Fine needle aspiration cytology of hepatic metastases of neuroendocrine tumors: a 20-year retrospective, single institutional study.

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Abstract and Keywords:

Aspiration – Cytology – Metastasis – Neuroendocrine - Liver

Background: Fine needle aspiration (FNA) is considered an excellent technique for documenting metastatic neuroendocrine tumors. This study aims to evaluate the accuracy of FNA in diagnosing metastatic NETs to the liver and determining the grade and origin of these metastases.

Methods: Our laboratory information system was searched from 1997 to 2016 to identify all cases of metastatic NETs to the liver that were sampled by FNA. The cytopathology and surgical pathology reports as well as the patients' electronic medical records were reviewed. The cytohistologic type and grade of the metastatic NETs, as well as the site of the patient's primary were recorded.

Results: High-grade NETs, including small cell and poorly differentiated neuroendocrine carcinomas, constituted 62% (167/271) of the cases, while low grade NETs, including well differentiated NET (grade1 and grade 2), pheochromocytomas, paragangliomas and carcinoid tumors of lung, constituted 38% (104/271) of cases. The most common diagnosis was metastatic small cell carcinoma accounting for 45% (122/271) of cases. The most common primary sites were lung (44%; 119/271) followed by pancreas (19%; 51/271). The FNA diagnosis was confirmed by histopathology in 121 cases that had a concurrent biopsies or resection specimens.

Conclusions: FNA is an accurate method for diagnosing metastatic NETs to the liver. There were significantly more high-grade (62%) than low-grade (38%) metastatic NETs to the liver. In our practice, lung (44%) and pancreas (19%) were the most common primary sites of metastatic NETs involving the liver. In 16% of the cases, a primary site could not be established.

Introduction:

Neuroendocrine tumors are defined as neoplasms with predominant neuroendocrine differentiation.(1, 2) They are a diverse group of tumors that most commonly arise from the lung and gastrointestinal tract, but can arise from virtually any organ in the body.(3) Neuroendocrine tumors are a heterogeneous group of neoplasms for which varying terminologies are employed depending upon the specific anatomic site that is primarily involved. These terms include, but are not limited to, carcinoid tumors, islet cell tumors, small cell carcinomas, large cell carcinomas, pheochromocytomas, paragangliomas, and medullary carcinomas of the thyroid gland (1, 2). In the United States, neuroendocrine tumors are more commonly seen in African Americans and among males. Among Caucasians, the lung is the most common site for neuroendocrine tumors (32%) while the rectum is the most common site in blacks (4).

The duality of blood supply to the liver makes it a favorable site for metastatic malignancies including metastatic neuroendocrine tumors. Neuroendocrine tumors (NETs) of the liver represent 1-5% of all liver tumors. The liver is the most common site for metastasis of neuroendocrine tumors originating from the gastrointestinal tract, pancreas and lung (2). With rare exceptions such as pancreatic neuroendocrine microadenomas, NETs are usually considered to be tumors with malignant potential (3, 5). NETs from the gastrointestinal tract, liver and pancreas are graded according to their mitotic count and/or Ki-67 proliferation index as: grade 1 (less than two mitosis and/or Ki-67 < 3 %); grade 2

(2- 20 mitosis and/or Ki-67 3- 20%) and grade 3 (> 20 mitosis and/or Ki-67 > 20%) (5, 6). NETs of the lung are classified into low grade (typical carcinoid tumors), intermediate grade (atypical carcinoid tumors) and high grade (small and large cell neuroendocrine carcinomas) based on the mitotic count (<2, 2-10 and >10), tumor necrosis and tumor morphology (small cells vs. large cell) (6, 7).

The aim of this study is to evaluate the diagnostic accuracy of FNA in diagnosing metastatic NETs to the liver determining the grade and origin of these metastases.

Material and methods:

This research protocol was reviewed by our institute review board (IRB) and was approved with an exemption from full review, since the study was a retrospective analysis of clinical work that had been previously performed, involved no patient contact, and utilized de-identified patient data. Our laboratory information system was searched from 1997 to 2016 to identify all cases of metastatic NETs to the liver that were sampled by FNA. The search was conducted to include all cases that had been diagnosed as neuroendocrine tumors, neuroendocrine carcinomas, small cell carcinomas, large cell carcinomas, carcinoid tumors, insulinomas, paragangliomas and pheochromocytomas. The cytopathology reports and correlating surgical pathology reports, including concurrent biopsies and/or resections, when available, were reviewed. The cytohistologic type and grade of the metastatic NETs, as well as the site of the patient's primary were recorded. The site of the patient’s primary tumor was determined by reviewing all

of the pathological, clinical and radiological data for each case.

