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Research Article

Liver Needle Biopsies are Poor Predictors of Histologic Tumor Grade for Midgut Neuroendocrine Tumors

Robert A. Ramirez[1*], David T. Beyer[2], Irma Oliva[1], Brianne Voros[2], Ioni Kokodis[3], Ramcharan Thiagarajan[2], M. Jennifer Ricks[1], Yvette Bren-Mattison[2], J. Philip Boudreaux[2], Yi-Zarn Wang[2] and Eugene A. Woltering[2]

[1]*Ochsner Medical Center, Kenner, LA, USA*
[2]*Louisiana State University Health Sciences Center, New Orleans, LA, USA*
[3]*Ochsner Clinical School, The University of Queensland School of Medicine, New Orleans, LA, USA*

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ABSTRACT

Background: Our group has previously shown than neuroendocrine tumors (NETs) are heterogeneous neoplasms having histologic and functional differences between their primary tumor, lymph node, and hepatic metastases. Due to the heterogeneity of these malignancies, we hypothesized that there would be discordance between the histologic grade of surgical specimens and that predicted by preoperative biopsies.

Methods: Twenty consecutive patients diagnosed with NETs of the ileum and hepatic metastasis were included. Ki-67 proliferative index and WHO 2010 histologic grade were recorded for preoperative hepatic needle biopsy and subsequent tissue-matched surgical specimens. Concordance between sample values was determined.

Results: Ten males and 10 females were included in this analysis. Five and 15 patients had fine-needle aspirate (FNA) and core needle biopsies, respectively. Preoperative biopsies predicted the histologic grade of subsequent tissue-matched surgical specimens in only 65% of samples (13/20). Of the 7 values that changed grade (7/20, 35%), 4 went from intermediate (G2) to low (G1) grade [1 FNA and 3 core biopsies] and 3 went from low (G1) to intermediate (G2) grade [1 FNA and 2 core biopsies]. The corresponding inter-rater agreement statistic (K) was 0.251±0.230 (95% CI: -0.199-0.702), with 0.21<K<0.40 indicating fair strength of agreement.

Conclusion: Preoperative fine-needle aspirates and core needle biopsies of hepatic metastasis have a 35% error rate in predicting the histologic grade from subsequent tissue-matched surgical NET specimens. Clinicians should be cognizant of this error rate when making decisions on systemic treatment and consider repeat needle biopsy or open biopsy if the actual clinical course does not match predicted behaviour.

Introduction

Neuroendocrine Tumors (NETs) are rare, indolent, and heterogeneous neoplasms with a poorly understood natural history [1]. Due to the indolent nature and rarity of this neoplasm, the "watch and wait" method is commonly practiced by community physicians. As a result, patients with NETs commonly present at advanced stages, with recent studies reporting up to 65-95% of patients with gastroenteropancreatic NETs (GEP-NETs) present with hepatic metastasis [2, 3]. Since surgical resection is the only known curative procedure for NETs and not all patients are good surgical candidates, there is a need for an accurate preoperative indication of the proliferative behavior of these malignancies in order to develop an optimal treatment plan for these patients [4]. These data are commonly obtained via preoperative biopsy of metastatic hepatic lesions.

[*]*Correspondence to: Robert A. Ramirez, D.O., F.A.C.P., 200 West Esplanade Avenue, Suite 200, Kenner, LA 70065, USA; Tel: 5044648500; Fax: 5044648525; E-mail: robert.ramirez@ochsner.org*

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GEP-NETs are heterogeneous neoplasms with multiple classification systems proposed by national and international societies, including the World Health Organization (WHO), European Neuroendocrine Tumor Society (ENETS), and American Joint Committee on Cancer (AJCC). The histologic grading system for NETs proposed by WHO in 2010 used both Ki-67 proliferative index and mitotic count to predict clinical outcome (Table 1) [5]. In this system, Grade I lesions (G1) were defined as having a Ki-67 less than or equal to 2% and a mitotic count less than 2 mitoses per 10 high power fields (HPFs), grade II lesions (G2) had Ki-67 values ranging from 3% to 20% and a mitotic count from 2 mitoses per 10 HPF to 20 mitoses per 10 HPF, and grade III lesions (G3) had Ki-67 values greater than 20% and a mitotic count greater than 20 mitoses per 10 HPF. Several studies have validated the significance of Ki-67 in predicting the clinical outcomes of patients with NETs, even in Stage IV disease [6, 7]. This further highlights the importance of accurately reporting Ki-67 indices.

