI, Kidd M, et al. Neuroendocrine tumor epidemiology: contrasting Norway and North America. *Cancer*. 2008;113:2655–2664.

3. Yao JC, Hassan M, Phan A, 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;26:3063–3072.

4. Araujo PB, Cheng S, Mete O, et al. Evaluation of the WHO 2010 grading and AJCC/UICC staging systems in prognostic behavior of intestinal neuroendocrine tumors. *PLoS One*. 2013;8:e61538.

5. Kliron D, Modlin IR, Coppola D, et al. The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas*. 2010;39:707–712.

6. Rihimaki M, Hemminki A, Sundquist K, et al. The epidemiology of metastases in neuroendocrine tumors. *Int J Cancer*. 2016;139:2679–2686.

7. Catena L, Bichisio E, Milione M, et al. Neuroendocrine tumors of unknown primary site: gold dust or misdiagnosed neoplasms? *Tumori*. 2011;97:564–567.

8. Scoza JY, Couvelard A, Monges G, et al. Professional practices and diagnostic issues in neuroendocrine tumour pathology: results of a prospective one-year survey among French pathologists (the PRONET study). *Neuropathologica*. 2017;105:67–76.

9. Bergsland EK, Nakakura EK. Neuroendocrine tumors of unknown primary: is the primary site really not known? *JAMA Surg*. 2014;149:889–890.

10. Wang SC, Parekh JR, Zaraek MB, et al. Identification of unknown primary tumors in patients with neuroendocrine liver metastases. *Arch Surg*. 2010;145:276–280.

11. Schreiter NF, Bartels AM, Froeling V, et al. Searching for primaries in patients with neuroendocrine tumors (NET) of unknown primary and clinically suspected NET: Evaluation of Ga-68 DOTATATE PET/CT and In-111 DTPA octreotide SPECT/CT. *Radiol Oncol*. 2014;48:339–347.

12. Hainsworth JD, Johnson DH, Greco FA. Poorly differentiated neuroendocrine carcinoma of unknown primary site. A newly recognized clinicopathologic entity. *Ann Intern Med*. 1988;109:364–371.

13. Stoyianni A, Pentheroudakis G, Pavlidis N. Neuroendocrine carcinoma of unknown primary: a systematic review of the literature and a comparative study with other neuroendocrine tumors. *Cancer Treat Rev*. 2011;37:358–365.

14. Kerr SE, Schnabel CA, Sullivan PS, et al. A 92-gene cancer classifier predicts the site of origin for neuroendocrine tumors. *Mod Pathol*. 2014;27:44–54.

15. Kaltas GA, Besser GM, Grossman AB. The diagnosis and medical management of advanced neuroendocrine tumors. *Endocr Rev*. 2004;25:458–511.

16. Shah MH, Goldner WS, Halfdanarson TR, et al. NCCN guidelines insights: neuroendocrine and adrenal tumors, version 2.2018. *J Natl Compr Canc Netw*. 2018;16:693–702.

17. Strosberg JR, Nasir A, Hodul P, et al. Biology and treatment of metastatic gastrointestinal neuroendocrine tumors. *Gastrointest Cancer Res*. 2008;2:113–125.

18. Kunz PL, Reidy-Lagunes D, Anthony LB, et al. Consensus guidelines for the management and treatment of neuroendocrine tumors. *Pancreas*. 2013;42:557–577.

19. Tiros A, Papadakis GZ, Millo C, et al. Association between neuroendocrine tumors biomarkers and primary tumor site and disease type based on total 68Ga-DOTATATE-Avid tumor volume measurements. *Eur J Endocrinol*. 2017;176:575–582.

20. Koo J, Dhall D. Problems with the diagnosis of metastatic neuroendocrine neoplasms. Which diagnostic criteria should we use to determine tumor origin and help guide therapy? *Semin Diagn Pathol*. 2015;32:456–468.

