Algorithm for diagnosing primary neuroendocrine neoplasms of the lung

Marija Gomerčić Palčić, Marija Perić, Luka Vrbanić, Nevenka Piskač Živković

1 Department of Clinical Immunology, Pulmonology and Rheumatology, University Hospital Center “Sestre milosrdnice”, Medical School, University of Zagreb, Zagreb, Croatia
2 Department of Pulmonary Diseases, University Hospital Dubrava, Zagreb, Croatia

Abstract
Pulmonary neuroendocrine neoplasms (PNEN) represent 25% of primary lung neoplasms. Diagnosing PNEN presents a significant challenge because of the heterogeneous clinical, radiographic and endoscopic presentations as well as varying degrees of malignant potential. The incidence of PNEN is expected to increase as a result of better diagnostic methods. Methods used in the diagnosis and staging of PNEN are similar to methods used in other types of lung cancers. Differentiation of PNEN from other types of lung tumors and between PNEN subtypes is difficult, which results in misdiagnosis, inadequate treatment and poor prognosis. Imaging methods represent a gold standard in diagnosing and staging PNEN. Almost half of pulmonary carcinoids (PC) are incidentally discovered on standard chest X-rays. Computed tomography (CT) of the chest and upper abdomen is required to define the primary tumor and its local and distant extent. When a lung tumor is suspected based on imaging methods, appropriate additional endoscopic and/or imagining methods need to be selected. It is of great importance to choose an adequate method for disease staging because the utility of 18F-fluorodeoxyglucose (18F-FDG) and isotope-labeled somatostatin analogues varies according to tumor histology. Positron emission tomography (PET)-CT of the chest and upper abdomen is required to define the primary tumor and its local and distant extent. When a lung tumor is suspected based on imaging methods, appropriate additional endoscopic and/or imagining methods need to be selected. It is of great importance to choose an adequate method for disease staging because the utility of 18F-fluorodeoxyglucose (18F-FDG) and isotope-labeled somatostatin analogues varies according to tumor histology. Positron emission tomography (PET)-CT plays an important role in identifying the tumor, its localization, size, invasion, as well as staging, and new radionuclide tracers are the future of PNEN diagnostics. Besides the use of biopsies and cytological findings in the classification of PNEN, immunohistochemical markers have an important role in the differential diagnosis of these tumors, and are used to help pathologists diagnose various subtypes of PNEN. The aim of this review is to present available biochemical, imaging and endoscopic markers/methods used in the diagnosis of PNEN and to provide a suitable diagnostic algorithm.

Key words: pulmonary neuroendocrine neoplasms, small cell lung cancer, large cell neuroendocrine carcinoma, atypical carcinoid, typical carcinoid, lung neoplasms diagnostics
1. Introduction

Primary neuroendocrine neoplasms of the lung (PNEN) encompass a heterogenic group of tumors that include poorly-differentiated (high-grade) small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC), as well as well-differentiated neoplasms, that include intermediate-grade atypical (AC) and low-grade typical carcinoids (TC). One fourth of primary lung neoplasms are PNEN with SCLC accounting for 15-20%, LCNEC 3%, and carcinoids 1-2%, but this number is expected to increase as a result of better detection due to sophisticated imaging methods, new radiotracers and immunohistochemical markers [1, 2]. Although methods used in the diagnosis and staging of PNEN are quite similar to other types of lung cancers, some differences and specificities do exist. Differentiation of PNEN from other types of lung tumors and between their subtypes is often difficult, resulting in misdiagnosis, inadequate treatment and poor prognosis. Carcinoids are usually diagnosed in early stages, have low metastatic potential, and are in most cases resectable in contrast to SCLC that are diagnosed in late stages, with distant metastases, and are inoperable at the time of diagnosis. The aim of this review is to present available biochemical, imaging and endoscopic markers/methods for diagnosing PNEN and to provide a suitable diagnostic algorithm to help clinicians make informed decisions when choosing the right marker and/or method.