Table 1: ENETS/WHO 2010 Classification Guidelines for Gastrointestinal Neuroendocrine Tumors [5].

Grade	Ki-67 index	Mitotic Count	Differentiation
1	≤2%	< 2 per 10 HPF	Well differentiated
2	3-20 %	2-20 per 10 HPF	Well differentiated
3	> 20%	> 20 per 10 HPF	Poorly differentiated

Our group has previously shown that there are functional and behavioral differences between primary NETs and liver metastasis [8]. Another study by Yang et al. found that nearly half of well-differentiated NETs with hepatic metastasis illustrated intratumoral heterogeneity in Ki-67 indices that resulted in a discordant Ki-67 grade [9]. Since only a portion of a single tumor is usually biopsied preoperatively, the clinician may not obtain an accurate representation of the proliferative nature of the disease. Due to the heterogeneous nature of NETs, we hypothesized that there would be discordance between the histologic grade of the tissue-matched surgical specimen and that predicted by preoperative biopsies.

Methods

Data from all patients seen by the New Orleans Louisiana Neuroendocrine Tumor Specialists (NOLANETS) are entered into a Velos electronic database (VELOS Inc. Freemont, CA) for quick identification and analysis. This database was queried for patients with a diagnosis of primary NET of the ileum with liver metastasis. Patients were included in this study who initially had fine-needle aspiration (FNA) or core needle biopsy performed on their metastatic liver lesion and subsequently underwent surgical cytoreduction. Patient demographics, Ki-67 proliferative indices, and method of biopsy were analyzed. Grading was established according to the WHO 2010 histologic grading criteria [5]. Ki-67 indices were quantified by calculating the percent of at least 500 tumor cells (as per The College of American Pathologist guidelines) in areas of highest nuclear labeling with MIB-1 antibody. Histologic results were evaluated by board certified pathologists at a high-volume neuroendocrine center, and the needle biopsy and surgical specimens were compared. Concordance was calculated via the inter-rater agreement statistic kappa (K). Table 2 shows a scale described by Altman to interpret the numeric value of kappa [10]. This study was given institutional review board (IRB) approval from the Louisiana State University Health Sciences Center and the Ochsner Clinical Foundation, New Orleans, LA. Statistical analyses were performed using MedCalc for Windows, Version 15.6.1 (Medcalc Software, Ostend, Belgium).

Table 2: Scale to evaluate the value of the kappa (K) statistic, as described by Altman [10].

Value of K	Strength of Agreement
<0.20	Poor
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Good
0.81-1.00	Very Good

Results

Twenty consecutive patients (10 males and 10 females) with primary NETs of the ileum who underwent a preoperative biopsy of their metastatic liver lesion at our institution from October 2012 to October 2015 were included for analysis. A total of 5 FNA (5/20, 25%) and 15 core needle biopsies (15/20, 75%) were performed. Preoperative biopsy for each patient resulted in 5 specimens with Grade 1 (5/20, 25%), 14 specimens with Grade 2 (14/20, 70%), and 1 specimen with Grade 3 (1/20, 5%). Post-operative histologic evaluation resulted in 6 lesions with Grade 1 (6/20, 30%), 13 lesions with Grade 2 (13/20, 65%), and 1 lesion with Grade 3 (1/20, 5%). Figure 1 demonstrates an example of the observed discordance between the hematoxylin and eosin (H&E) stain expression values in the preoperative liver biopsy and the corresponding liver resection specimen. Figure 2 demonstrates an example of the observed discordance in the Ki-67 expression values between the preoperative liver biopsy and the corresponding liver resection specimen. Preoperative biopsy predicted the subsequent tissue-matched surgical specimen grade in only 65% of the samples (13/20).

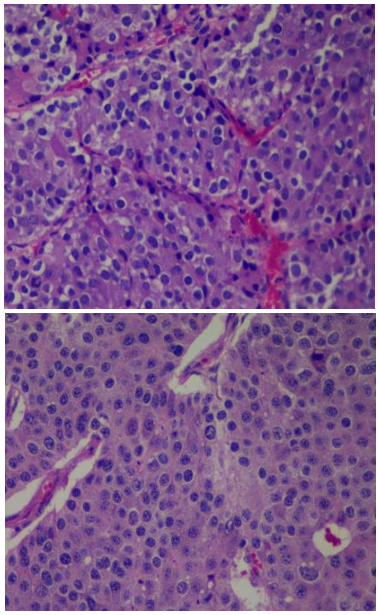

Figure 1: Example of discordance between H&E stain (40x) expression values from **A)** liver biopsy (Ki-67: <2%, low grade) and **B)** the corresponding liver resection specimen (Ki-67: 23%, high grade).