21. Barnes J, Johnson SJ, French JJ. Correlation of Ki-67 indices from biopsy and resection specimens of neuroendocrine tumours. *Ann R Coll Surg Engl*. 2017;99:193–197.
22. Khan MS, Luong TV, Watkins J, et al. A comparison of Ki-67 and mitotic count as prognostic markers for metastatic pancreatic and midgut neuroendocrine neoplasms. *Br J Cancer*. 2013;108:1838–1845.

23. Bellizzi AM. Assigning site of origin in metastatic neuroendocrine neoplasms: a clinically significant application of diagnostic immunohistochemistry. *Adv Anat Pathol*. 2013;20:285–314.

24. Chan ES, Alexander J, Swanson PE, et al. PDX-1, CDX-2, TTF-1, and CK7: a reliable immunohistochemical panel for pancreatic neuroendocrine neoplasms. *Am J Surg Pathol*. 2012;36:737–743.

25. Heverhagen AE, Geis C, Fendrich V, et al. Embryonic transcription factors CDX2 and Oct4 are overexpressed in neuroendocrine tumors of the ileum: a pilot study. *Eur Surg Res*. 2013;51:14–20.

26. Sangeor A, Ogami RS, Pai RK, et al. PAX8 expression reliably distinguishes pancreatic well-differentiated neuroendocrine tumors from ileal and pulmonary well-differentiated neuroendocrine tumors and pancreatic acinar cell carcinoma. *Mod Pathol*. 2011;24:412–424.

27. Brachtel EF, Opera RN, Sullivan PS, et al. Molecular classification of cancer with the 92-gene assay in cytology and limited tissue samples. *Oncotarget*. 2016;7:27220–27231.

28. Anderson GG, Weiss LM. Determining tissue of origin for metastatic cancers: meta-analysis and literature review of immunohistochemistry performance. *Appl Immunohistochem Mol Morphol*. 2010;18:3–8.

29. Weiss LM, Chu P, Schnedder BE, et al. Blinded comparator study of immunohistochemical analysis versus a 92-gene cancer classifier in the diagnosis of the primary site in metastatic tumors. *J Mol Diagn*. 2013;15:263–269.

30. Yu R, Wachsman A. Imaging of neuroendocrine tumors: indications, interpretations, limits, and pitfalls. *Endocr Relat Cancer*. 2017;46:795–814.

31. Armbruster M, Zech CJ, Sourbron S, et al. Diagnostic accuracy of dynamic gadobetate-contrast-enhanced MRI and PET/CT compared in patients with liver metastases from neuroendocrine neoplasms. *Magn Reson Imaging*. 2014;40:457–466.

32. Dromain C, de Baere T, Lumbroso J, et al. Detection of liver metastases from endocrine tumors: a prospective comparison of somatostatin receptor scintigraphy, computed tomography, and magnetic resonance imaging. *J Clin Oncol*. 2005;23:70–78.

33. Etchebehere EC, de Oliveira Santos A, Guntz B, et al. 68Ga-DOTATATE PET/CT, 99mTc-HYNIC-octreotide SPECT/CT, and whole-body MR imaging in detection of neuroendocrine tumors: a prospective trial. *J Nucl Med*. 2014;55:1598–1604.

34. Santhanam P, Chandramahalathi S, Kroiss A, et al. Nuclear imaging of neuroendocrine tumors with unknown primary: why, when and how? *Eur J Nucl Med Mol Imaging*. 2015;42:1144–1155.

35. Sadowski SM, Neychev V, Millo C, et al. Prospective study of 68Ga-DOTATATE positron emission tomography/computed tomography for detecting gastro-entero-pancreatic neuroendocrine tumors and unknown primary sites. *J Clin Oncol*. 2016;34:588–596.

36. Hofmann M, Maecke H, Börner R, et al. Biokinetics and imaging with the 68Ga-DOTA(TC)PET:CT in patients with metastatic neuroendocrine tumor. *J Nucl Med*. 2017;58:1054–1057.

37. Naswa N, Sharma P, Kumar A, et al. (68)Ga-DOTANOC PET/CT in patients with carcinoma of unknown primary of neuroendocrine origin. *Clin Nucl Med*. 2012;37:245–251.

