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Correlation of Ki-67 indices from biopsy and resection specimens of neuroendocrine tumours

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ABSTRACT

INTRODUCTION Neuroendocrine tumours (NETs) are a heterogeneous group of tumours with a highly variable presentation and prognosis. Management decisions are complex. Ki-67 levels in tissue samples are a key indicator used to grade tumours and guide treatment. This study assessed whether the Ki-67 index and tumour grade generated from tissue samples correlated with that assessed in resection specimens.

METHODS This was a retrospective cohort analysis of all patients who had both a tissue sample and a resection specimen analysed in our trust, a tertiary referral centre, during 2012 and 2013.

RESULTS Data from 36 patients were reviewed. Ki-67 indices from tissue samples and resection specimens showed strong correlation (r=0.95, $p<0.001$). Tumour grading was the same in the tissue sample and resection specimens for 22 patients (61.1%). In four patients (11.1%), the tissue sample overestimated the grade while in ten (27.8%), the sample underestimated the grade.

CONCLUSIONS In most cases, the Ki-67 index and tumour grade from the tissue sample matched that of the resection specimen. However, in nearly 40% of cases, the tissue sample grading did not match the resection tumour grading. In the majority of these, the tissue sample underestimated disease activity. A low Ki-67 index in a tissue sample should therefore be taken as provisional and should not, in isolation, persuade clinicians to choose a more conservative treatment approach if there is clinical, biochemical or radiological evidence suggestive of a more aggressive disease pathology.

KEYWORDS

Neuroendocrine tumours – Ki-67 – Diagnostic techniques and procedures – Pathology – General surgery

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Neuroendocrine tumours (NETs) are a rare and heterogeneous group of neoplasms[1–3] that present a diagnostic and therapeutic challenge. NETs are derived from the diffuse neuroendocrine cell system[4,5] and can occur throughout the body, most commonly in the gastrointestinal system or the lungs. NETs affect both sexes across all age groups. Although predominantly sporadic, they may occur in association with genetic disorders including multiple endocrine neoplasia type one and von Hippel–Lindau syndrome.[6,7]

NETs often present with vague, varied and non-specific symptoms.[7] Symptoms depend on the disease stage and whether the tumour produces hormones (functional) or not (non-functional).[8] NETs may present with symptoms related to hormonal function (eg insulinoma) or with those of mass effect.[3,4,8,9] Typically, they remain asymptomatic for a long period of time as they are often small and inactive in the early stages.[9] In a growing number of patients, NETs remain completely asymptomatic and are only found incidentally. The delay in diagnosis from first presentation is in the region of 5–7 years.[3,7,9] This makes clinical detection difficult and means that patients often present late with metastases. In specialist centres, up to 70% of cases present with metastases. These are predominantly to the liver.[6,8,10]

The annual incidence of NETs is approximately 2.5–5 per 100,000.[7,11] Although rare, the incidence and prevalence are increasing.[3,4,9,12] NET incidence is thought to be growing at a rate of 3–10% per annum,[9] a rate of increase faster than any other malignancy.[13] This may in part be attributable to better diagnostic techniques and increased awareness.[7]

Diagnosis poses not just a clinical but also a radiological challenge, with the primary tumour site remaining unknown even after investigation in 20–50% of cases.[4] For this reason, a multimodal diagnostic approach is required. Diagnostic and staging investigations include gut hormones, urinary screening, cross-sectional imaging, nuclear medicine studies and endoscopic ultrasonography in some cases.[4,6] Tissue sampling is also required to diagnose and then grade the tumour, and is a key part of treatment planning.