2. Imaging and endoscopic methods

Imaging methods represent a gold standard in diagnosing and staging PNEN. A nonspecific pathological finding of pulmonary parenchyma on chest X-ray such as a peripheral lesion or an isolated hilar or perihilar mass often rises the suspicion of a PNEN [3, 4]. Almost half of pulmonary carcinoids (PC) are incidentally discovered on standard chest X-rays. When a suspicious lesion is detected on chest X-ray, computed tomography (CT) of the chest and upper abdomen is required to define the primary tumor, as well as its local and distant extent. Additionally, CT is more sensitive than chest X-ray. This imaging method provides additional information regarding tumor size, characteristics, local extent, and distal metastases. Radiological features depend on the carcinoid type. TC present as a nodule with smooth margins, are highly vascularized and centrally located, while AC have irregular margins and are peripherally located [5]. TCs, in almost half of cases, are centrally-located endobronchial tumors, usually in the main, lobar and segmental bronchi, resulting in parenchyma consolidation, lobar atelectasis, air trapping, obstructive pneumonitis, and even lung abscesses. Twenty percent of TC are accompanied by local lymphadenopathy, which is mostly caused by reactive inflammation, while 50% of AC present with lymph node metastases. Tumor calcifications as well as tumor vascularity can help us diagnose PCs; centrally located tumors that narrow the endobronchial lumen with punctate or diffuse calcifications are highly suggestive for carcinoids and vascularity in peripheral lesions can help us distinguish carcinoids from benign states such as mucoid impactions [6].

Almost 90-95% of SCLC cases present radiologically as a large mass originating from the main or lobar bronchus. The mass is composed of the primary tumor and enlarged lymph nodes that are secondarily changed due to metastases, which obstructs the bronchi. Also, pleural effusions can commonly be seen in up to 20-38% of cases, depending on study results, and this disease presentation can help confirm the diagnosis [7]. In a small number of cases, an isolated peripheral nodule (5-10%), without lymph node metastases can be seen [8]. The radiological presentation of LCNECs is nonspecific, in three quarters of cases they are peripherally located, usually visualized as a well-defined and lobulated tumor with no air bronchograms and inhomogeneous enhancement caused by intratumor necrosis. Calcifications can be seen in 10% of cases as well as pleural effusions or surrounding emphysema [9].

When a lung tumor is suspected based on imaging methods, further adequate imagining or endoscopic methods need to be selected and applied. CT- or ultrasound-guided transthoracic needle biopsy (TTNB) are preferred methods for peripheral lung lesions, and in cases with negative results, video-assisted thoracic surgery or an open thoracotomy are alternative options.

Flexible fiberoptic bronchoscopy with bronchial brushing and forceps biopsy is preferred for central tumors. Although risks from bronchial brushing and bronchial biopsy in PNENs are similar to bronchoscopy in
general, in some cases with macroscopically definitive findings of extensively vascularized endobronchial tumors, this preoperative biopsy may be avoided. Just as radiological appearances differ among subgroups of PNEN, endobronchial findings do also. Carcinoids are in 75% of cases visible with bronchoscopy because they are centrally located, endobronchial well-demarcated yellow colored tumors [10]. In order to confirm the diagnosis, brush cytology or biopsy should be done. TC and AC can, in most cases, be distinguished only based on resected material. The rest of the tumors are peripherally located, without communication with bronchi, which represents an obstacle for endoscopic diagnosis. Although the majority of PC are vascular tumors, serious hemorrhage is reported in less than 1% of cases. If serious hemorrhage is a concern, rigid bronchoscopy should be performed [11]. LCNECs are mainly peripherally located, but when located centrally, as seen in a quarter of cases, they can be seen by fiberoendoscopy as a tan-colored polyp, protruding into the bronchial lumen. The majority (~90%) of SCLCs present as a large, centrally located submucosal tumor with little or no exophytic endobronchial extension. Diagnosis of SCLC is usually made by cytology, which is superior to a small biopsy because of the crush artifact, limiting small biopsy interpretation, which is minimized in cytology specimens [12, 13]. Although reports say that cytology can be appropriate for diagnosing LCNEC some studies implicate that they can frequently be falsely recognized as SCLC [14]. New and advanced methods, such as endobronchial ultrasound (EBUS) with real-time transbronchial needle aspiration (TBNA) enables more accurate and sufficient specimen acquirement, resulting in earlier diagnosis, reduction of repeated bronchoscopy procedures and additionally, lower morbidity. EBUS-TBNA is particularly useful in centrally located peritracheal, peribronchial and paraoesophageal PNENs, which are not visible by conventional bronchoscopy and are unreachable on CT-guided TTNB. Staging of the disease, in terms of lymphatic spread, is usually done with EBUS-guided TBNA and endoscopic ultrasound-guided (EUS) fine needle aspiration (FNA) if those are available.

