

Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript

Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

Title: Endocrine and neuroendocrine cytopathology.

Authors: Bongiovanni M, La Rosa S, Sykiotis GP

Journal: Minerva endocrinologica

Year: 2018 Sep

Issue: 43

Volume: 3

Pages: 294-304

DOI: 10.23736/S0391-1977.17.02744-4

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

Endocrine and neuroendocrine cytopathology

Massimo Bongiovanni^{1*}, Stefano La Rosa¹, Gerasimos P. Sykiotis²

¹Service of Clinical Pathology, Lausanne University Hospital, Institute of Pathology, Lausanne, Switzerland.

²Service of Endocrinology, Diabetology and Metabolism, Lausanne University Hospital, Lausanne, Switzerland.

^{1*}Corresponding author:

Massimo Bongiovanni, MD

Service of Clinical Pathology

Lausanne University Hospital

Institute of Pathology

25, rue du Bugnon

CH-1011 Lausanne, Switzerland

Tel.: +41 21 314 72 02;

Fax: +41 21 314 72 05;

E-mail: massimo.bongiovanni@chuv.ch

Abstract

Cytology is an easily accessible, cost-effective and safe procedure for the initial evaluation of most endocrine/neuroendocrine lesions. Both fine-needle aspiration cytology and exfoliative cytology have shown good sensitivity and specificity in detecting endocrine/neuroendocrine benign proliferations and malignancies. Thanks to its utility for early diagnosis, cytology has contributed to the decline in mortality of endocrine/neuroendocrine neoplasms.

The endocrine system comprises different endocrine organs, such as the thyroid, adrenal glands, paraganglia, parathyroid, pancreas, hypothalamus, pituitary gland, ovaries and testes, which can give rise to non-neoplastic, benign and malignant proliferations. In addition, several neuroendocrine cells do not form specific endocrine organs, but are widely present along other systems, notably in the lungs and in the gastrointestinal tract. The general diagnostic approach to proliferations originating from neuroendocrine cells is similar to that of endocrine organs.

In this review we concentrate on the cytological features of neuroendocrine proliferations, with particular emphasis on their most common sites of origin, i.e. the thyroid, pancreas, lungs and skin. We also discuss ancillary approaches applied to cytological material to improve the diagnosis.

Key words: Endocrine – Neuroendocrine - Cytology - Fine-needle aspiration.

Introduction

The endocrine system can be virtually divided into the neuroendocrine (NE) and non-neuroendocrine (non-NE) system. The latter includes follicular thyroid cells, adrenal cortical cells, and endocrine cells of the ovaries and testes. The NE system consists of hormone or amine secreting cells showing a neural-like phenotype. NE cells are typically immunoreactive for general neuroendocrine markers including chromogranin A (ChA), synaptophysin (Syp), CD56, and neuron-specific enolase (NSE).^{1, 2} They can be structured to form specific NE organs like the pituitary gland, parathyroid, paraganglia, and adrenal medulla or are dispersed (gut, lungs, skin and thyroid neuroendocrine cells – C cells) or grouped in small clusters (pancreatic islets) in other exocrine organs. It was initially thought that all NE cells originate from the neural crest, but it is now recognized that NE cells located in the lung and digestive system derive from endodermal stem cells.³

Following the definition of the European Taskforce on Endocrine Cancer, endocrine (E) cancers are defined as malignant tumors arising in endocrine organs, like the thyroid, adrenal cortex, ovaries and the testes.⁴ Conversely, NE proliferations are defined as lesions originating from NE cells which give rise to lesions across the full spectrum applied to non-NE cells, ranging from hyperplasia and dysplasia to low grade malignant tumors and high grade neuroendocrine carcinomas, which are associated with different clinical aggressiveness and consequent therapeutic approach.

Cytological examination is usually the first and most frequently used approach to obtain a clear diagnosis for any suspected proliferation or mass in the human body and this can also be

applied to NE proliferations. Cells can physiologically shed or desquamate within existing cavities or organs and can then be collected naturally or with aspiration (urine, sputum, ascites, cerebrospinal fluids), or they can be forced to desquamate with various procedures, such as in the case of bronchial aspiration/brushing or biliary duct brushing (exfoliative cytology). A sample of a lesion can also be collected by inserting a needle into it: this is the basis of fine-needle aspiration cytology (FNAC), which is the most frequently used technique to investigate NE proliferations. In expert hands, FNAC is a safe, cost-effective and valuable approach.⁵ The accuracy of FNAC is higher if it performed under guidance by ultrasound (US) or computed tomography (CT).

In this review we address the following topics: (i) We examine the morphological appearance of NE proliferations in endocrine organs and in non-endocrine organs, with particular attention to cases where cytology plays an important role in the diagnostic process; (ii) we analyze the most recent technical advances in FNAC; (iii) we discuss the role of ancillary techniques in the diagnostic process; and, finally, (iv) we discuss the classification cytology systems and their impact on the management of NE lesions.

1. Morphological aspects of NE proliferations.

Apart from rare cases, such as in patients with known thyroid nodules associated with elevated plasma calcitonin levels or in patients with symptoms of specific endocrine syndromes (insulinoma, glucagonoma, carcinoid, Zollinger-Ellison, etc.), the clinical diagnosis of NE tumors is very rarely done pre-operatively. Thus cytopathologists should be aware of this possibility when examining cytological specimens, and possess a certain level of suspicion for these rare tumors.

