# Reliable pathology reporting of extra-pulmonary large cell neuroendocrine carcinoma: pertinence of WHO 2010 guideline

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*Submitted*

## ABSTRACT

**Introduction:** Neuroendocrine carcinomas (NEC) are diagnosed through a combination of immunohistochemistry (IHC) and morphology according to WHO guidelines. A reliable diagnosis is critical for treatment selection and interpretation of trials. Aim of this study is to assess the effect of WHO 2010 on pathology reporting for NEC and on prognostic significance.

**Methods:** Patients registered with a NEC (gastrointestinal or unknown origin) in the Netherlands Cancer Registry (NCR) between 2008 and 2012 were included. Local pathology reports were reviewed for reporting of morphology and IHC comparing 2008-2010 (baseline) with 2011-2012. The diagnosis of NEC was confirmed according to WHO 2010, if synaptophysin or chromogranin were positive in a majority of cells and Ki67 or mitotic count confirmed a grade 3 tumour.

**Results:** 591 patients were registered with a NEC in the NCR. 436 pathology reports were reviewed. 63.2% of reports described morphology, IHC and grading in accordance with WHO 2010. Reporting of these parameters increased from 50.0% in 2008 to 70.6% in 2012. Large cell NEC could be confirmed in 45.0% of patients, increasing from 31.7% in 2008 to 56.7% in 2012 (p=0.02). Other diagnoses included NET G1/2 13.3%, small cell carcinoma 2.8%, no NEN 17.7%, NEN grade unknown 21.3%. Mean survival was 1.1 years in large cell NEC versus 2.2 years in NET G1/2 (p=0.005).

**Conclusion:** Implementation of the WHO 2010 guideline resulted in a significant increase of reporting parameters needed for classification. This has important implication for prediction of survival.

## INTRODUCTION

Histopathology is fundamental for the diagnosis of neuroendocrine neoplasms (NENs). Biomarkers and imaging can certainly provide circumstantial evidence but a biopsy is needed to confirm the diagnosis and for prognostic stratification.[1] The correct diagnostic process is of great importance for several reasons: firstly, for the individual patient as diagnosis and grading is critical for the selection of correct treatment. Secondly, a uniform diagnosis is needed to interpret clinical trials and cohort studies. It is often assumed that publication of a guideline ensures this much needed correct and uniform diagnosing. Specifically for NENs several guidelines or classifications on histopathology have been published over time. In the last decades the classification of NENs has evolved from a classification based on embryological origin[2] via a classification based on morphology (well versus poorly differentiated) and size[3] to a classification based on proliferative activity.[4] In the most recent WHO classification for neuroendocrine tumours of the pancreas of 2017 high grade tumours are separated again based on morphology.[5] In 2010 the classification of NENs changed, parting with a system defining a high-grade malignant group based on metastases, invasion and differentiation. Simultaneously, the pathology reports can be expected to have evolved along with the evolution of NEN classification. Therefore we set out to investigate whether the implementation of the WHO 2010 guideline actually resulted in a change in pathology reporting for non-lung neuroendocrine carcinoma's (NEC) in the Netherlands Cancer Registry (NCR) around the period of WHO 2010 publication. To evaluate the pertinence of the guideline relation with survival was investigated.

## METHODS

In the Netherlands, information on all patients with cancer is recorded in the Netherlands Cancer Registry (NCR), which covers 95% of all cancers. Primary notification occurs through the histopathological diagnosis made by the local pathologist. Demographics, tumour characteristics and treatment are also registered. Morphology and topography of the tumour is recorded using the International Classification of disease for Oncology third edition (ICD-O3). The datamanager of the NCR is obligated to follow the conclusion of the local pathologist. The datamanager selects an ICD-O3 code based on the histopathological conclusion by the pathologist. For this study all patients with a large cell extra-pulmonary neuroendocrine carcinoma (ICD-O3 code M8013) or a neuroendocrine carcinoma not otherwise specified (ICD-O3 code M8246) were included if registered from 2008 to 2012. Only tumours from the gastrointestinal tract or unknown primary tumour were included. From the NCR, vital status, extent of disease, primary origin, age at diagnosis and gender were collected. Using an anonymized link via a third trusted party between PALGA[6] (the nationwide network and registry of histo- and cytopathology in the Netherlands) and the NCR, pathology reports of

these patients were obtained from the time of diagnosis. Microscopy text was reviewed for morphological appearance, immunohistochemistry, Ki-67 index, mitotic figures, necrosis and differentiation.