Positron emission tomography (PET)-CT plays an important role in identifying the tumor, its localization, size and invasion, as well as staging. The most frequently used method for further evaluation of pulmonary nodules is 18F-fluorodeoxyglucose (18F-FDG)-PET scan, before the histological diagnosis is confirmed, although in case of a solitary pulmonary node the diagnosis of carcinoid is most likely and conventional somatostatin receptor scintigraphy is the preferred method. Staging using 18F-FDG-PET was found to be less crucial in well-differentiated PNEN; therefore, preoperative 18F-FDG-PET is considered optional in biopsy proven, well-differentiated PNENs. These recommendations are based on the results of the first study on a small number of patients, which implicated that 86% of carcinoids were not detectable using FDG PET/CT, although later studies revealed higher sensitivity, up to 75%, and even more if cut-off values of the maximal standardized uptake value (SUVmax) were set to be lower [15, 16].

It is important to mention that carcinoid tumors show variable FDG uptake according to mitotic potential and tumor proliferation, especially if Ki-67 index is over 10-15%, depending on study results. In contrast to ambivalent results in case of carcinoids, 18F-FDG-PET is a valuable tool for the preoperative assessment of disease extent in patients with intermediate- and high-grade tumors. In one study, LCNEC showed high FDG uptake on PET. For SCLC, in a study by Pandit N et al., 18F-FDG-PET demonstrated a very high sensitivity (100%) and was shown to be a valuable prognostic tool for staging and follow up as well [17]. Additionally, it was shown that maximum SUVs are significantly different when comparing LCNECs and SCLCs, and a high maximum SUV is associated with a shorter survival [18]. We can conclude that PET is a valuable method in all subtypes of PNEN in diagnosing, initial staging and treatment planning.

Another method for identifying metastatic PNEN is somatostatin receptor scintigraphy with radionuclide-coupled octreotide, which binds to somatostatin receptors (SSTR) found on tumor cells. SSTR are expressed in approximately 80-90% of PNEN, predominantly SSTR2 [19]. Synthetic analogues mainly bind to SSTR2, and much less to SSTR5. In contrast to well-differentiated neuroendocrine tumors, in undifferentiated tumors expression of SSTR is less frequent and lower in density [20, 21]. Therefore, most TC as well as AC and rarely LCNEC express SSTR that can be adequately verified by isotope-labelled somatostatin analogues. Righ
et al. compared the immunohistochemical expression pattern of SSTR in PNENs against SSTR scintigraphy on 218 patients with PNEN and showed that immunohistochemistry is less sensitive as compared to SSTR scintigraphy. The correlation between immunohistochemical SSTR expression and SSTR scintigraphy was found in 70% of PNENs [22]. In a study by Genestreti et al., SSTR scintigraphy showed lower accuracy (65% for primary tumors, 62% for lymph node involvement, and 52% for distant metastases) in comparison with standard radiological staging in SCLC, but patients with a positive SSRS had better disease control on applied therapy with equal overall survival [23]. SSTR scintigraphy is suggested in preoperative staging and in postoperative follow-up of LCNEC, although evidence supporting its use in clinical practice is still missing.

