

Differential diagnosis of neuroendocrine carcinoma

- 1) Case 1 - A biopsy, cytology sample or resection can have a clearly recognizable non-small cell component. This type of sample raises a complex differential diagnosis that can be resolved a variety of ways, but also leads to some areas of diagnostic uncertainty.

A key question is whether the cells represent a relatively homogenous population of tumor cells. This question focuses on 2 potential heterogeneous tumors, combined small cell carcinoma and combined adenocarcinoma (or squamous carcinoma) with large cell neuroendocrine carcinoma. Often there is a crushed cellular population that may represent crush in the non-small cell carcinoma pattern, or alternatively can represent a small cell component. In this scenario the heterogeneity focus on artifact versus true heterogeneity. In the second scenario, the focus is on the subjective impression of what represents neuroendocrine morphology.

The presence of a crushed tumor population within what is partly a non-small cell carcinoma requires attention. The characterization of the tumor needs to focus on morphologic well preserved areas that have unequivocal small cell morphology. This includes salt and pepper chromatin, relatively scant cytoplasm, nuclear molding, frequent apoptotic debris and mitoses; the crush artifact is already a given to lead into this discussion. The chromatin pattern and high nuclear to cytoplasmic ratio are critical (as is molding), but apoptotic debris and mitoses are also a part of large neuroendocrine carcinoma. In the absence of well-preserved morphology in this scenario, other features may be helpful. The pattern of cytokeratin can be very helpful. Small cell carcinoma often have weak cytokeratin staining that is often dot-like and not membranous. Large cell neuroendocrine carcinoma is more often membranous. However more important than the pattern itself is the recognition of 2 patterns, supporting the impression of heterogeneous population. Neuroendocrine markers may also be of interest here, as these will also show variability in the populations, especially synaptophysin staining.

Some other considerations include ruling out squamous carcinoma, as some cases can have two populations based on differentiation, including basaloid differentiation, and degree of keratinization. If the tumor is diffusely p40 positive, this homogenous staining supports a squamous carcinoma, albeit with different growth patterns.

Assuming a heterogeneous population as defined morphologically and immunohistochemically, crush artifact, the exclusion of squamous cell carcinoma and the impression of a small cell component, such a tumor should be classified as small cell carcinoma, combined type.

If the population is not determined to be heterogeneous, then the non-small cell component needs to be properly evaluated. Assuming squamous cell carcinoma has

been excluded as noted above, the remaining broad differential includes large cell neuroendocrine carcinoma, solid type and cribriform adenocarcinoma, and perhaps large cell carcinoma. This assumes a focus on tumors of lung primary. For this differential diagnosis, the morphology once again becomes critical. Does the tumor have neuroendocrine morphology? This is subjective, but presence of rosettes, an organoid pattern, a trabecular pattern or cellular features of relatively uniform nuclei with small nucleoli and moderate amounts of cytoplasm all are in support of neuroendocrine morphology. These nuclei can have coarse chromatin but macronucleoli with a vesicular chromatin background are not usually seen in larger numbers.

If determined to be neuroendocrine morphology, immunohistochemistry for neuroendocrine markers is needed to confirm this. Synaptophysin, chromogranin and CD56 are generally used, with CD56 having highest sensitivity and lowest specificity. If not neuroendocrine morphology, then neuroendocrine markers should not be performed.

This leads to several options. Large cell neuroendocrine morphology with positive markers as large cell neuroendocrine carcinoma. This will leave some cases that have neuroendocrine morphology but negative neuroendocrine markers. The presence of mucicarmine staining or any glandular component may move this into the adenocarcinoma category; TTF1 reactivity may also move this into the solid adenocarcinoma category. In the absence of these findings, such tumors are large cell carcinoma, in this case with neuroendocrine morphology. If the tumor does not have neuroendocrine morphology, IHC for these markers should not be performed. However, in the event that such markers are performed, these tumors should be classified as adenocarcinoma or squamous carcinoma and the neuroendocrine differentiation by IHC noted in a comment. This latter finding is of uncertain significance. In the unusual event that a large cell carcinoma is encountered that does not have neuroendocrine morphology but is positive for IHC markers of NE differentiation, a careful consideration of the morphologic features is needed to rule out neuroendocrine morphology in such cases.