NE tumors present as a variable spectrum of lesions; from benign or low-grade malignant tumors to highly aggressive forms. Cytology, either alone or in conjunction with proliferation markers such as the Ki-67 index, can easily differentiate between the different forms. In general, cytomorphological features for NE tumors are well defined.^{6,7}

For benign/low malignant tumors (variably called carcinoids, atypical carcinoids, or well differentiated NE tumors, according to the organ or origin and the classification system used) the most striking cytological feature is the presence of monotonous groups of cells with “salt and pepper” chromatin and absence of necrosis. Cells are monotonous in the sense that they are medium-sized and similarly shaped along the whole surface of the cytology slides. They are round, cuboidal or columnar in shape and can contain slightly granular and eosinophilic cytoplasm. Nuclei are round to oval, with a distinct nuclear membrane and characteristic “salt-and-pepper” chromatin, defined as granular, hyperchromatic chromatin. Nuclear pleomorphism is moderate and molding (conformity of adjacent cell nuclei to one another) is usually absent. NE cells present predominantly as isolated cells or in small groups that are

defined as being trabecular, solid, or rosette-like. Trabecular groups are elongated structures of cells with slightly angulated borders. Solid groups are densely packed aggregates of cells with no visible lumen. Rosette-like groups are oval structures composed at the periphery by circularly positioned NE cells surrounding a lumen, thus resembling a rosette. Finally, the background of the slide is either clean or hemorrhagic. The key features which define low-grade malignant proliferations are the absence of necrosis, which confers a clean aspect to the slide, and the absence of mitotic activity (Table 1).

High-grade malignant lesions (poorly differentiated NE carcinomas, small-cell and large-cell type) also show characteristic morphological features. The slide background is dirty and occupied by necrotic debris, cellular ghosts and apoptotic bodies. Necrosis can be so abundant as to be the only feature present on the slide. Cells are usually isolated and discohesive; they can be small as well as large, spindle or fusiform. Cytoplasm is scant and the nuclear/cytoplasmic (N/C) ratio is increased, except in the large-cell variant. Nuclei are large with condensed and coarsely granular chromatin, that can present multinucleation and can show abundant mitotic figures, molding, apoptotic bodies and prominent nucleoli. Crushing artifacts are typical in more aggressive forms (Table 2).

2. Technical advances in FNAC of NE neoplasms

The investigation of superficial and palpable lumps (especially in the thyroid) has traditionally been done with FNAC performed by simple palpation. However, the yield in terms of FNAC sampling was not very satisfactory. The advent of ultrasound (US) examination not only increased the detection of thyroid (including medullary thyroid carcinomas, MTC) and parathyroid nodules, but it also radically increased the yield of diagnostic material.⁸ US-FNAC is currently the standard of care for the initial investigation of thyroid and liver nodules.⁹ Reported sensitivity and specificity in distinguishing benign from malignant thyroid lesions was 65% to 98% and 72% to 100%, respectively.¹⁰

For the investigation of pulmonary and pancreatic masses, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound FNA (EUS-FNA), have been successfully introduced into daily clinical practice; these procedures are safe and they are increasingly replacing CT-guided FNAC.¹¹ The sensitivity of EBUS-TBNA in the diagnosis of lung cancer is reported to be up to 90% after a necessary learning curve, while its diagnostic accuracy is higher than that of CT and PET (98% vs. 60.8% and 72.5%, respectively).¹²¹³ The sensitivity of EUS-FNAC in diagnosing NE pancreatic lesions ranges from 84 to 94% and its specificity is as high as 95%.^{14, 15} The highest complication rate reported is 1.5% for EBUS-TBNA. Both procedures are minimally invasive, safe, cost-effective and are now considered the standard of care for the staging of lung cancers, and for the investigation of lung and pancreatic masses.¹⁶⁻¹⁹ The availability of skilled endoscopists is of primary importance to obtain high-quality material. In our hospital, both EBUS-TBNA and EUS-FNA are performed in conjunction

with the cytological evaluation of the aspirated material by a cytopathologist. This approach is called rapid on-site evaluation (ROSE). In our experience, this procedure increases the yield of the collected material and permits the best triage of the specimen. This is particularly important in case of suspected NE morphology during the rapid evaluation: in such a case, the cytopathologist asks for additional passes to realize a cytoblock, which is treated as a small biopsy and permits the testing for multiple immunocytochemical markers that are needed to confirm the NE diagnosis.¹³ Moreover, the adequate routing of aspirated material done during the ROSE, permits the time before the final diagnosis to be shortened and the patient can be directed to the appropriate specialist more quickly. In our daily practice we use a linear endosonographic device with a 22- or 25-gauge needle. The first pass is used to prepare a smear that is rapidly (10 seconds) stained with toluidine-blue staining; we assess the quality of material and the possible diagnosis. In case of a suspected NE neoplasm, we use the second, third and even fourth pass to enrich the yield for the production of a cytoblock.

Cytological examination is less reported for the identification of NE lesions originating from other NE organs. Pituitary and hypothalamic lesions are rarely diagnosed by cytology in the first instance; interestingly, we identified the cells of a pituitary carcinoma by cytological examination of cerebrospinal fluid, during an investigation for an invalidating headache (Figure 1). Adrenal, ovarian and testicular proliferations can undergo US-guided FNA. Although pheochromocytomas and paragangliomas can be easily identified by cytology²⁰, hypersecretion of catecholamines should be excluded before any biopsy of an adrenal mass or possible paraganglioma to avoid an adrenergic crisis.

3. Ancillary techniques in the diagnostic process

Due to the complexity and peculiarity of the biology and treatment of NE neoplasms, a panel of immunocytochemical analyses should always confirm the cytological diagnosis.²¹ Immunocytochemistry can be performed on classical smears, liquid-based preparations or cytoblocks. The first step of the diagnostic work-up in case of suspected NE lesions, irrespective of the site of origin, is to confirm the epithelial nature of the proliferation. For this, large-spectrum cytokeratins are used, such as AE1/AE3. In poorly differentiated forms, such as pulmonary small-cell carcinomas, the lymphocytic marker CD45 is often used as well, as the differential diagnosis with a lymphoproliferative disorder may be difficult. The second step is the demonstration of a NE differentiation. The most frequently used NE markers are ChA and Syp; they are always used together and variably combined with CD56 or NSE. Among them, ChA is the most specific whilst synaptophysin is the most sensitive. CD56 and NSE have low sensitivity since they are expressed in several non-NE neoplasms (Figure 2 and 3). In selected cases, the Ki-67 proliferative marker is used to assess the fraction of proliferating cells, as this information is important for the classification (i.e. pancreatic NE tumors)(Figure 4).²²

