

Upper GI biopsies for Adenocarcinoma – how many biopsies should endoscopists take?

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Abstract

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Introduction

HER 2 testing is a requirement for a significant proportion of gastro-oesophageal adenocarcinomas as response to Trastuzumab is dependent on positive expression¹. However, expression of HER2 may vary significantly within a tumour and in a gastric resection specimen a threshold of 10% strong positive staining is regarded as positive while in biopsies, a cluster of 5 positive cells showing strong membranous staining is regarded as positive². Initial guidelines which were based on no specific evidence recommended “an adequate number of viable biopsy specimens (ideally 6-8) are required”³. The recently published BSG quality standards in upper gastrointestinal endoscopy⁴ have also recommended a minimum of 8 biopsies. Reported concordance between biopsy and resection cases varies quite markedly in the literature between 45-85%⁵⁻⁸. Two studies in particular, both published in 2015, have focussed on how many biopsies are required to ensure maximum correlation between biopsies and the resected specimen. Ahn et al⁹ compared 702 paired biopsy and resection cases. They found a high degree of biopsy heterogeneity, but using ROC analysis concluded that a minimum of 4 biopsies were required to minimise discrepancy. Tominaga et al¹⁰ used a different methodology by examining 84 gastric cancer resection cases and assessing 6 “virtual biopsies” distributed evenly through the luminal compartment of the tumour. They concluded that 5 biopsies would be sufficient for complete correlation with the gastrectomy specimen. Interestingly, they also showed preferential expression in the luminal and lateral aspects of the tumour.

These studies provide vital evidence, but neither have considered the real world scenario where many of the biopsies which are taken do not contain viable tumour. The object of this audit study was to look at the actual number of targeted biopsies taken and determine how many of these fragments actually contain viable tumour (i.e. diagnostic). Our aims was to see if the minimum standard for the recommended number of biopsies is being adhered to, and more importantly, determine the minimum number of biopsies required to get diagnostic material and reach the recommended requirement for HER2 testing.

Methods

This audit was registered with the NUH Trust online Clinical Audit system. 105 consecutive cases of biopsy proven upper GI cancer during 2016 were retrieved using the Winpath laboratory information system. Cases were diagnostic biopsies with a proven diagnosis of upper GI cancer. We therefore did not include cases where the diagnosis of cancer had been missed, or not proven. The cases were then reviewed and the following recorded:

- The total number of endoscopic biopsies taken.
- The total number of endoscopic biopsies containing viable tumour.
- The total number of endoscopic biopsies containing necrotic tumour.
- The anatomical location i.e. oesophageal or gastric.

The total number of biopsies and percentage with viable tumour was calculated, which enabled determination of the number of biopsies required to be taken to establish a diagnosis of cancer. Further, the data was inspected to determine the number of biopsies required to yield 4 or 5 viable tumour fragments. Further, the number of biopsies required for viable tumour fragments was also calculated mathematically.

Results

We assessed 105 diagnostic upper gastrointestinal biopsies. 35 cases (33%) met the minimum national standards of at least 8 biopsies. The relationship between total number of biopsies and viable tumour fragments is shown in table 1. A total of 667 biopsies were taken of which 471 yielded viable tumour. On average, the chance of getting viable tumour with a single biopsy is therefore around 70%, and there is a 30% chance of missing tumour with each biopsy. The chance of missing tumour with multiple biopsies is therefore 0.3^n etc. This translates to a 97% chance of getting at least one viable tumour biopsy with 3 biopsies

However, for optimal gastric HER 2 testing, evidence suggests that at least 4 or possibly 5 tumour biopsies are required. 70/105 (67%) of cases had at least 4 viable tumour biopsies, but only 47/105 (45%) had 5 or more.

Inspection of our data showed that if only 4 biopsies were taken 7/15 had 4 biopsies with tumour, 7/14 with 5 biopsies, 9/12 with 6 biopsies, 16/17 with 7 biopsies (table 2a). Taking further biopsies beyond 7 did not appear to increase the likelihood of reaching 4 tumour viable biopsies. If however 5 viable tumour biopsies are required, at least 10 biopsies were required for greater than 90% chance of 5 viable biopsies (table 2b). Assuming a fixed probability for a viable tumour biopsy of 0.7 we have constructed a probability tree using the binomial distribution to calculate a 90% chance of getting at least 4 viable tumour biopsies. This amounted to 8 biopsies being required for a 94% chance of obtaining at least four viable tumour biopsies. With

seven biopsies the probability of at least four viable tumour biopsies is less than 90%. This theoretical approach assumes a fixed probability for each biopsy and as a model system could be refined with the collection of more data.

Discussion

Trastuzumab (Herceptin) has become standard of care for first line chemotherapy in patients with metastatic gastro-oesophageal cancer for the small proportion of tumours which strongly express HER2¹¹. Most of these patients will have had biopsies only and will not have undergone prior gastrectomy. Recent studies^{9,10} suggest that 4 or 5 viable biopsies are required to reach optimal concordance for HER2 status between biopsy and gastrectomy specimen.

This audit study was undertaken to determine how frequently we met this standard, and how many biopsies were required to be taken to reach this number. BSG guidelines recommend taking 8 tumour biopsies, but no evidence currently exists to support this⁴.