Case 2 – grading neuroendocrine tumors – when it is not a carcinoma?

In good quality samples, it is relatively straightforward to evaluate neuroendocrine tumors. Uniform nuclei, salt and pepper chromatin and relatively abundant cytoplasm (that can vary in staining quality) with architectural patterns that are organoid and trabecular lead us to the correct morphologic diagnosis which can be confirmed by immunohistochemistry. However, when evaluating atypical carcinoid and ultimately, higher grade neuroendocrine carcinomas such as small cell carcinoma and large cell neuroendocrine carcinoma require an assessment of necrosis and mitotic activity.

The presence of necrosis is helpful in that it moves a neuroendocrine proliferation out of the lung carcinoid category. However the behavior of atypical carcinoids, while more aggressive than carcinoids, is not that of small cell carcinoma or LCNEC. In small samples especially, morphology can be crushed and necrosis can obscure the morphology. Also, while mitotic counts are the agreed upon definition to distinguish atypical carcinoid from the higher grade categories, these can be difficult to count in samples with limited morphologic preservation. I find that apoptotic debris easier to identify than mitoses, and that these are a clue that a crushed proliferation is high grade.

This is where Ki67 is particularly useful. While the precise cut-off values by category have not been definitively established, low values are not consistent with high grade carcinomas, and most high grade carcinomas have Ki67 values over 50%. This approach can help avoid over diagnosis of high grade carcinomas on small samples with crush artifact, which tends to be the problem on such samples.

Case 3 – Unusual neuroendocrine tumors, and tumors with positive CD56 which are not neuroendocrine.

The use of CD56 as a neuroendocrine marker is widely established and this is the most sensitive marker in this evaluation. As a result, this will be the sole positive marker in a proportion of truly neuroendocrine tumors. In morphologically consistent cases, CD56 is very helpful as a result. However, the use of CD56 also raises problems, especially in morphologic mimickers of neuroendocrine tumors, and also in small samples in which the morphology is limited or relatively undifferentiated.

For example, CD56 can be positive in squamous carcinomas, and when these tumors are non-keratinizing, this can be a challenge. Including p40 in such cases is very helpful. Some adenocarcinomas of the lung are CD56 positive. CD56 is positive in plasma cell and natural killer cells tumors; once again a panel approach to immunohistochemistry can avoid incorrect diagnosis. Smooth muscle tumors, sex cord stromal tumors, endometrial tumors including stromal sarcomas are also routinely positive. All of these possibilities must be considered when CD56 is the sole positive neuroendocrine marker.

These are unusual neuroendocrine tumors that can involve the lung and thorax. Primitive neuroectodermal tumor (PNET) is a small round blue cell tumor of bone and soft tissue of children and young adults which is histologically and molecularly similar to Ewing's sarcoma and belongs in the same family. While some cases can have morphologic similar to small cell carcinoma, the epidemiology generally differs in that PNET tumors occur in younger individuals and small cell in patients over 40. Some overlap does occur.

Primary neuroblastoma of the lung is rare, but has been reported, largely in patients over the age of 20 (in contrast to abdominal cases). These rare pulmonary cases have been ganglioneuroblastomas. Primary pulmonary paraganglioma is a very rare tumor and its diagnosis requires the application of strict criteria; these should be cytokeratin negative and have S100 sustentacular cells

It is also important to keep in mind that small cell carcinoma can also have a variety of other mimickers that include lymphomas, desmoplastic small round cell tumor and rhabdomyosarcoma. The use of morphology – cohesion and organoid patterns in distinction from lymphoma, desmoplastic stroma in DSRCT and rhabdomyoblasts – and immunohistochemistry are important adjuncts to consider. Desmin, for example, is not positive in small cell carcinoma, and cytokeratin is not positive in most lymphomas. The use of immunohistochemistry panels in cases that present in unusual sites, age group or have atypical morphology is essential in these difficult cases.