Besides these general epithelial and NE markers, more specific organ-related immunocytochemical markers can be useful and applied routinely. In the thyroid, immunostaining for calcitonin (CT), carcinoembryonic antigen (CEA) and thyroglobulin (Tg) are mandatory to confirm the diagnosis (Figure 5). Indeed, MTC is positive for CT and CEA and negative for Tg, even if some rare exceptions do exist (i.e. the small-cell variant of MTC stains negatively for CgA).²³ Moreover, the Congo red cytochemical staining for amyloid is very useful

on cytoblock material. In the lung, NE proliferations can secrete a variety of hormones that cause clinically evident paraneoplastic syndromes (syndrome of inappropriate secretion of antidiuretic hormone, syndrome of ectopic secretion of corticotropin-releasing hormone and syndrome of ectopic secretion of adrenocorticotrophic hormone, ACTH). Some of these hormones can also be detected immunocytochemically in the NE cells such as ACTH, CT and the CT gene-related peptide, bombesin. Pancreatic NE tumors can be functioning or nonfunctioning. However, independently of the clinical presentation, a variety of hormones can be immunocytochemically detected in NE cells: the ones most frequently used in cytology are insulin, glucagon, somatostatin, gastrin, serotonin, ACTH and vasoactive intestinal peptide (VIP). In pancreatic NE tumors, somatostatin receptor type 2A can also be detected in cytological specimens.²⁴ In the study of suspected parathyroid lesions (parathyroid adenomas and carcinomas), immunohistochemistry for parathyroid hormone (PTH) is very useful, as the morphological aspect of FNAC of parathyroid lesions is indistinguishable from the most common follicular-patterned thyroidal lesions. It is worth noting that routine FNAC of parathyroid lesions is not recommended as it can cause disseminated seeding of the cells leading to inoperable residual disease, termed "parathyromatosis". In paragangliomas and pheochromocytomas, attention should be given to the negative expression of cytokeratins, which are conversely expressed by almost all other NE tumors. A positive immunocytochemical staining for S-100 protein, which is expressed in sustentacular cells, confirms the diagnosis.²¹ In the skin, Merkel cell carcinomas shows a characteristic paranuclear and dot-like cytokeratin-20 expression, as well as negativity for TTF-1.

In cases of metastatic NE tumors, notably to the liver, and in the absence of a known primary lesion, a combination of different immunocytochemical markers and hormonal markers can be helpful to establish the site of origin of the metastasis. Duan et al. proposed an algorithmic approach for the determination of the site of origin in cases of well differentiated NE tumors, combining thyroid transcription factor 1 (TTF-1), caudal type homeobox 2 (CDX-2), insulin gene enhancer protein (Islet-1), pancreatic and duodenal homeobox 1 (PDX-1) and prostatic acid phosphatase (PAP).²¹ However, these markers are only useful in the diagnostic work-up of well differentiated neoplasms, because their expression in poorly differentiated neuroendocrine carcinomas does not respect the site of origin.

4 . Cytological classification and management of NE tumors

An internationally accepted cytology classification scheme exists only for pancreatic and thyroid tumors.^{25, 26} The Papanicolaou Society of Cytopathology recommended the use of a six-tiered diagnostic category system (Table 3) for pancreatic lesions. It is also recommended to adhere to this classification for NE pancreatic tumors. In the majority of cases, well differentiated pancreatic NE tumors are classified in the “neoplastic category”, under the specification “other”. One motivation for not classifying well differentiated/low grade pancreatic NE tumors in either the benign or the malignant diagnostic category is to allow a broad choice of management options for these lesions in accordance with the clinical-pathological situation (e.g. age and general health condition of the patient, clinical hormonal symptoms, size and proliferation rate of the lesion, compression of other structures). In cases

of suspected more aggressive neoplasms, showing necrosis, mitotic activity or high grade atypia, the most appropriate cytological diagnosis is “suspicious” or “positive”.

For MTC, as for the other thyroids neoplasms, the classification system widely used is The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). MTC are usually classified into the diagnostic category V (suspicious for malignancy) or VI (malignant) (Table 4). Both categories have high malignancy risks (60 to 75% and 97 to 99%, respectively) that justify a surgical approach.

The management of others NE tumors varies according to the site of origin. Several guidelines have been published by expert pathologists and clinicians, and are available on the website of the European Neuroendocrine Tumor Society (ENETS).²⁷ A consensus on the diagnosis and treatment has been difficult to reach in some cases, due to the limited evidence available in the literature for these rare tumors. Generally, if the tumor is resectable, surgery is the first line of treatment. In advanced cases, other options for local and/or systemic therapy can be considered.

It is noteworthy to remember that in several cases, the cytological material may represent the only source of tumor tissue for diagnostic purposes; thus appropriate biobanking and tissue preservation is strongly recommended to facilitate the use of new diagnostic tools and patient-tailored future therapies.

5. Conclusions

In conclusion, cytology remains the most accurate (and sometimes the only) diagnostic approach to the initial management of NE lesions. Furthermore, the application of

immunocytochemical staining and the use of the proliferation index is fundamental for the appropriate classification and consequent management, for patients suffering NE diseases.

Figure legends

Figure 1. Pituitary carcinoma with growth hormone expression in cerebrospinal fluid (CSF).

This was the case of a 16-year-old female presenting with severe headaches, vertical diplopia, and nausea for the previous 6 days. Neurological examination showed partial deficit of the third pair of cranial nerve ptosis and diplopia. Endocrinological examination revealed hypocorticism and hypothyroidism. A sellar IRM was performed showing a 2.7 cm sellar mass. Clinically, the possible diagnoses were hypophysary apoplexia or a craniopharyngioma. Cerebrospinal fluid analysis and resection of the mass were performed. **A.** CSF examination showed a monotonous group of bland-appearing cells (arrows) along with amorphous debris (Cytospin preparation, Papanicolaou staining, 200x). **B.** The cell block preparation showed the same population of cells with abundant eosinophilic cytoplasm (Hematoxylin and Eosin staining, 200x). **C.** Cells in the cytoblock were intensely positive for the growth hormone and only scattered cells showed prolactin expression (Inset, immunohistochemical staining, 200x). LH, TSH, ACTH and HCG were all negative. The final cytological diagnosis was: cerebrospinal fluid infiltrated by a pituitary carcinoma with GH diffuse expression in immunocytochemistry. **D.** The resection specimen showed a monomorphic proliferation of cells with a lack of a reticulin network among neoplastic cells and was consistent with a pituitary carcinoma with GH expression (Hematoxylin and Eosin staining, 100x).