38. Prasad V, Ambrosini V, Homann M, et al. Detection of unknown primary neuroendocrine tumours (CUP-NEG) using (68)Ga-DOTA-NOC receptor PET/CT. *Eur J Nucl Med Mol Imaging*. 2010;37:67–77.

39. Sharma P, Arora S, Mukherjee A, et al. Predictive value of 68Ga-DOTANOC PET/CT in patients with suspicion of neuroendocrine tumors: is its routine use justified? *Clin Nucl Med*. 2014;39:37–43.

40. Yu R, Wachsman A. Imaging of neuroendocrine tumors: indications, interpretations, limits, and pitfalls. *Endocr Relat Cancer*. 2017;46:795–814.

41. Kroiss A, Putzer D, Decristoforo C, et al. 68Ga-DOTA-TOC uptake in neuroendocrine tumour and healthy tissue: differentiation of physiological uptake and pathological processes in PET/CT. *Eur J Nucl Med Mol Imaging*. 2013;40:514–523.

42. Howe JR, Cardona K, Fraker DL, et al. The surgical management of small bowel neuroendocrine tumors: consensus guidelines of the North American Neuroendocrine Tumor Society. *Pancreas*. 2017;46:715–731.

43. Frilling A. Determining tissue of origin for metastatic cancers: meta-analysis and literature review of immunohistochemistry performance. *Appl Immunohistochem Mol Morphol*. 2010;18:3–8.

44. Kroiss A, Putzer D, Decristoforo C, et al. 68Ga-DOTA-TOC uptake in neuroendocrine tumour and healthy tissue: differentiation of physiological uptake and pathological processes in PET/CT. *Eur J Nucl Med Mol Imaging*. 2013;40:514–523.

45. Howe JR, Cardona K, Fraker DL, et al. The surgical management of small bowel neuroendocrine tumors: consensus guidelines of the North American Neuroendocrine Tumor Society. *Pancreas*. 2017;46:715–731.
59. Vinik AI, Raymond E. Pancreatic neuroendocrine tumors: approach to treatment with focus on sunitinib. *Therap Adv Gastroenterol*. 2013;6:396–411.

60. Wells SA Jr., Robinson BG, Gagel RF, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol*. 2012;30:134–141.

61. Bunn PA Jr., Minna JD, Augustyn A, et al. Small cell lung cancer: can recent advances in biology and molecular biology be translated into improved outcomes? *J Thorac Oncol*. 2016;11:453–474.

62. Kaufman HL, Russell J, Hamid O, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol*. 2016;17:1374–1385.

63. Kotteas EA, Syrigos KN, Safi MW. Profile of capecitabine/temozolomide combination in the treatment of well-differentiated neuroendocrine tumors. *Onco Targets Ther*. 2016;9:699–704.

64. Schnabel CA, Erlander MG. Gene expression-based diagnostics for molecular cancer classification of difficult to diagnose tumors. *Expert Opin Med Diagn*. 2012;6:407–419.

65. Pillai R, Deeter R, Rigl CT, et al. Validation and reproducibility of a microarray-based gene expression test for tumor identification in formalin-fixed, paraffin-embedded specimens. *J Mol Diagn*. 2011;13:48–56.

66. Rosenwald S, Gilad S, Benjamin S, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. *Mod Pathol*. 2010;23:814–823.

67. Erlander MG, Ma XJ, Kesty NC, et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. *J Mol Diagn*. 2011;13:493–503.

68. Kerr SE, Schnabel CA, Sullivan PS, et al. Multisite validation study to determine performance characteristics of a 92-gene molecular cancer classifier. *Clin Cancer Res*. 2012;18:3952–3960.

69. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon Research Institute. *J Clin Oncol*. 2013;31:217–223.

70. Hendifar A, Soifer HS, Israel MA, et al. Molecular classification of neuroendocrine tumors: clinical experience with the 92-gene assay in >24,000 cases. *Pancreas*. 2017;47:332–361.

71. Chauhan A, Farooqui Z, Silva SR, et al. Integrating a 92-gene expression analysis for the management of neuroendocrine tumors of unknown primary. *Asian Pac J Cancer Prev*. 2019;20:113–116.