Results:

Of the 271 patients included in the study, 160 were males (59%) and 111 were females (41%) with ages that ranged from 7 to 88 years. **Table 1**. High-grade NETs, including small cell and poorly differentiated neuroendocrine carcinomas, constituted 62% (167/271) of the cases, while well differentiated pancreatic and gastrointestinal NET (grade1 and grade 2), pheochromocytomas, paraganglioma and carcinoid tumor of lung, constituted 38% (104/271) of cases. The most common diagnosis was metastatic small cell carcinoma accounting for 45% (123/271) of the cases, followed by low-grade neuroendocrine tumors (G1, G2) 37% (61/271), while pheochromocytoma and paraganglioma represented only 1% (3/271) and 0.4% of cases (1/271) respectively. **Table 2**.

Low grade well differentiated NETs are composed of uniform, monomorphic, small to medium sized cells arranged in loosely cohesive groups and single cells. The tumor cells are round or cuboid to plasmacytoid with eccentric or central nuclei and containing smooth nuclear membrane and uniformly and coarsely granular chromatin (salt and pepper chromatin). Nucleoli maybe present. The cytoplasm is scant to moderate and granular. Mitosis and necrosis is usually not present in the low grade NETs. (Figure 1) High grade NETs tends to show tumor cells with marked nuclear atypia, necrosis and frequent mitosis,but the nuclei still demonstrate a salt and pepper chromatin pattern. Small cell carcinomas consist of small to medium sized tumor cells arranged in a prominent single cell

dispersed cellular pattern with occasional loosely cohesive groups. There is scant cytoplasm. The nuclei are oval, pleomorphic with salt and pepper chromatin without distinct nucleoli. There is prominent nuclear molding with frequent apoptosis and mitosis. (Figure 2)

The most common primary sites were lung (44%; 119/271), followed by pancreas (19%; 51/271) and small intestine (8%; 21/271). **Table 3**. Among metastatic low-grade neuroendocrine tumors, which included low-grade pancreatic and gastrointestinal neuroendocrine tumors (G1, G2), carcinoid and atypical carcinoid tumors, the pancreas was the most common primary site (37, 37%) followed by metastasis from unknown primary (23, 23%), and the small intestine (21, 21%). **Table 4.** The FNA diagnosis was confirmed by histopathology in all 121 cases that had concurrent surgical biopsies or resections. Concurrent core biopsies were performed on selected cases. In the majority of cases, the diagnoses were rendered based on direct smears with or without cell blocks and supported by ancillary immunocytochemical stains that were either performed on the cell block or on the direct smears through cell transfer technique. Chromogranin, synaptophysin and Ki67 were routinely performed on cases of which the tumor cells demonstrating neuroendocrine morphology.

Discussion:

Tumors with neuroendocrine differentiation are a heterogeneous group of tumors

that include carcinoid tumors, islet cell tumors, small cell carcinomas, large cell neuroendocrine carcinomas, pheochromocytomas, paragangliomas, medullary carcinomas of the thyroid, and others. According to the WHO classification, the term neuroendocrine tumor (NET) is the preferred term to refer to both carcinoid tumors of the gastrointestinal tract and islet cell tumors of the pancreas (6). However, carcinoid and atypical carcinoid tumors are still commonly used terms to describe low and intermediate grade neuroendocrine tumors of the lung (7).

Two important somewhat overlapping pathologic parameters that must be evaluated for neuroendocrine tumors are grade and differentiation. Neuroendocrine tumors are considered well differentiated when they have a recognizable neuroendocrine architecture such as an organoid or nested growth pattern, contain neuroendocrine nuclear features characterized by finely granular chromatin as well as smooth nuclear membranes, and granular cytoplasm. These features are almost always associated with intense expression of the neuroendocrine immunohistochemical markers, synaptophysin and chromogranin. On cytology, these tumors are formed of loosely cohesive, monotonous, plasmacytoid cells with finely granular chromatin. As tumors become poorly differentiated, they lose the characteristic growth pattern and cellular morphology, start to resemble poorly differentiated non-neuroendocrine tumors, and exhibit weaker immunostaining for the neuroendocrine markers, especially for chromogranin (1). It is apparent that high-grade tumors, evident by high mitosis with or without necrosis, are also more likely to be poorly differentiated in nature. However, some tumors do show a well differentiated

architecture, while expressing a high mitotic count (>20/HPF) and a high Ki-67 proliferation index (>20%) The term high-grade well-differentiated neuroendocrine tumors has been proposed for this situation (1, 8).

For many years, fine needle aspiration cytology alone or in combination with core biopsy specimens has been used to diagnose both primary and metastatic liver tumors. According to Kuo et al (9) the sensitivity of FNA for the diagnosis of liver tumors is 78% while the specificity is 97%, with improvements in sensitivity to 85% and 99% when FNA is combined with histological examination of core biopsies. According to that study, the reduced accuracy of FNA was attributed to difficulty in differentiating hepatocellular carcinoma from benign lesions. Tsai et al (10), however, reported a lower false negative rate in diagnosing liver malignancy compared to histology (12% and 16% respectively). Similarly, Chhieng claimed that FNA has almost 100% specificity and is superior to biopsy alone (11).