Figure 2. Immunocytochemical panel used as the first line diagnosis of neuroendocrine lung tumors in cases of suspicious morphology.

In this case, a 71-year-old male was investigated for multiple mediastinal adenopathy following weight loss and cough. **A.** EBUS-TBNA of lymph node station 11R was performed and showed a densely packed group of small cells, with scant cytoplasm and dark nuclei. Associated are small lymphocytes (arrows) and macrophages (arrow head) demonstrating that the FNA was performed in a lymph node (Papanicolaou staining, Liquid-based preparation, 600x). **B.** The cell block preparation showed the same population of small cells with some more spindled elements, a high mitotic activity (arrow heads indicate mitosis) necrosis (asterisks) and some scattered lymphocytes (arrows). The differential diagnosis is between a small cell lung carcinoma metastatic to a lymph node and a lymphoma (Cell block preparation, Hematoxylin and Eosin staining, 600x). **C.** shows dot-like positivity (arrows) of malignant cells for cytokeratin MNF116, an intense cytoplasmic positivity of normal bronchial cylindrical cells (asterisks) for the same immunocytochemical marker and negativity of all other cells present (lymphocytes and macrophages) (immunocytochemical staining, 600x). **D.** Conversely, CD45 shows negativity

in malignant cells (asterisks) and scattered positive lymphocytes (arrows) demonstrating the epithelial nature of the malignant proliferation (immunocytochemical staining for CD45, 600x). Other markers should then be used to prove the neuroendocrine nature of the malignant cells, such as **E.** Chromogranin A (immunocytochemical staining, 400x), **F.** Synaptophysin (immunocytochemical staining, 600x), both with granular para-nuclear positivity and **G.** CD56 (immunocytochemical staining, 600x). **H.** In the case of lung neuroendocrine carcinoma, the proliferation index (ki-67) is not necessary for classification, but is used to demonstrate the highly proliferative activity of the lesion (in this case 50% of cells are proliferating), thus confirming the diagnosis (immunocytochemical staining, Mib-1 antibody, 600x). The final cytopathological diagnosis was: metastatic small cell neuroendocrine carcinoma.

Figure 3. Large cell neuroendocrine carcinoma of the lung.

A 65-year-old female with a history of smoking and chronic obstructive pulmonary disease was discovered with a lung mass and multiple adenopathies and investigated with EBUS-TBNA of Baretty's space lymph nodes. **A.** Cytological smears showed isolated malignant large cells, with scant cytoplasm, dark and nucleated nuclei (Smear, Papanicolaou staining, 600x). **B.** A different smear showed the typical polymorphous and atypical nuclei and the molding aspect of the delicate chromatin (Smear, Papanicolaou staining, 600x). **C.** Smears can also be used to perform immunocytochemical staining, showing in this slide positive para-nuclear and dot-like expression for Chromogranin A (immunocytochemical staining, 600x). **D.** The cell block was used to biobank the material in case of future additional testing. Large cells were present (arrows) as well as necrosis (asterisk) and normal lymphocytes (arrow heads) (Cell block preparation, Hematoxylin and Eosin staining, 600x). The final cytopathological diagnosis was: metastatic large cell neuroendocrine carcinoma.

Figure 4. Pancreatic neuroendocrine proliferations.

A 72-year-old male with chronic diarrhea was discovered to have a pancreatic mass in the head. EUS-FNAC with ROSE evaluation by a cytopathologist was performed in the pancreatic lesion. **A.** Cytological ROSE smears showed isolated tumoral cells with scant cytoplasm, and nucleated nuclei. Blood is present in the background of the slide. Note the poor quality of the staining, being performed in 10 seconds for rapid evaluation and assessment. Following the cytological ROSE examination (suspicion of a neuroendocrine proliferation), additional passes were performed to create a rich cell block (Smear, Toluidine blue, 600x). **B.** The cell block preparation showed the same monotonous proliferation of cells, with moderate cytoplasm. Nucleoli are present and necrosis is absent (Cell block preparation, Hematoxylin and Eosin

staining, 600x). **C.** The neuroendocrine nature of the lesion was demonstrated by the intense and cytoplasmic expression for Chromogranin A (immunocytochemical staining, 600x). **D.** the proliferation index (ki-67) is necessary for grading and classification. In this case less than 1% of cells are proliferating, thus confirming the low grade nature of the tumor (immunocytochemical staining, Mib-1 antibody, 100x). The final cytopathological diagnosis was: pancreatic well differentiated neuroendocrine tumor (NET), G1. Inset shown for comparison: a different case of pancreatic neuroendocrine proliferation with Ki-67 index around 80%. In this case the diagnosis is pancreatic poorly differentiated neuroendocrine carcinoma (NEC), G3 (immunocytochemical staining, Mib-1 antibody, 200x).

Figure 5. FNAC of a medullary thyroid carcinoma.

A 37-year-old female with an unremarkable medical history presented with a small pre-tracheal nodule, probably originating from the thyroid. It was described as “not very suspicious” by the endosonographer. **A.** The smear was hypercellulated (Smear, Papanicolaou staining, 100x) and also contained scattered amorphous material, suspicious to be amyloid (Inset, Papanicolaou staining, 600x). **B.** Malignant cells were large with abundant and granular cytoplasm, organized in ribbons and nuclei presented pseudoinclusions (arrows). The chromatin of the other cells was granular (Smear, Papanicolaou staining, 600x). **C.** The cell block showed the same morphological characteristics of the smear and an organoid growth pattern. Immunocytochemical staining performed on the smears showed **(D)** intense expression for Calcitonin and **(E)** focal expression for Chromogranin A (immunocytochemical staining, 200x). The final cytopathological diagnosis was: medullary thyroid carcinoma. **F.** The histological specimens showed a trabecular / insular pattern of growth (Hematoxylin and Eosin staining, 200x) and an intense expression for calcitonin, while the surrounding normal thyroid parenchyma was negative (asterisks) (Inset, immunohistochemical staining, 100x).