As the aim of this study was to demonstrate a change in pathology reporting after the implementation of WHO 2010, we used pathology reports from the period 2008-2010 as baseline to compare with reports after WHO 2010 in the period 2011-2012. This was possible because the ICD-O3 codes did not change in 2010. Percentages of cases with complete reporting of neuroendocrine markers, mitotic index and Ki-67 index were calculated for the period 2008-2010 (baseline) and 2011-2012 to potentially demonstrate difference in diagnostic strategy.[7] Thereafter we aimed to study if the implementation of the WHO 2010 resulted in a more uniform diagnostic pattern. Using the reports from patients registered in the NCR as well as the information from the original pathology report we classified Neuroendocrine Carcinomas (M8013 or M8246) using the flow-chart shown in Figure 1. We aimed to reproduce the diagnosis of a NEC with use of the described morphology, IHC and grading. First only patients concluded to have a NEC by the local pathologist were included. Pathology conclusions diagnosing small cell carcinoma, low-grade NEN or carcinoma with neuroendocrine differentiation were regarded to be registered incorrectly . They were excluded because the quality standard defined for NEC did not apply for these tumours.

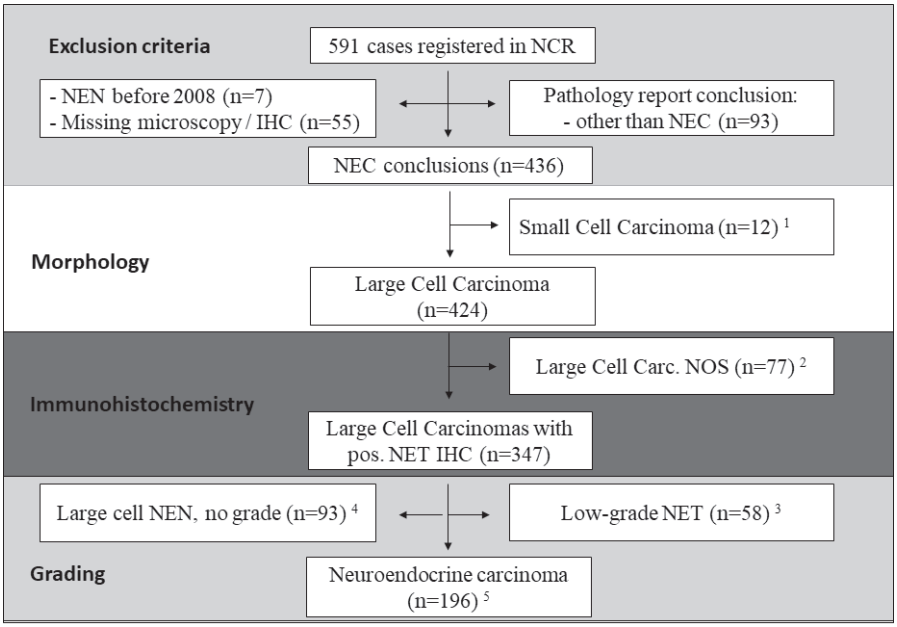


**Figure 1:** Flow chart of neuroendocrine carcinoma diagnosis

1: Small cell carcinoma; 2: Large Cell Carcinoma, Not Otherwise Specified (Negative IHC), 3: Low grade NET (Pos IHC, Ki-67< 21% or Mitotic Count < 21 per 10 HPF), 4: Large Cell NEC, not graded (Pos IHC, missing Ki-67 and Mit Count) 5: True NEC (pos IHC and Ki-67>20% or Mitotic Count >20 per 10 HPF)

If no immunohistochemistry was described at all, the patient was excluded, as immunohistochemistry might have been performed but could not be tracked with the method used. Then, reports were classified as reporting a small cell or large cell carcinoma, either on the basis of the morphological description or histopathological conclusion. Small cell neuroendocrine carcinomas can be (focal/faint) positive or negative for neuroendocrine immunohistochemistry.