Prognosis is highly variable owing to the variability in underlying cellular pathology, tumour grade and the range of stages at presentation. In local disease, the five-year

survival rate is around 70–80% while in metastatic disease, this figure is thought to be closer to 20–30%.[14] A large meta-analysis of European studies showed the five-year survival rate for gastroenteropancreatic NETs (GEP-NETs) to be almost 60% for well differentiated tumours (low grade), regardless of whether patients had metastatic disease, while it was less than 10% for small cell carcinomas (poorly differentiated, high grade).[15] This extreme difference in prognosis based on grade (and morphology) is also seen in non-GEP-NETs (eg lung).[16]

In 2010 the World Health Organization produced a new histological grading system for NETs of the digestive system (GEP-NETs).[5,17] It divides tumours into three grades based on their mitotic rate and Ki-67 index.[18] Tumours are therefore graded as:

- G1 (low grade, well differentiated, Ki-67 <3%, mitotic count <2 per 10 high power fields)
- G2 (intermediate grade, moderately differentiated, Ki-67 3–20%, mitotic count 2–20 per 10 high power fields)
- G3 (high grade, poorly differentiated, Ki-67 >20%, mitotic count >20 per 10 high power fields)

Ki-67 is an immunohistochemical marker of cellular proliferation[7] as it is expressed exclusively by cells in the S, G_2 and M phases of the cell cycle.[9] It is a more accurate marker of tumour proliferation and prognosis of NETs than other previously used indices, including mitotic count.[19,20] Another advantage of Ki-67 is the consistent interobserver reproducibility of results.[21] In practice, the Ki-67 index should be obtained from the areas of highest disease activity with a minimum of 2,000 cells used as the denominator.[22,23]

Ki-67 levels should be measured as part of the initial diagnostic workup, either via core biopsy or fine needle aspiration (FNA). This allows grading to be established early and treatment directed appropriately. Treatment options are varied; the only curative option is surgical resection[17] with a clear margin of excision.[4,8,20] For patients presenting with advanced disease, this may not be appropriate. Other treatment options include radiofrequency ablation, chemoembolisation, radioembolisation, palliative surgery (eg debulking), systemic chemotherapy (for high grade tumours) and medical therapy (eg somatostatin analogues) to reduce symptoms in functional tumours.[4,8,9] It is essential that tumours are graded accurately at initial diagnosis to ensure that patients with low grade tumours are not put through unnecessary treatments, that those with aggressive disease are not undertreated[24] and that those for whom curative therapy exists, receive it.

Tumours can, however, show variation in Ki-67 with areas of higher and lower activity. This may be noted and corrected for when analysing a whole excised specimen but it can lead to sampling error in initial biopsies. Ideally, Ki-67 indices from tissue samples should accurately represent the actual tumour indices if they are to be used to guide treatment. To date, no study has assessed to what degree Ki-67 indices from tissue sampling correlate with excised tumour Ki-67 expression.

The objective of this study was therefore to investigate whether Ki-67 levels from NET core biopsy/FNA samples correspond accurately to those found in the tumour on resection and if not, whether tissue samples tended to over or underestimate disease activity. A secondary aim was to assess whether there was any difference in accuracy of grading for samples taken from FNA versus [core] biopsy and for samples taken from a metastasis versus those from the primary tumour.

Methods

This study was a retrospective cohort analysis. A list was obtained from the trust's pathology database of all patients who had had tissue samples analysed at our trust's cellular pathology laboratory between 1 January 2012 and 31 December 2013 as part of the investigation for a NET. Inclusion criteria were confirmed diagnosis of NET and both the tissue sample and resection specimen being analysed in our trust's laboratory by a specialist NET pathologist.

Data were collected from the trust database and electronic patient records. Diagnosis, tumour site, Ki-67 assessment from tissue sampling, the sampling method used and Ki-67 assessment from the subsequent resection specimen were recorded. Where Ki-67 was given as a range of values, a mean was generated. Tumour grades were then generated from the Ki-67 levels for both the biopsy and resection specimen.

Statistical analysis was undertaken using Fisher's exact test and Pearson's correlation coefficient. A p-value of <0.05 was deemed statistically significant.

Results

A total of 208 individual patients were identified from the database. Of these, 106 had had just tissue sampling, 66 had had just resection tissue analysed, and 36 had had both tissue sampling and resection of a NET.

Of these 36 patients included in the study, 15 (41.7%) were female. The mean age was 56 years (standard deviation [SD]: 16 years). The mean period between tissue sampling and surgical resection was 114 days (SD: 94 days). The longest period between tissue sampling and resection was 359 days.