References

1. Cutz E. Neuroendocrine cells of the lung. An overview of morphologic characteristics and development. *Exp Lung Res.* 1982;3: 185-208.
2. Szczyrba J, Niesen A, Wagner M, Wandernoth PM, Aumuller G, Wennemuth G. Neuroendocrine Cells of the Prostate Derive from the Neural Crest. *J Biol Chem.* 2017;292: 2021-2031.
3. Rosai J. The origin of neuroendocrine tumors and the neural crest saga. *Mod Pathol.* 2011;24 Suppl 2: S53-57.
4. Jervis N, Bouvier C. Cancerous neuroendocrine vs endocrine tumours: broad commonalities and differences. 19th European Congress of Endocrinology. Lisbon, Portugal: bioscientifica, 2017.
5. Roskell DE, Buley ID. Fine needle aspiration cytology in cancer diagnosis. *Bmj.* 2004;329: 244-245.
6. Nicholson SA, Ryan MR. A review of cytologic findings in neuroendocrine carcinomas including carcinoid tumors with histologic correlation. *Cancer.* 2000;90: 148-161.
7. 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: 292-300.
8. Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. *Nat Rev Endocrinol.* 2016;12: 646-653.
9. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. *Acta Cytol.* 2012;56: 333-339.
10. Gharib H, Goellner JR. Fine-needle aspiration biopsy of the thyroid: an appraisal. *Ann Intern Med.* 1993;118: 282-289.
11. Jhala D, Eloubeidi M, Chhieng DC, et al. Fine needle aspiration biopsy of the islet cell tumor of pancreas: a comparison between computerized axial tomography and endoscopic ultrasound-guided fine needle aspiration biopsy. *Ann Diagn Pathol.* 2002;6: 106-112.
12. Yasufuku K, Nakajima T, Motoori K, et al. Comparison of endobronchial ultrasound, positron emission tomography, and CT for lymph node staging of lung cancer. *Chest.* 2006;130: 710-718.
13. Righi L, Franzi F, Montarolo F, et al. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)-from morphology to molecular testing. *J Thorac Dis.* 2017;9: S395-s404.
14. Bernstein J, Ustun B, Alomari A, et al. Performance of endoscopic ultrasound-guided fine needle aspiration in diagnosing pancreatic neuroendocrine tumors. *Cytojournal.* 2013;10: 10.
15. Lee LS. Diagnosis of pancreatic neuroendocrine tumors and the role of endoscopic ultrasound. *Gastroenterol Hepatol (N Y).* 2010;6: 520-522.
16. VanderLaan PA, Wang HH, Majid A, Folch E. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA): an overview and update for the cytopathologist. *Cancer Cytopathol.* 2014;122: 561-576.
17. Manta R, Nardi E, Pagano N, et al. Pre-operative Diagnosis of Pancreatic Neuroendocrine Tumors with Endoscopic Ultrasonography and Computed Tomography in a Large Series. *J Gastrointestin Liver Dis.* 2016;25: 317-321.
18. Kann PH, Moll R, Bartsch D, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) in insulinomas: Indications and clinical relevance in a single investigator cohort of 47 patients. *Endocrine.* 2017;56: 158-163.
19. Vaidya PJ, Munavvar M, Leuppi JD, Mehta AC, Chhajed PN. Endobronchial ultrasound-guided transbronchial needle aspiration: Safe as it sounds. *Respirology.* 2017;22: 1093-1101.

20. Jimenez-Heffernan JA, Vicandi B, Lopez-Ferrer P, Gonzalez-Peramato P, Perez-Campos A, Viguer JM. Cytologic features of pheochromocytoma and retroperitoneal paraganglioma: a morphologic and immunohistochemical study of 13 cases. *Acta Cytol.* 2006;50: 372-378.
21. Duan K, Mete O. Algorithmic approach to neuroendocrine tumors in targeted biopsies: Practical applications of immunohistochemical markers. *Cancer.* 2016;124: 871-884.
22. Carlinfante G, Baccarini P, Berretti D, et al. Ki-67 cytological index can distinguish well-differentiated from poorly differentiated pancreatic neuroendocrine tumors: a comparative cytohistological study of 53 cases. *Virchows Arch.* 2014;465: 49-55.
23. Yerly S, Triponez F, Meyer P, Kumar N, Bongiovanni M. Medullary thyroid carcinoma, small cell variant: a diagnostic challenge on fine needle aspiration. A case report and review of the literature. *Acta Cytol.* 2010: In press.
24. Papotti M, Croce S, Bello M, et al. Expression of somatostatin receptor types 2, 3 and 5 in biopsies and surgical specimens of human lung tumours. Correlation with preoperative octreotide scintigraphy. *Virchows Arch.* 2001;439: 787-797.
25. Pitman MB, Centeno BA, Ali SZ, et al. Standardized terminology and nomenclature for pancreatobiliary cytology: the Papanicolaou Society of Cytopathology guidelines. *Diagn Cytopathol.* 2014;42: 338-350.
26. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. *Thyroid.* 2009;19: 1159-1165.
27. https://www.enets.org/enets_guidelines.html. (Accessed 15.08.2017).

Table 1: Main cytomorphological features of benign/low malignant potential neuroendocrine tumors.

Background	Clean or hemorrhagic
Cellularity	Highly cellular smear, monotonous cells isolated or in aggregates (trabecular, solid, rosette-like)
Cytoplasm	Granular and eosinophilic; low N/C ratio
Cell	Medium sized cells with polygonal/cuboidal appearance
Nuclei	Nuclei round to oval with “salt & pepper” chromatin, regular nuclear membrane

N/C: nuclear/cytoplasmic

Table 2: Main cytomorphological features of well differentiated/malignant neuroendocrine carcinomas.