As a third step, large cell carcinomas required a report of a majority of tumour cells positive with immunohistochemical staining for either chromogranin or synaptophysin. Immunohistochemical staining for CD56 was also registered, but was not used for classification as NEN as it is an adhesion molecule and not a neuroendocrine protein.[8] If a neuroendocrine stain was not described, while other (non-neuroendocrine) markers were described, the neuroendocrine marker was assumed not to be performed. If all neuroendocrine stains were negative or weakly positive the diagnosis was revised to "Large cell carcinoma not otherwise specified" (LCC NOS).

As a fourth step proliferative indexes, mitosis and Ki-67 index, were evaluated. The Ki-67-staining was required to be higher than 20% or the mitotic count needed to be higher than 20 per 10 high-power fields (HPF) in accordance with ENETS/WHO 2010 grading[9], for the tumours to be classified as neuroendocrine carcinoma (NEC). When there was a discrepancy between Ki-67 and mitotic count, the highest grade was used for stratification. Patients with Ki-67 index smaller than 20% and mitotic count below 20 per 10 HPF were recorded as a low-grade neuro-endocrine tumour (low-grade NET). If no Ki-67 was done and mitotic index not mentioned NENs were classified as "NEN, unknown grade". In certain cases de Ki-67 or mitotic count were described subjectively (e.g. high/low or abundant). Cases with high or abundant proliferation parameters were classified as NEC and with low levels as NET.

To determine the adherence to the WHO 2010 guideline, percentages of cases with complete reporting of neuroendocrine markers, mitotic index and Ki-67 index were calculated. A Chi-square test was performed to test if the proportion of cases in which all WHO 2010 parameters were reported increased for the years 2008 through 2012. To validate the model, survival of the different diagnostic groups was estimated with a Kaplan Meier and difference in survival was tested with a log-rank test. A univariate analysis was performed to calculate hazard ratios.

## RESULTS

From 2008 through 2012 a total of 591 patients were registered in the NCR with a large cell extra-pulmonary neuroendocrine carcinoma (M8013) or a neuroendocrine carcinoma not otherwise specified (M8246) from the gastro-intestinal tract or unknown origin. Seven patients were excluded because of a NEN being reported in the PALGA pathology registry

before 2008 and another 55 patients were excluded due to missing microscopy or immunohistochemistry and therefore were not applicable for this study. Of the remaining 529 NEC cases in the NCR, 436 (82.4%) were concluded to be a NEC in the local pathology report conclusions. The 93 NCR cases for which the local pathology report assigned different conclusions included small cell carcinoma (n=15, 2.8%), carcinoma with neuroendocrine differentiation (n=29, 5.5%), low-grade NET (n=42, 7.9%) or other carcinomas (n=7, 1.3%). These tumours were regarded to be misclassified. As the defined quality criteria do not apply for these (non-NEC) tumours, only the 436 patients with a confirmed NEC conclusion in the pathology report were included in the further assessments. These patients were on average 66.8 years old and 56.2% was male. Most patients had a primary tumour in the colon, pancreas or of unknown primary origin (respectively 16.3%, 16.1% and 41.3% of cases, Table 1).

**Table 1:** Demographic and disease characteristics

| | |
|---|---|
| Age | 66.7 ± 12.6 |
| Male; *n* (%) | 245 (56.2) |
| Primary; *n* (%) | |
| Oesophagus | 20 (4.6) |
| Gastroduodenal | 48 (11.0) |
| Small intestine | 15 (3.4) |
| Colon | 71 (16.3) |
| Rectum | 32 (7.3) |
| Pancreas | 70 (16.1) |
| Unknown | 180 (41.3) |
| Extent of disease; *n* (%) | |
| Localized | 89 (20.4) |
| Advanced | 168 (38.5) |
| Unknown | 179 (41.1) |

**Table 1:** Characteristics of 436 cases with a conclusion of neuroendocrine carcinoma in local pathology report. Age: mean ± SD

| Table 2: diagnosis per year | 2008 | 2009 | 2010 | 2011 | 2012 | Total |
|---|---|---|---|---|---|---|
| Neuroendocrine Carcinoma; *n (%)* | 19 (31.7) | 35 (40.7) | 35 (40.7) | 48 (48.0) | 59 (56.7) | 196 |
| Low-grade Neuroendocrine Tumour; *n (%)* | 10 (16.7) | 20 (23.3) | 8 (9.3) | 12 (12.0) | 8 (7.7) | 58 |
| Small Cell Carcinoma; *n (%)* | 0 | 4 (4.7) | 5 (5.8) | 1 (1.0) | 2 (1.9) | 12 |
| Neuroendocrine neoplasia, no grade; *n (%)* | 15 (25.0) | 18 (20.9) | 21 (24.4) | 23 (23.0) | 16 (15.4) | 93 |
| Large Cell Carcinoma NOS; *n (%)* | 16 (26.7) | 9 (10.5) | 17 (19.8) | 16 (16.0) | 19 (18.3) | 77 |
| Total | 60 | 86 | 86 | 117 | 104 | 436 |