In our study, only 45% of the cases (121/271) had correlating surgical specimens (including concurrent biopsies or later resections). In all 121 FNA cases with histologic follow-up, the histopathologic diagnoses agreed with the FNA diagnoses, confirming the reliability of FNA in diagnosing metastatic neuroendocrine tumors to the liver. There were no discrepancies between the FNA and histologic diagnoses in these cases.

High-grade NETs, including small cell and poorly differentiated neuroendocrine carcinomas, constituted greater than 60% of the cases, with small cell carcinoma being the most common diagnosis (45%). According to the literature, the lung is

the most common site of neuroendocrine tumors among white Americans, while the rectum is the most common site among blacks. Despite the fact that neuroendocrine tumors are more prevalent among blacks, given that 72% of Americans are white (72%, 2010 census)(12), the lung still accounted for the most common primary site for metastatic neuroendocrine tumors in the United States (3). Our study also confirmed that the lung is the most common primary origin of metastatic NETs to the liver (44%), far more common than the pancreas (19%), which was the second most common primary site. In this study, the pancreas was the most common primary site of the low-grade metastatic neuroendocrine tumors to the liver. Approximately 16% of metastatic NETs to the liver in this series had an unknown primary, which is in keeping with the range of 11% to 18% reported in the literature (11).

Conclusion

FNA is an accurate method for the diagnosis of metastatic NETs to the liver. In fact, for those cases with follow-up histology, there were no discrepancies between the FNA and histologic diagnoses. There were significantly more high-grade (62%) than low-grade (38%) metastatic NETs to the liver. In our practice, lung (44%), pancreas (19%) and small intestine (8%) were the most common primary sites of origin of NETs that metastasized to the liver. As reported by others, a primary site could not be established in a significant minority of patients (16%)

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Figure legends:

Figure 1. Smears of well-differentiated pancreatic NET predominantly show-dispersed single cells with moderate vacuolated cytoplasm (A, Diff-Quik stain). The tumor cells have eccentrically located nuclei with a characteristic finely stippled chromatin pattern (B, Papanicolaou stain). A similar morphology is seen in carcinoid tumors of the lung. The cells are loosely cohesive, with plasmacytoid appearance and granular cytoplasm (C, Papanicolaou stain). A common feature of low-grade neuroendocrine tumors is the formation of small clusters or rosettes of cells as seen in this NET of small intestine (D, Papanicolaou stain).

Figure 2. Smears of small cell neuroendocrine carcinomas show extensive nuclear molding, crush artifact and frequent apoptotic bodies (A, Diff-Quik stain). The powdery and evenly distributed chromatin is characteristic (B, Papanicolaou stain). Large cell neuroendocrine carcinomas show cohesive sheets and single cells with large irregular nuclei, prominent nucleoli and more cytoplasm than is seen in small cell carcinoma (C, Papanicolaou stain). Cell block slides from large cell neuroendocrine carcinoma stain positively for synaptophysin and show a high Ki-67 proliferation index (D, E respectively).

Table 1
Demographic Characteristics of the Patients with Metastatic Neuroendocrine Tumors to the Liver

	Number	Percent
Gender		
Male	160	59%
Female	111	41%
Ethnic group		
White	241	88.9%
African American	22	8.1%
Hispanic	1	0.4%
Other	3	1.1%
Unknown	4	1.5%
Age		
Minimum	7 years	
Maximum	88 years	
Mean (SD)	64 (12) years	

Table 2
The Cytohistological Type of Metastatic Neuroendocrine Tumors to the Liver

Grade and Subtype	Number	Percent
High Grade		
Small cell carcinoma	123	45.4 %
Large cell carcinoma	12	4.4 %
Neuroendocrine tumor, high grade (G3)	30	11.1%
Mixed small cell and large cell	2	0.7%
Subtotal	167	61.6%
Low Grade		
Neuroendocrine tumors, low grade (G1, G2)	100	36.9%
Pheochromocytoma	3	1.1 %
Paraganglioma	1	0.4 %
Subtotal	104	38.4%
Total	271	100 %

Table 3
Primary Site for Metastatic Neuroendocrine Tumors to the Liver

	Number	Percent
Lung	119	43.9 %
Pancreas	51	18.8 %
Small intestine	21	7.8 %
Large Intestine	8	3.0 %
Adrenal	3	1.1 %
Prostate	5	1.8 %
Vagina	1	0.4 %
Appendix	1	0.4 %
Stomach	2	0.7 %
Esophagus	1	0.4 %
Paraortic	1	0.4 %
Soft palate	1	0.4 %
GIT NOS	2	0.7 %
Testes	1	0.4 %
Bladder	2	0.7 %
Liver	1	0.4 %
Unknown	43	15.9 %
Unavailable data	8	3.0 %

Table 4
Primary Site for Metastatic Low-Grade (G1, G2)
Neuroendocrine Tumors to the Liver

	Number	Percent
Lung	6	6%
Pancreas	37	37%
Small intestine	21	21%
Large Intestine	3	3%
Vagina	1	1%
Appendix	1	1%
Stomach	2	2%
GIT NOS	2	2%
Liver	1	1%
Unknown	23	23%
Unavailable data	3	3%