This audit has shown that in our centre which is likely to be representative of the UK in general, only 33% of all endoscopies met the required standard of 8 biopsies. 70% of targeted biopsies contained diagnosable cancer which all things being equal would translate to 3 biopsies being required for a 97% chance of diagnostic success. Few previous studies have looked at this. Choi et al¹² in a prospective study showed very high diagnostic rates when 3-4 biopsies were taken in gastric and colorectal cancer but a previous study in rectal cancer showed less than 80% diagnostic rate in colorectal cancer with 6 biopsies¹³ while an even older upper GI study recommended 7 biopsies for a greater than 98% chance of diagnosis¹⁴.

We are not aware of any studies which have looked at the optimal number of biopsies to be taken to ensure that the target of 4 or 5 viable tumour biopsies are collected for HER2 testing. The evidence here suggests that a minimum of 8 targeted biopsies should yield at least 4 viable tumour biopsies in more than 90% of cases while 5 viable biopsies requires at least 10 biopsies to be taken for a greater than 90% chance of success. Although there is some variation in the literature on the optimal number of viable biopsies needed, the current BSG upper GI quality standards guidelines recommendation of 8 biopsies now has solid evidence to support it. The German guidelines similarly recommend 8 biopsies be taken¹⁵.

This study was limited by not including cases of intended tumour biopsy which did not yield a positive diagnosis at all and had to be repeated. However, it is likely that the same principle will apply to these cases and that taking at least 8 biopsies will avoid the requirement for repeat endoscopy other than in exceptional cases. It is likely that

the biopsy yield for colorectal cancer will be even lower, as these tumours often have a large residual adenomatous component which may interfere with sampling the invasive component . It would certainly be worth doing a similar study on lower GI cancer.

It is clear that there are multiple factors related to the chance of getting an adequate diagnostic biopsy. These include both tumour characteristics as well as endoscopist skill and experience. This study could not account for these factors, but gives a snapshot of actual practise in our institution where endoscopies are performed by a very wide range of individuals. The evidence presented here suggests that taking at least 8 biopsies will accomplish the task satisfactorily in the vast majority of cases. This study may also be of use to those planning the acquisition of fresh biopsy tissue for research, in particular the 100 000 genome project and other large studies seeking to obtain high quality genetic material. As mentioned, the chance of getting viable tumour with one targeted biopsy is no better than 70% so when harvesting upper GI tumour tissue for this purpose at least two and preferably 3 biopsies should be obtained.

In summary, the results of this audit show that we are currently reaching the national guideline target in only a third of cases, and that only 67% of our cases had sufficient tumour tissue to accurately grade HER2 staining at the 4 viable tumour biopsy threshold (and only 45% at the 5 biopsy mark). Furthermore, we have shown that taking at least 8 biopsies would likely increase our yield of required tissue to over 90%, supporting the national guidance.

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Philip Kaye devised study and wrote the paper

Daniel Lindsay collected data and analysed it

Craig Platt did mathematical calculations and edited paper

Ravinder Vohra, James Catton, Srinivasan Madhusudan and Krish Rangunath, edited paper.

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Tables

Table 1: Relationship between total number of biopsies and viable tumour fragments

	Viable tumour present										Total	
	1	2	3	4	5	6	7	8	9	13		
Total 1	4	0	0	0	0	0	0	0	0	0	0	4
Biopsies 2	1	3	0	0	0	0	0	0	0	0	0	4
3	1	2	2	0	0	0	0	0	0	0	0	5
4	3	3	2	7	0	0	0	0	0	0	0	15
5	1	3	3	3	4	0	0	0	0	0	0	14
6	2	0	1	3	5	1	0	0	0	0	0	12
7	0	1	0	6	4	1	5	0	0	0	0	17
8	0	1	0	2	1	0	1	5	0	0	0	10
9	1	0	0	2	1	3	1	3	2	0	0	13
10	0	0	0	0	0	1	0	1	2	0	0	4
11	0	0	0	0	1	0	1	1	0	0	0	3
12	0	0	1	0	0	2	0	0	0	0	0	3
13	0	0	0	0	0	0	0	0	0	0	1	1
Total	13	13	9	23	16	8	8	10	4	1	105	

Table 2 Relationship between number of biopsies and cases with >4 (table 2a) and >5 (table 2b) viable tumour fragments

2a

Total Number of biopsies	Number of cases	Number with ≥ 4 tumour biopsies
4	15	7 (47%)
5	14	7 (50%)
6	12	9 (75%)
7	17	16 (94%)
≥ 8	32	29 (91%)

2b

Total Number of biopsies	Number of cases	Number with ≥ 5 tumour biopsies
5	14	4 (29%)
6	12	6 (50%)
7	17	10 (59%)
8	10	7 (70%)
9	13	10 (77%)
>9	11	10 (91%)

Figure Legend

Figure 1 a: 5 gastric biopsies were taken. Only one (circled) shows cancer. b: Another case with three biopsies taken; one is normal, one is necrotic tumor (blue circle) and only one is viable tumour (red circle) c: high power of viable tumour biopsy d: Her 2 staining on this case is negative (weak staining in benign epithelium) but not sufficient for a conclusive answer