## Pathology reporting

Of all 436 patients it was possible to deduce the cell type from either the histopathological conclusion or the morphological description in the pathology report. Of these 436 patients the morphology of 12 (2.8%) tumours was described as a small cell neuroendocrine carcinoma in the pathology report.

A synaptophysin stain was reported in 366 patients (83.9%) and chromogranin staining was reported in 371 (85.1%) patients (Figure 2). Of 408 (93.6%) tumours at least one neuroendocrine marker was reported. Grade was reported in smaller amounts of patients: Ki-67 was reported in 191 (43.8%) patients and mitotic rate was reported in 183 (42.0%) patients. Sixty-eight percent of cases could be graded because either Ki-67 (n=107, 24.5%), mitotic rate (n=115, 26.3%) or both (n=76, 17.4%) were reported. Altogether, in 271 (62.2%)

patients all necessary biomarkers for diagnosis and grading could be assessed. In 2008 50.0% of reports included IHC and grading, increasing to 69.2% in 2012 (p=0.02). This was mainly determined by the reporting of grade (mitosis or Ki-67), increasing from 63.4% in 2008-2010 to 74.0% in 2011-2012 (Figure 2, p=0.02). The increase of Ki-67 reporting alone was larger: from 35.3% in 2008-2010 to 53.4% for 2011-2012 (p<0.001). Necrosis and differentiation were seldom reported (25.0% and 24.1%).

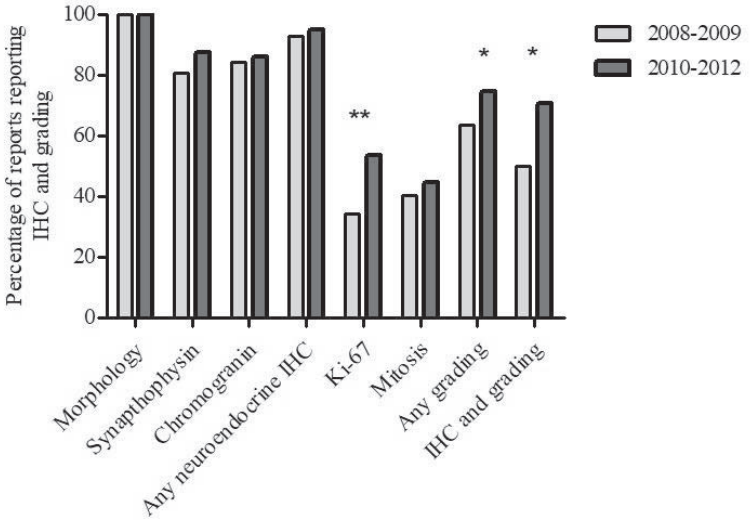


**Figure 2:** Completeness of pathology reports of neuroendocrine carcinomas from 2008 to 2012 (percentage)
IHC: immunohistochemistry
* p<0.05
**p<0.001
* Any neuroendocrine IHC: either synaptophysin or chromogranin was reported

### Classification

Reviewing conclusions and description of morphology in the pathology reports demonstrated that 196 (45.0%) patients were diagnosed with positive neuroendocrine markers and either mitosis or Ki-67 compatible with a NEC in accordance with WHO 2010 guidelines (Figure 1). Other diagnoses included low-grade NETs (n=58, 13.3%) and small cell carcinoma (n=12, 2.8%). Also, 77 (17.7%) had a large cell carcinoma but the neuroendocrine differentiation could not be confirmed with IHC (LCC NOS), because IHC was negative (n=23), IHC was only described as weakly positive (n=28), or no neuroendocrine markers were described at all (n=26). However, CD56 was positive in 64.9% of the LCC NOS (50/77), possibly explaining why these tumours were (incorrectly) classified as neuroendocrine. In 93 patients with a NEN (21.3%), grading was not possible due to missing Ki-67 or mitotic count.