Seven of the tissue samples were from FNA while 29 were from core biopsy. Table 1 gives a breakdown of the tumours by primary tumour site and by technique used to obtain the samples.

Tumour grading

Based on the resection specimens, 15 (41.7%) of the tumours were graded as G1, 18 (50.0%) as G2 and three (8.3%) as G3. Table 2 shows the correlation between the grades as evaluated from biopsies and the grades as evaluated from surgical resection.

Table 3 illustrates how the grading of tissue samples related to that of resected specimens. The difference in grading accuracy between FNA and core biopsy samples (71.4% vs 58.6%) was not statistically significant (p=0.68).

Table 1 Site of tumour biopsy and tissue acquisition method

Site of primary tumour	*n*	Core biopsy	Fine needle aspiration
Pancreas	10	3	7
Large bowel	7	7	0
Gastric	6	6	0
Lung	4	4	0
Liver	3	3	0
Terminal ileum	3	3	0
Larynx	1	1	0
Ovary	1	1	0
Testes	1	1	0
Total	**36**	**29**	**7**

Table 2 Comparison of neuroendocrine tumour grade for tissue samples and resection specimens

Grade on resection	*n*	Tissue sample grading relative to resection specimen		
		Underestimated	Same	Overestimated
G1	15	3*	9	3
G2	18	6	11	1
G3	3	1	2	–
Total	**36**	**10**	**22**	**4**

*Sample suggested tumour was benign

Table 3 Comparison of neuroendocrine tumour grade for tissue samples and resection specimens by tissue sampling method and location

Sample subgroup	*n*	Tissue sample grading relative to resection specimen		
		Underestimated	Same	Overestimated
Method				
Core biopsy	29	8 (27.6%)	17 (58.6%)	4 (13.8%)
Fine needle aspiration	7	2 (28.6%)	5 (71.4%)	0 (0%)
Total	**36**	**10 (27.8%)**	**22 (61.1%)**	**4 (11.1%)**
Site				
Primary tumour	31	9 (29.0%)	18 (58.1%)	4 (12.9%)
Metastasis	5	1 (20.0%)	4 (80.0%)	0 (0%)
Total	**36**	**10 (27.8%)**	**22 (61.1%)**	**4 (11.1%)**

For five of the biopsies, the sample was taken from a metastasis rather than the primary site. In two of these cases, resection involved removal of the metastasis that was sampled along with the primary tumour (with/without further debulking). In all five cases, the Ki-67 value generated from the resection specimen was based on the primary tumour (not the sampled metastases).

Biopsy samples taken from a metastasis matched resection specimen grading in 4 of 5 cases (80.0%) while the grading of those extracted from the primary tumour matched in 18 of the 31 cases (58.1%). This difference was not statistically significant (p=0.63).

Overall, Ki-67 indices in the tissue samples and resection specimens had a correlation coefficient of 0.89 (p<0.001). The correlation coefficient for Ki-67 indices for core biopsy samples and for resected tumour specimens was 0.95 (p<0.001). When comparing only FNA samples with resected tumour specimens, it was 0.74 (p=0.09). The Ki-67 indices for tissue samples taken from metastases and those for resected tumours had a correlation coefficient of 0.48 (p=0.052) while the correlation coefficient for indices from samples taken from the primary tumour and for those from resection specimens was 0.89 (p<0.001).

The grading of the tissue sample differed from that of the resection specimen in 14 cases. In three of these cases, the biopsy report stated specifically that the sample was, in some way, difficult to assess and the result may not be completely representative of actual disease activity. In one sample, there was noted to be a 'scanty amount of tissue' while in another, there was only a 'small amount of tissue' with tumour cells bordering other cells that also showed 'prominent nuclear expression'. The third sample was noted to show highly 'degenerate' cells with 'indistinct boundaries'. In a further fourth case where the sample and resection grading did not match, although the grading was clear, the pathologist noted that the microscopic findings did not fit well with what had been reported on endoscopy. As a result, caution was advised in interpretation of the findings.