Background	Hemorrhagic and/or necrotic
Cellularity	Highly cellular smear, polymorphous cells isolated or in irregular aggregates
Cytoplasm	Inconspicuous with high N/C ratio in small cell carcinomas; abundant in large cell carcinomas with low N/C ratio
Cell	All possible sized cells with monstrous appearance
Nuclei	Nuclei enlarged, polymorphous, bizarre, multinucleated, “salt & pepper” chromatin, irregular nuclear membrane, multiple mitotic figures

Table 3: The classification systems for reporting pancreatobiliary cytology. Most well differentiated NE tumors fall into the neoplastic category, while poorly differentiated NE tumors fall in the suspicious or positive/malignant category (modified by: Pitman MB et al.).²⁵

Diagnostic category
Non-diagnostic
Negative
Atypical
Neoplastic (benign or other)
Suspicious
Positive/malignant

Table 4: The classification systems for reporting thyroid cytology know as The Bethesda System for Reporting Thyroid Cytopathology. Thyroid medullary carcinomas are usually diagnosed in the diagnostic category V and VI (modified by Ali and Cibas).²⁶

Diagnostic category	Risk of malignancy, %	Usual management
I. Non-diagnostic or unsatisfactory	1-4	Repeat FNA with ultrasound guidance
II. Benign	0-3	Clinical follow-up
III. AUS/FLUS	5-15	Repeat FNA
IV. FNS/SFN	15-30	Lobectomy
V. Suspicious for malignancy	60-75	Near-total thyroidectomy or lobectomy
VI. Malignant	97-99	Near-total thyroidectomy

Figure 1. Pituitary tumor with growth hormone expression in CSF

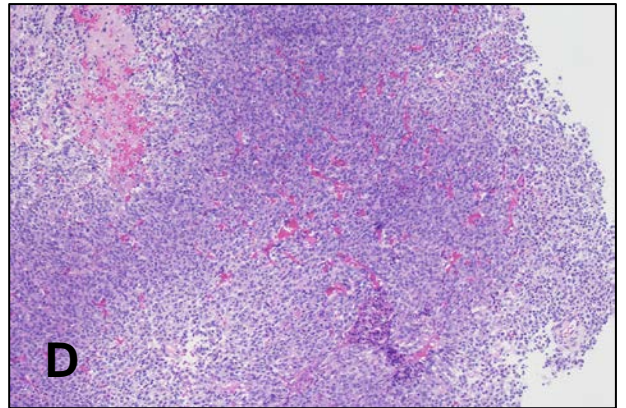
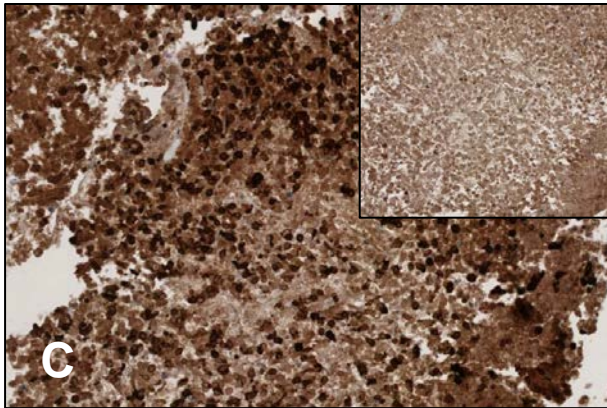
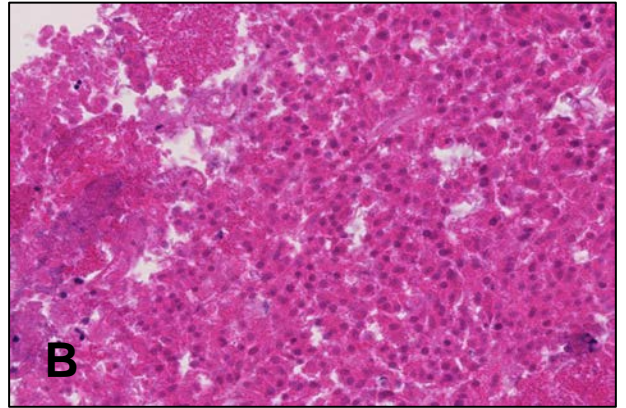
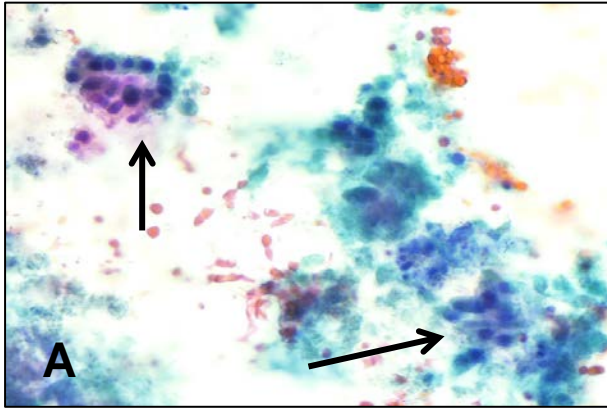