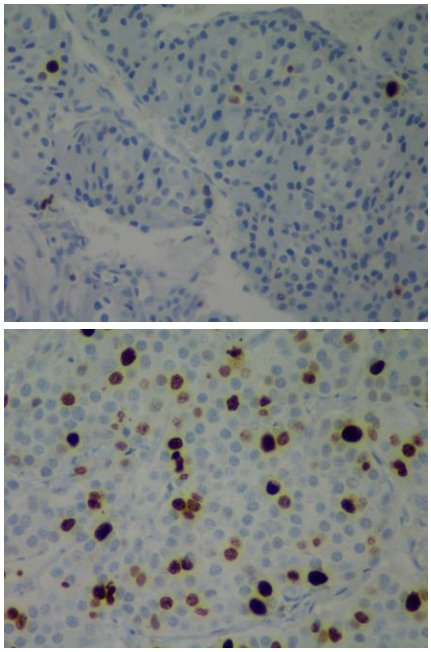

Figure 2: Example of discordance between Ki-67 (40x) expression values from **A)** liver biopsy (Ki-67: <2%, low grade) and **B)** the corresponding liver resection specimen (Ki-67: 23%, high grade).

The corresponding inter-rater agreement kappa statistic (K) was 0.251 ± 0.230 (95% CI: -0.199-0.702), with 0.21<K<0.40 indicating fair strength of agreement. Of the 7 values that changed grade (7/20, 35%), 4 went from intermediate (G2) to low (G1) grade [1 FNA and 3 core biopsies], 3 went from low (G1) to intermediate (G2) grade [1 FNA and 2 core biopsies], and 1 went from low (G1) to high (G3) [1 core biopsy] (Table 3).

Table 3: Direction of Change in Preoperative Biopsy Grade to Surgical Specimen Grade (n=7).

Direction of Change	**N (%)**	**Surgical Specimen Type**
Low to High	**1** (14%)	1 Core biopsy
Low to Intermediate	**2** (29%)	1 FNA & 1 Core biopsy
Intermediate to Low	**4** (57%)	1 FNA & 3 Core biopsies

Discussion

Neuroendocrine tumors are a rare malignancy, which are often heterogeneous in nature [1]. There is also a poor understanding of the natural history of NETs, which, in part, explains why many patients are diagnosed with advanced disease at the time of initial presentation [2, 3]. The patients' treatment modalities are constructed based on the grade of their tumor, making accurate diagnosis and staging paramount. Our study evaluates the accuracy of fine needle aspiration and/or core needle preoperative biopsy against post-operative histological evaluation in patients with primary ileal NETs with metastatic liver lesions. We found that there was an error in grading approximately 35% of the time, which may indicate both prognostic and therapeutic implications in about one-third of the patient population diagnosed [9].

Histological proliferative grading for NETs is currently based on the ENETs guidelines that have also been adopted by both the WHO and AJCC. [It is important to note that this study was completed prior to the new WHO 2017 grading criteria, and therefore, follows the guidelines established in the WHO 2010 grading system (Table 1) [11]. It is also important to note that while significant changes were introduced in the WHO 2017 guidelines (most notable the Ki-67 cut-off for a G1 NET was changed from ≤2% to <3%; and Ki-67 >20% tumors were subdivided into well differentiated G3 NETs and poorly differentiated G3 NECs), these changes did NOT alter the conclusions of this study, i.e., the observed discordance between the histologic grade of the preoperative biopsies and the tissue-matched surgical specimen.] These guidelines use both Ki-67 index and mitotic index to group tumors into low (G1), intermediate (G2), or high grade (G3) categories. The current guidelines do not suggest a preference for either the Ki-67 percentage or the mitotic count. Thus, in many instances, treatment is based on clinician opinions and patient factors, such as the ability to withstand surgical resection or biopsy. In many situations, core needle biopsy or fine needle aspiration samples are of limited size and may lack the 40-50 high power fields that are required to establish a mitotic count. There have also been studies establishing that the Ki-67 index itself is a prognostic factor in patients with NETs, with those having lower values demonstrating higher overall survival [3]. This may suggest an increased importance in the Ki-67 index over mitotic count; though further investigation is warranted as there is no current data to support this.