Discussion

This study of NETs confirms that Ki-67 values obtained from tissue samples have a strong correlation with those from subsequent resection specimens. This is reassuring because tumour grade is a key variable used during treatment planning. It is therefore essential that cellular proliferation seen on tissue samples represents actual disease activity accurately.[21]

However, for nearly 40% of patients, the grade generated from the tissue sample did not match the grade for the resected tumour; in the majority of these cases, the sample results underestimated actual disease activity. This suggests that a low Ki-67 index from an initial tissue sample should not be completely relied on when grading the tumour, especially if there are contradictory clinical or radiological features.

It is possible that the trend for results from tissue samples leading to underestimation rather than overestimation of

true disease activity may reflect natural tumour progression during the time interval between biopsy and surgical resection but this is considered unlikely given the typically slow growth of NETs and the relatively short period between tissue sampling and resection in the study cases. It is more likely, in our opinion, that any discrepancies represent variation of proliferative activity in the tumour, which can be allowed for in resection samples when assessing Ki-67.

There was a statistically significant correlation between the Ki-67 indices for core biopsy samples and those for resected tumours, but this was not the case for FNA samples. This may indicate that core biopsy facilitates more accurate Ki-67 analysis than FNA. Nevertheless, as only seven samples were taken via FNA, it is difficult to draw meaningful conclusions and these results should be viewed with caution. Interestingly, there was no significant difference in the accuracy of grading of tumours between each sampling modality. This is an area where further study would be beneficial.

In cases where the sample was taken from a distant metastasis, 80% of the sample grades agreed with the grade for the resected tumour (based on grading from the primary tumour). There was no significant difference between the accuracy of tissue sample grading versus resection specimen grading for samples taken from a metastasis compared with those from the primary tumour. On the other hand, Ki-67 indices from primary tumour samples did show a much stronger correlation with resection specimen indices than that seen in samples from a metastasis. As with the FNA samples, however, these results are based on a very small sample size (five metastasis samples), which prevents any meaningful conclusions being drawn. Despite this, it is interesting that tissue samples from metastases do seem to give an accurate prediction of tumour grade at resection and this is a further area in which future study would be of value.

These conclusions are limited by the small sample size and diverse range of underlying primary tumour sites reviewed. A larger study of tumours arising from different sites would be useful to replicate these findings and assess grading accuracy at different tumour sites. Further study looking at accuracy of grading generated from sampling of metastases versus primary tumour sites and from FNA versus core biopsy would also be valuable in optimising NET management.

A final result that merits discussion is the time taken from tissue sampling to surgery. In our study, the shortest period was 15 days, with 15 patients (41.7%) being operated on within 12 weeks and 24 (66.7%) being operated on within 18 weeks. This time lag is likely to reflect the extensive workup patients often need before surgery. For example, patients often require an extensive and time consuming biochemical and endocrine workup, multiple sets of imaging (some functional) and often significant anaesthetic preassessment.

The remaining 12 patients (33.3%) underwent surgery between 18 and 52 weeks after initial tissue sampling. As NETs are generally slow growing and sometimes diagnosed incidentally, a 'watchful waiting' approach is frequently employed, particularly if the tumour is small and the patient asymptomatic with multiple co-morbidities (making surgery high risk). The 12 patients who were operated on between 18 and 52 weeks are likely to be individuals who were originally assigned to surveillance rather than surgery, but (owing to some clinical, radiological or biochemical change) were then operated on, accounting for the long delay between tissue sampling and resection.

Conclusions

Although further work is needed, these results have an important clinical message. NET grades generated from Ki-67 values from tissue samples correlate strongly with the final resection tumour grades. However, a minority of biopsy samples may not accurately represent the resected tumour grade, more often underestimating disease activity. So, if there is clinical suspicion to the contrary, a low Ki-67 index from an initial sample should not be relied on to guarantee a low grade of tumour activity. Ki-67 results from tissue samples are useful but should only be interpreted in the context of other findings.

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