Based on the before mentioned study results, great attention was dedicated to the invention of a combined method that would include higher spatial resolution than conventional scintigraphy, radiotracers with higher affinity for SSTRs, and tools for quantifying radiotracer uptake. PET with somatostatin analogs was shown to be preferable over standard imaging and SSTR scintigraphy, as well as to influence positively on chosen therapy modalities and prognostic accuracy. These advantages are extremely important for low-grade tumors not only for staging but also for planning therapy [24]. Introducing new radiotracers, 68Ga-labeled somatostatin analogues for PET/CT, made this method cheaper and shorter. The main difference among these three tracers (68Ga-DOTA-TOC (1,4,7,10-tetraazacyclododecane-NI,NII, NIII,NIIII-tetraacetic acid - Thy3-octreotide), 68-Ga-DOTA-NOC (68Ga-DOTA - 1-Nal(3)]-octreotide), and 68Ga-DOTATATE (68Ga-DOTA - Tyr3-octreotate)) is their variable affinity to SSTR subtypes. Kayani et al. conducted the first study on 18 patients with PCs comparing PET somatostatin receptor ligand 68Ga-DOTATATE and 18F-FDG [25]. Typical carcinoids showed significantly higher uptake of 68Ga-DOTATATE and significantly less uptake of 18F-FDG than tumors of higher grade (P = 0.002 and 0.005) and additionally 68Ga-DOTATATE was superior in distinguishing endobronchial tumors from distal collapsed lung (P = 0.02). It is interesting that no false-positive uptake of (68)Ga-DOTATATE was seen while 3 sites of 18F-FDG uptake were due inflammation.

Additionally, it was noticed that SUVmax in typical carcinoids on 68Ga-DOTATOC-PET/CT was significantly higher (SUVmax, 8.8-66) compared with atypical carcinoids (SUVmax, 1.1-18.5; P = 0.002) [26]. Ambrosini V et al. compared 68Ga-DOTANOC PET/CT to CT in 11 patients with PCs. The methods were discordant in a surprisingly high number of patients (8 of 11), whereas in only three patients both provided similar results. 68Ga-DOTANOC PET/CT detected more lesions than CT (37 versus 21) and provided additional information in 82% of patients changing the clinical management in one third of cases [27]. Additional studies confirmed high sensitivity by using somatostatin receptor PET, 100% for TC and 80% for AC [11, 28, 29, 30, 31]. Poorly-differentiated (high-grade) tumors showed great avidity for 18F-fluorodeoxyglucose and low avidity for 68Ga-DOTATATE, while the opposite was noticed in low grade tumors. In a study by Rodrigues M et al., the authors reported that 111In-DOTATOC and 111In-DOTALAN were suitable for imaging tumor lesions in patients with NETs and could detect lesions that may not be seen by conventional imaging and 18F-FDG-PET [32]. Compared with 111In-DOTALAN, 111In-DOTATOC was superior in diagnosing liver metastases, but inferior for confirming metastases in the mediastinum and bone. Sollini et al. evaluated the usefulness of PET/CT with 68Ga-labeled SST-analogues in 24 patients with metastatic SCLC, to select patients for subsequent peptide receptor radionuclide therapy (PRRT) and compared 68Ga-labeled SST-analogues PET/CT results to contrast-enhanced CT findings. PET/CT was positive in 83% of patients and concordant to CT findings for all the sites of disease in 37.5% of cases [33]. A pilot study using 68Ga-Pentixafor as a novel PET tracer in SCLC might be useful for confirmation of CXCR4 expression as a prerequisite for the potential chemokine receptor 4 (CXCR4)-directed treatment including PRRT [34].

In conclusion, different uptake patterns and SUVs on 18F-FDG and 68Ga-SST-analogue PET could be the tool for distinguishing subtypes of pulmonary carcinoids. 68Ga-SST-analogue PET/CT proved to be effective for staging and electing adequate treatment options, including PRRT. Therefore, staging of patients with well- and moderately differentiated PNEN should include PET with somatostatin analogs together with diagnostic
CT of the chest and abdomen. In high-grade PNEN, an 18F-FDG-PET scan with integrated diagnostic CT is the first method of choice. Somatostatin receptor scintigraphy should be included in the diagnostic work up to evaluate presence of SSTR and usefulness of PRRT, or as part of follow-up. 68Ga-DOTATATE with integrated PET/CT, although not yet available in many centers, can be useful in the preoperative workup of pulmonary carcinoids and for evaluating recurrence of low-grade carcinoid tumors.