Introduction of the WHO 2010 resulted in clear increase of reproducibility of NEC diagnoses. From 2008-2010 31.7-40.7% of patients could be classified as NEC with positive IHC and grading. This increased to 48.0 and 56.7% in 2011 and 2012 (p=0.02, Table 2).

### Overall survival and prognostic factors

Overall survival patterns are in accordance with the histopathological classifications (Figure 3). Low-grade NETs were associated with the longest survival (mean survival 2.2 years) and small cell carcinomas with a short mean survival of 1.1 years (p=0.02). Only low-grade NET was a significant predictor in a univariate analysis with a hazard ratio of 0.61 (95% CI: 0.44-0.83). Small cell carcinoma (HR 0.95, 95% CI: 0.52-1.75), LCC NOS (HR 1.0, 95% CI: 0.77-1.36) and NEN, no grade (HR 1.1; 95% CI: 0.82-1.38) showed similar overall survival when compared to NEC.

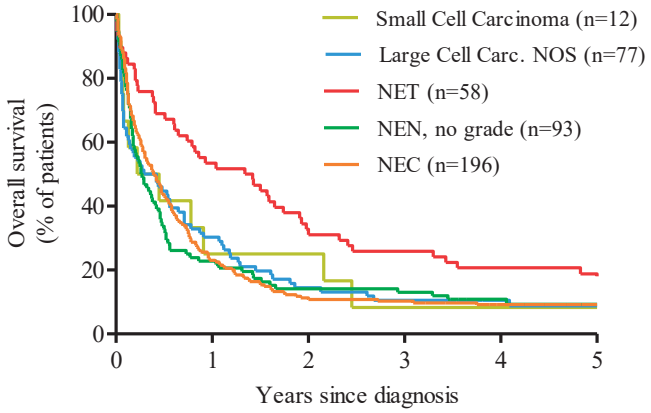


**Figure 3:** overall survival stratified for diagnosis)

## DISCUSSION

Diagnostic standards for NEN are described in the WHO 2010 and 2017 guideline for the classification of tumours and the new *Standard of Care* for pathology by ENETS.[5,10] The main changes in the WHO 2017 guideline for neuroendocrine tumours of the pancreas, which will probably also be adopted for neuroendocrine tumours of the gastrointestinal tract, are the slight change in cut-off between grade 1 and 2 NET and the introduction of a further stratification in high-grade NEN. This study demonstrates that implementation of new guidelines for reporting is an effective measure. Implementation of the WHO 2010 was associated with an increase of the use of grading based on Ki67 or mitotic count from 60.9% to 70.6%. But still many important parameters are lacking in the pathology reports. Even after 2010, only 48.0-56.7% of NEC were classified with reporting of all the necessary parameters.

Incorrect classification in the NCR seemed to be present. In 93 (17.5%) patients the conclusion in the pathology report stated a different diagnosis than NEC. Discrepancies in the written information in the patient files apart from pathology reports, are a challenge for NCR data managers to report these rare types of cancers correctly. Additionally due to this diversity it is difficult to build on expertise for the 168 NCR data mangers as NEN can be diagnosed in all Dutch hospitals.

Secondly, a large number of pathology reports did not describe all necessary parameters to diagnose a NEC. This has also been observed in several other cancers. Completeness of pathology reports varies between 10 to 100%, but is often around 30%.[11] For example, in a recent Italian study, pathology reports for cutaneous melanoma were complete in 77.8% of cases.[12] Thyroid cancer pathology reports were complete in an Australian study in only 36.4 % of cases.[13] In that perspective, adherence to pathology guidelines for NEN seems comparable with other cancers.

The pathology reports in this study were all written as a narrative, but this way of reporting is famed for missing parameters and thus misinterpretation.[14] Since 2010, in the Netherlands, synoptic reporting is available and widely used since 2013. These synoptic reports contain standardized reporting language and mandatory parameters. This style of reporting has been shown to significantly increase completeness of pathology reports to nearly 100% for various cancers.[11,15] The next *Standard of Care* could suggest such a standardized report.