Figure 2. Lung small cell NE carcinoma, EBUS

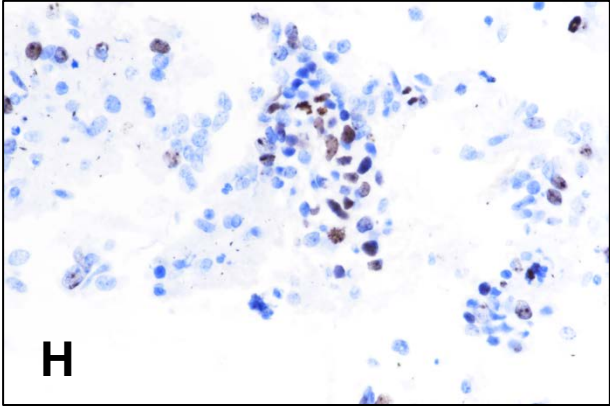
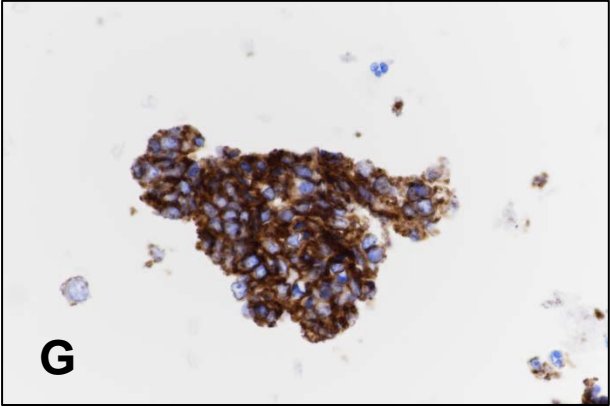
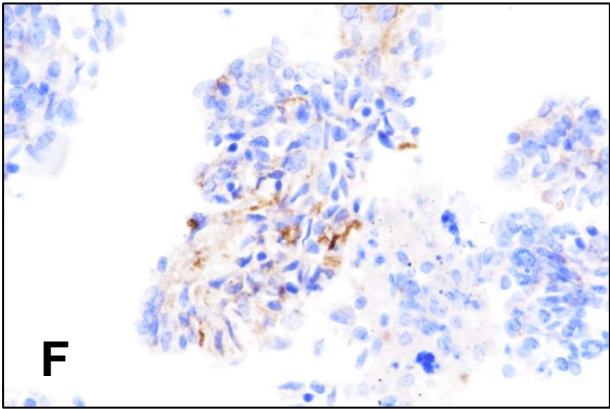
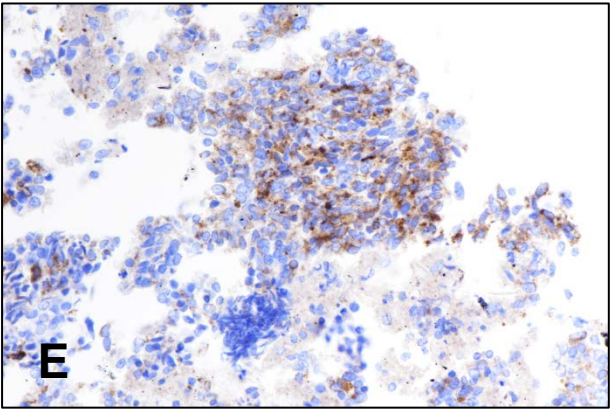
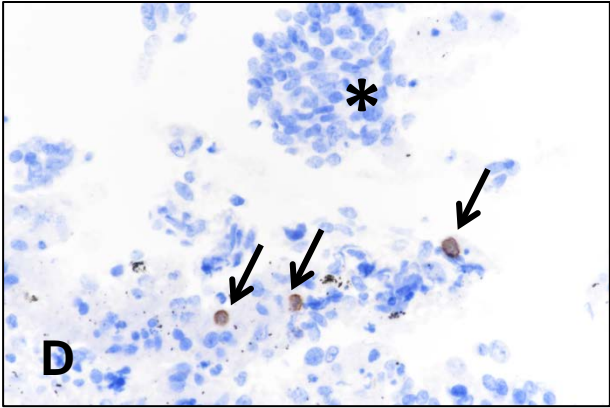
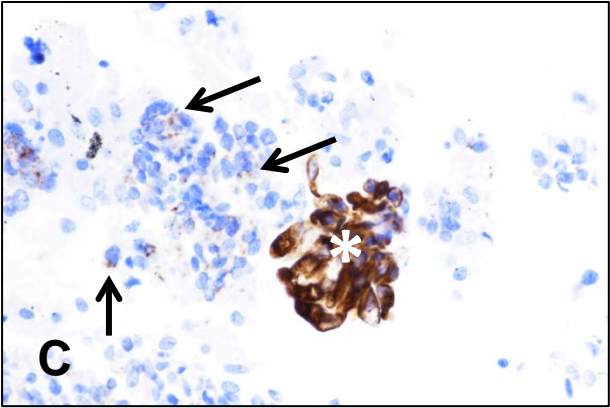
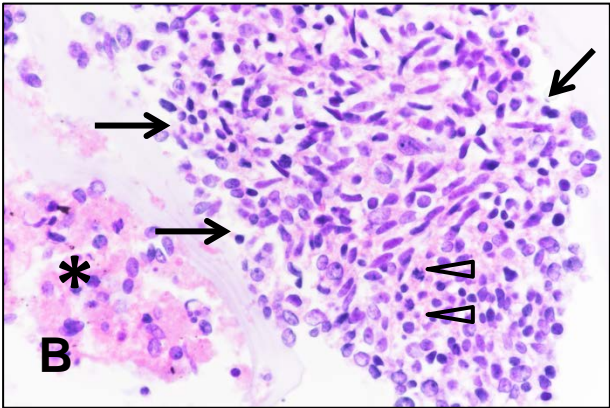
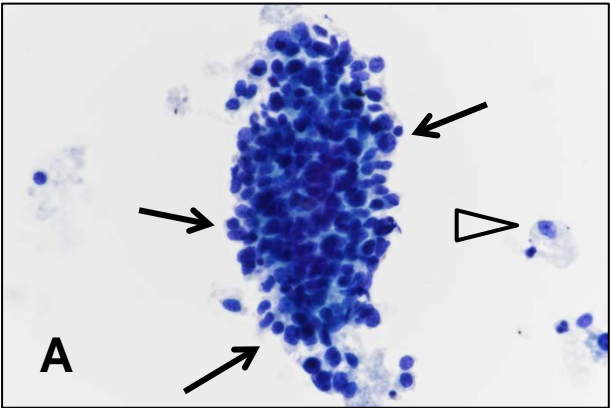