References

- Aslan DL, Gulbahce HE, Pambuccian SE, Manivel JC, Jessurun J. Ki-67 immunoreactivity in the differential diagnosis of pulmonary neuroendocrine neoplasms in specimens with extensive crush artifact. *Am J Clin Pathol* 2005; **123**(6): 874-8.
- Pelosi G, Rodriguez J, Viale G, Rosai J. Typical and atypical pulmonary carcinoid tumor overdiagnosed as small-cell carcinoma on biopsy specimens: a major pitfall in the management of lung cancer patients. *Am J Surg Pathol* 2005; **29**(2): 179-87.
- Travis WD, Linnoila RI, Tsokos MG, et al. Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma. An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am J Surg Pathol* 1991; **15**(6): 529-53.
- Yang YJ, Steele CT, Ou XL, Snyder KP, Kohman LJ. Diagnosis of high-grade pulmonary neuroendocrine carcinoma by fine-needle aspiration biopsy: nonsmall-cell or small-cell type? *Diagn Cytopathol* 2001; **25**(5): 292-300.
- Hiroshima K, Abe S, Ebihara Y, et al. Cytological characteristics of pulmonary large cell neuroendocrine carcinoma. *Lung Cancer* 2005; **48**(3): 331-7.
- Hoshi R, Furuta N, Horai T, Ishikawa Y, Miyata S, Satoh Y. Discriminant model for cytologic distinction of large cell neuroendocrine carcinoma from small cell carcinoma of the lung. *J Thorac Oncol* 2010; **5**(4): 472-8.
- Jimenez-Heffernan JA, Lopez-Ferrer P, Vicandi B, et al. Fine-needle aspiration cytology of large cell neuroendocrine carcinoma of the lung: a cytohistologic correlation study of 11 cases. *Cancer* 2008; **114**(3): 180-6.

Khalbuss WE, Yang H, Lian Q, Elhosseiny A, Pantanowitz L, Monaco SE. The cytomorphologic spectrum of small-cell carcinoma and large-cell neuroendocrine carcinoma in body cavity effusions: A study of 68 cases. *Cytojournal* 2011; **8**: 18.

den Bakker MA, Willemsen S, Grunberg K, et al. Small cell carcinoma of the lung and large cell neuroendocrine carcinoma interobserver variability. *Histopathology* 2010; **56**(3): 356-63.

Iyoda A, Hiroshima K, Toyozaki T, Haga Y, Fujisawa T, Ohwada H. Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. *Cancer* 2001; **91**(11): 1992-2000.

Zacharias J, Nicholson AG, Ladas GP, Goldstraw P. Large cell neuroendocrine carcinoma and large cell carcinomas with neuroendocrine morphology of the lung: prognosis after complete resection and systematic nodal dissection. *Ann Thorac Surg* 2003; **75**(2): 348-52.

Peng WX, Sano T, Oyama T, Kawashima O, Nakajima T. Large cell neuroendocrine carcinoma of the lung: a comparison with large cell carcinoma with neuroendocrine morphology and small cell carcinoma. *Lung Cancer* 2005; **47**(2): 225-33.

Visscher DW, Zarbo RJ, Trojanowski JQ, Sakr W, Crissman JD. Neuroendocrine differentiation in poorly differentiated lung carcinomas: a light microscopic and immunohistologic study. *Mod Pathol* 1990; **3**(4): 508-12.

Paci M, Cavazza A, Annessi V, et al. Large cell neuroendocrine carcinoma of the lung: a 10-year clinicopathologic retrospective study. *Ann Thorac Surg* 2004; **77**(4): 1163-7.