A third reason for the incorrect classification could lie in the previous classification (WHO 2000). This classification defined three groups, based on size, metastases and differentiation. All metastatic disease was classified as endocrine carcinoma with a further differentiation between low- and high-grade malignant behaviour based on differentiation. This could partly explain the pathology conclusions of neuroendocrine carcinoma before 2010, while with the current WHO guideline a low-grade NET would be diagnosed. However, the mandatory differentiation (poor versus well) was only reported in 28.4% of reports before 2010, further underlying the need for a standardized report.

The importance of correct diagnosing is illustrated by the clear difference in survival between low-grade NET and true NEC (Figure 3). While tumours were concluded to be a NEC by the local pathologist we recognised a NET grade 1 or 2 in 13.3% of patients due to the reported parameters in the report. These NETs had a significant longer survival confirming the heterogeneity in the cohort and the importance of uniform reporting and classification.

The current study showed that despite implementation of the WHO 2010 guideline, one or more items were missing for classification and grading in 29.4% of the pathology reports.

Although well differentiated NEN, especially in resection specimens can be readily diagnosed without immunohistochemistry as also stated in de paper of the Delphic consensus process,[16] poorly differentiated NEC lose their neuroendocrine morphology and can often only be recognized with neuroendocrine markers. Therefore the reporting of neuroendocrine markers is essential in this group of patients. Moreover as these tumours are morpho-

logically seen in a biologic continuum estimation of the proliferative activity by mitotic count and ki-67 staining are again shown to predict survival. Although publication of guidelines improves the use of essential parameters, these need several years to be applied universally. Synoptic reporting might give an important boost to meet requirements more quickly.

## REFERENCE


1. Hofland J, Zandee WT, de Herder WW. Role of biomarker tests for diagnosis of neuroendocrine tumours. *Nat Rev Endocrinol* 2018.
2. Williams ED, Sandler M. The classification of carcinoid tum ours. *Lancet* 1963; **1**(7275): 238-9.
3. Solcia E, Klöppel Gn, Sobin LH. Histological typing of endocrine tumours. New York: Springer; 2000.
4. Bosman FT, World Health O. WHO classification of tumours of the digestive system. Lyon, France.: IARC Press; 2010.
5. Lloyd RV, Osamura RY, Klöppel G, et al. WHO classification of tumours of endocrine organs. 4th edition. ed.
6. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cellular oncology : the official journal of the International Society for Cellular Oncology* 2007; **29**(1): 19-24.
7. Korse CM, Taal BG, van Velthuysen ML, Visser O. Incidence and survival of neuroendocrine tumours in the Netherlands according to histological grade: experience of two decades of cancer registry. *Eur J Cancer* 2013; **49**(8): 1975-83.
8. Garcia-Carbonero R, Sorbye H, Baudin E, et al. ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas. *Neuroendocrinology* 2016; **103**(2): 186-94.
9. Garcia-Carbonero R, Sorbye H, Baudin E, et al. ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas. *Neuroendocrinology* 2016; **103**(2): 186-94.
10. Perren A, Couvelard A, Scoazec JY, et al. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Pathology: Diagnosis and Prognostic Stratification. *Neuroendocrinology* 2017.
11. Sluijter CE, van Lonkhuijzen LR, van Slooten HJ, Nagtegaal ID, Overbeek LI. The effects of implementing synoptic pathology reporting in cancer diagnosis: a systematic review. *Virchows Arch* 2016; **468**(6): 639-49.
12. Tumino R, Minicozzi P, Frasca G, et al. Population-based method for investigating adherence to international recommendations for pathology reporting of primary cutaneous melanoma: Results of a EUROCARE-5 high resolution study. *Cancer Epidemiol* 2015; **39**(3): 424-9.
13. Kahn C, Simonella L, Sywak M, Boyages S, Ung O, O'Connell D. Postsurgical pathology reporting of thyroid cancer in New South Wales, Australia. *Thyroid* 2012; **22**(6): 604-10.
14. Powsner SM, Costa J, Homer RJ. Clinicians are from Mars and pathologists are from Venus. *Arch Pathol Lab Med* 2000; **124**(7): 1040-6.
15. Srigley JR, McGowan T, Maclean A, et al. Standardized synoptic cancer pathology reporting: a population-based approach. *J Surg Oncol* 2009; **99**(8): 517-24.
16. Klimstra DS, Modlin IR, Adsay NV, et al. Pathology reporting of neuroendocrine tumors: application of the Delphic consensus process to the development of a minimum pathology data set. *Am J Surg Pathol* 2010; **34**(3): 300-13.