Figure 3. Lung large cell NE carcinoma, EBUS

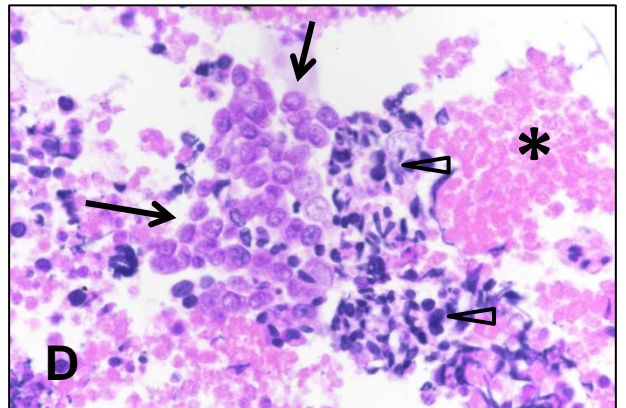
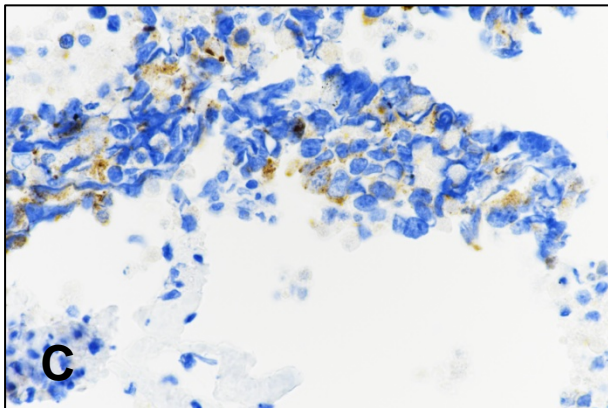
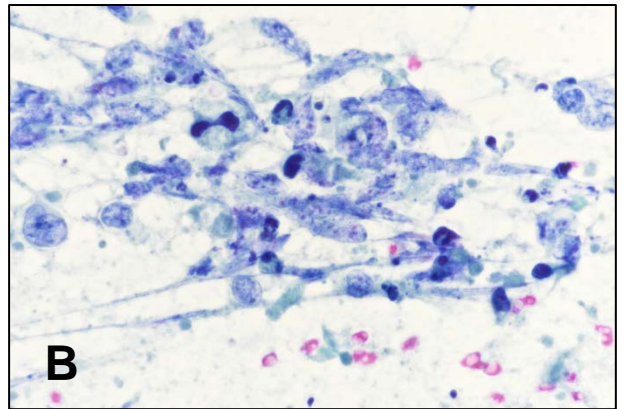
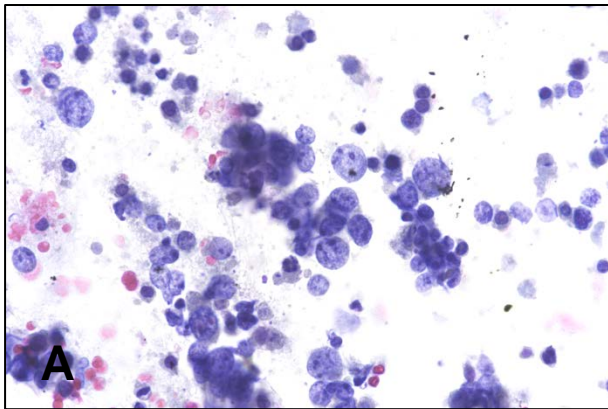


Figure 4. Pancreatic well differentiated NE tumor (NET), G1, EUS

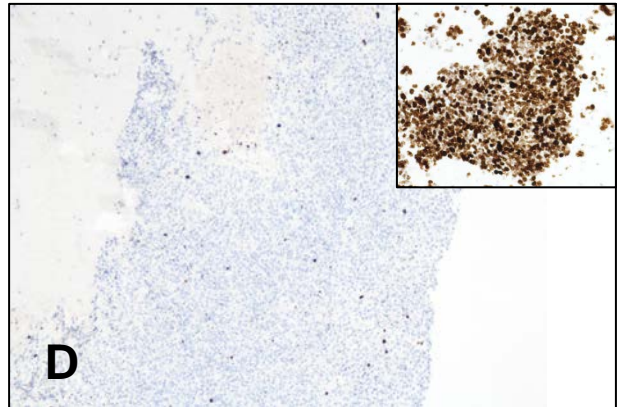
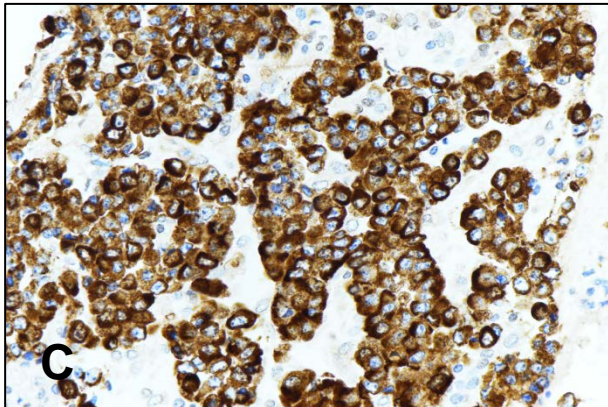
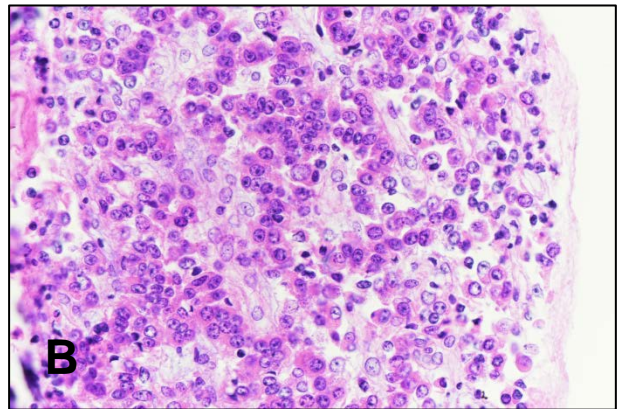
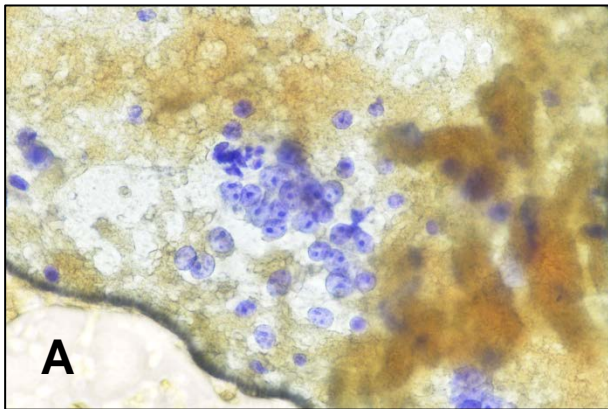


Figure 5. Thyroid medullary carcinoma, fine-needle aspiration