Thus, many researchers have been investigating the accuracy of the methods used to establish either the staging or grade for these tumors due to the importance of these methods. For example, a study by Piani et al., which examined 18 pancreatic NET patients, found that preoperative endoscopic ultrasonography-guided FNA cytology correctly predicted the Ki-67 index in 89% of patients (K =0.78) [12]. On the other hand, our data illustrates that preoperative liver biopsies correctly predicted the Ki-67 grade of the tissue-matched surgical specimens, only 65% of the time using the two different biopsy methods. This suggests that the accuracy in FNA or core needle biopsy in establishing grade for gastrointestinal NETs with hepatic metastasis may not be sufficient moving forward. This is a crucial finding as studies have shown overall histological grades after an aggressive surgical resection can affect a patient's prognosis [2].

The discrepancy in grading between the preoperative and post-operative biopsies may be explained by the heterogeneous nature of NETs. Not only has it been found that many NETs are heterogeneous within the primary tumor itself, but there has been research to suggest that heterogeneity exists between the primary tumor and its metastasis [13]. A study by Yang et al. found that when using the Ki-67 index, obtained from a core needle biopsy, to grade the hepatic metastasis, the samples were heterogeneous 47% of the time intratumorally [9]. It is possible that the preoperative biopsy methods in our study did not achieve the sample size or requirement of the most Ki-67-dense tissue suggested by current guidelines to establish the most accurate grading. Given our results, it is suggested that several FNA or core needle biopsies may be required to achieve an accurate Ki-67 grade if surgical resection and biopsy are not plausible. This is important moving forward as the change from G1 to G2 or reverse can have further implications in surgical or medical therapy. The potential limitations in our study include the

retrospective design and the small cohort size. In addition, not all patients had mitotic count performed during the immunohistochemical testing on their hepatic lesions. Therefore, we only used the Ki-67 proliferative index to determine the histologic grade. While the current guidelines suggest either index is acceptable, further research could be done to evaluate if there would be changes in results if both were calculated. In conclusion, our results found a difference in preoperative core needle biopsy or FNA when compared to post-operative biopsy of hepatic metastasis from primary ileum NETs resulting in 7 patients having the grade of their tumors changed. With an accuracy of 65% between the methods in establishing grading, we argue that clinicians need to be cognizant that the initial grading based on core needle biopsy or FNA may not be accurate. If possible, patients should undergo postoperative immunohistochemical testing or multiple core needle biopsies/FNAs to establish a final grade before proceeding with the formulation of a treatment plan.

Conclusion

Preoperative fine-needle aspirates and core needle biopsies of hepatic metastasis have a 35% error rate in predicting histologic grade from subsequent tissue-matched surgical NET specimens. Clinicians should be cognizant of this error rate when making decisions on systemic treatment and consider repeat needle biopsy or open biopsy if the actual clinical course does not match predicted behavior.

Author Contributions

Study conception and design: Ramirez, Beyer, Oliva, Voros, Kokodis, Thiagarajan, Ricks, Boudreaux, Wang, Woltering.
Acquisition of data: Ramirez, Beyer, Oliva, Voros, Kokodis, Thiagarajan, Ricks, Boudreaux, Wang, Woltering.
Analysis and interpretation of data: Ramirez, Beyer, Oliva, Voros, Boudreaux, Wang, Woltering.
Drafting of manuscript: Ramirez, Beyer, Voros, Woltering.
Critical revision: Ramirez, Beyer, Bren-Mattison, Woltering.

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None.

Conflicts of Interest

Robert A. Ramirez, D.O. serves as a consultant for Advanced Accelerator Applications, Novartis, and BioTheranostics, Inc., as well as a speaker for Ipsen Biopharmaceuticals, Inc., Merck & Co. Inc., Genentech/Roche, AstraZeneca, Plc., Guardant Health, Inc., and Advanced Accelerator Applications, and receives research funding from Merck & Co. Inc. Eugene A. Woltering, M.D. serves as a speaker and consultant for Ipsen Biopharmaceuticals, Inc., Interscience Institute, and Lexicon Pharmaceuticals, Inc. J. Philip Boudreaux, M.D. serves as a speaker and consultant for Ipsen Biopharmaceuticals, Inc. No other authors have conflicts of interest to disclose.

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