3. Immunohistochemistry

The classification of neuroendocrine lung tumors has been based on the evaluation of biopsies or cytological preparations. Some immunohistochemical markers obtained from other specimens are an important part of the differential diagnosis of these tumors and are used to help pathologists diagnose the various subtypes.

It is known that neuroendocrine tumors have the ability to synthesize, store and secrete neuroamines and neuropeptides that can be measured in the blood or urine. These neuroendocrine markers may help to confirm PNEN and epithelial differentiation, especially when it can be very difficult to establish a precise diagnosis. Commonly used markers include synaptophysin, chromogranin A (CgA), CD56 and neuron-specific enolase (NSE).

Carcinoid tumors are diffusely positive for Neuroendocrine (NE) markers; especially for synaptophysin and CgA. Synaptophysin has a higher sensitivity than CgA or NSE, so it is one of the most specific markers of neuroendocrine differentiation [35]. Serum CgA correlates with tumor volume, therefore it is mostly used in follow-up care (recurrence after radical surgery, response to treatment, in advanced or metastatic disease). A higher level of CgA may be associated with proton pump inhibitor use, but also with liver or renal failure, or chronic gastritis [36]. In a small part of carcinoid tumors, particularly in AC, not all NE markers may be expressed, therefore a panel approach is recommended [37]. According to the literature, about 80% of carcinoids are positive for cytokeratins, while about 50% are positive for thyroid transcription factor-1 (TTF-1), especially peripheral carcinoids [38, 39]. On the other hand, reactivity for synaptophysin and CgA is weak in SCLC, while CD56 is the most sensitive NE marker for SCLC. About 25% of SCLC are negative for both synaptophysin and CgA, but positive for CD56 [40]. In about 10% of SCLC, commonly used NE markers for SCLC are negative, especially in small biopsy and cytology specimens [40, 41, 42]. In these cases, recommendations are that if the morphology is typical for SCLC, the diagnosis should go in that way, despite negative NE markers [43]. TTF-1 reactivity in SCLC is expressed in about 90%, although it is not specific for lung origin (it is expressed in approximately 50% of LCNEC, that originate in lung, bladder, prostate). Because it is a maker of abnormal reactivation of primitive neural pathway, TTF-1 is expressed in 20% to 80% of small cell carcinomas of extrapulmonary origin (gastrointestinal tract, bladder, prostate, cervix) [44, 45, 46]. LCNEC is similar to SCLC, but for diagnosis of LCNEC, at least one NE marker is required. Synaptophysin and CgA are coexpressed in most LCNEC (about 70%) [14, 47]. As in SCLC, TTF-1 is not specific for lung origin (it is expressed in approximately 50% of LCNEC, that originate in lung, bladder, prostate). Cytokeratins (pool AE1/AE3) are mostly positive in SCLS and LCNEC though it may be negative in up to 20% of carcinoid tumors, as mentioned above [31].
Although the utility of Ki-67 (percentage of positive tumor cells) is not a part of immunohistochemical diagnostic criteria accepted in 2004, as opposed to gastrointestinal NETs, it is an important marker, especially in small biopsy and cytology. It is useful in distinguishing low-grade carcinoids from high-grade NE neoplasms, but more data is needed. According to the literature, TC have a Ki-67 rate of less than 2%, AC less than 20%, whereas Ki-67 rate in SCLS and LCNEC is higher than 20% (Ki-67 rate of less than 25% excludes the diagnosis of SCLC) [48].

In this article we have reviewed current modalities in diagnosing PNENs. We also provided an algorithm (Figure 1) that may help clinicians make the right diagnostic decisions when confronted with a patient with suspected PNEN.

Author contributions

MGP and MP wrote the manuscript. LV and NPŽ contributed in drafting the article and revising it critically.

References


