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Annals of Gastroenterology and the Digestive System

Open Access | Research Article

Optimising diagnostic biopsies for molecular analysis in oesophagogastric cancer

Sing Yu Moorcraft[1]; Shaila Desai[2]; Ujjwala Mohite[2]; Larissa S Teixeira Mendes[1]; Ruwaida Begum[1]; Isma Rana[1]; Carole Collins[2]; Ian Chau[1*]

[1]Department of Medicine, The Royal Marsden NHS Foundation Trust, UK

[2]West Middlesex University Hospital, UK

***Corresponding Author(s): Ian Chau**

Department of Medicine, Gastrointestinal and Lymphoma Unit, The Royal Marsden NHS Foundation Trust, Downs Road, Sutton SM2 5PT, UK

Tel: +44 208 915 6196; Email: ian.chau@rmh.nhs.uk

Received: Mar 28, 2018

Accepted: Jun 07, 2018

Published Online: Jun 13, 2018

Journal: Annals of Gastroenterology and the Digestive System
Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Gastric cancer; Oesophageal cancer; Therapeutic & Diagnostic endoscopy; Genetic testing

Abstract

Objective: To investigate the quality of tissue collection and preparation in patients undergoing diagnostic endoscopic biopsies for oesophagogastric cancer as DNA yield has implications for molecular analysis for precision oncology.

Design: Retrospective audit of patients undergoing diagnostic endoscopic biopsies between April 2013 and April 2015.

Setting: A District General Hospital, London, United Kingdom.

Patients: Fifty-seven patients diagnosed with localised or metastatic oesophagogastric cancer.

Interventions: Audit of routine clinical practice for biopsy sample collection and preparation.

Main outcome measures: The proportion of patients who had at least 6 biopsy samples taken and the time taken for each step of the tissue preparation pathway.

Results: Seventy percent of patients had ≥ 6 biopsy samples taken. Of the samples received by pathology, 67% contained tumour tissue. The median formalin fixation time was 1 day (range 0 – 4 days) and 11 samples (19%) spent ≥ 2 days in formalin. The median time from biopsy date to paraffin embedding was 2 days (range 0–7 days). The median formalin fixation time for biopsies taken on Friday was 3 days compared to 1 day for biopsies taken from Monday to Thursday.

Conclusion: Clinicians are not always taking the recommended number of diagnostic biopsies and tissue samples are frequently spending prolonged amounts of time in formalin, particularly if biopsies are performed on a Friday.

Cite this article: Moorcraft SY, Desai S, Mohite U, Mendes LST, Chau I, et al. Optimising diagnostic biopsies for molecular analysis in oesophagogastric cancer. Ann Gastroenterol Dig Syst. 2018; 2: 1007.

Introduction

In oncology, biomarkers are increasingly used to provide information on prognosis and/or identify patients who might be suitable for treatment with targeted anti-cancer therapies. For example, HER2 status is routinely tested in patients with metastatic gastric cancer in order to identify patients who are eligible for treatment with trastuzumab [1]. In addition, clinical trials often involve molecular analysis, either to determine eligibility for the trial or to stratify patients within the trial. Therefore patients' original biopsies are frequently not only used to make the initial diagnosis of cancer, but also for specialised tests (including analysis of mutations, copy number variations and protein expression) that can influence their subsequent treatment.

The evidence for the optimal number of biopsy samples required is limited [2], but studies have shown that the diagnostic sensitivity for gastric cancer is 79% for 1-2 biopsies, reaches >95% with 4 biopsies and 100% with 5-6 biopsies [3]. In contrast, in oesophageal cancer the diagnostic sensitivity is 96% for 1-2 biopsies, 98% for 4 biopsies and 100% for 6 biopsies [4]. Although it is unclear whether histology (adenocarcinoma versus squamous) affects the sensitivity of diagnostic biopsies, taking at least 5 biopsies is particularly important for infiltrative gastric lesions as these are more difficult to distinguish from normal mucosa [3]. Therefore conventional practice and current guidelines recommend taking a minimum of 6 biopsies for the diagnosis of Oesophagogastric (OG) cancer, [2,5] but this does not take into account the more recent use of biopsy samples for molecular analysis [4]. There is also limited guidance available regarding the collection and preparation of OG cancer samples for molecular analysis, but a set of 5 biopsy samples has been shown to have a sensitivity of 92% and specificity of 97% for HER2 assessment [6]. Furthermore, it has been suggested that gene expression profiling and DNA/RNA-based assays require 1–2 biopsies and variations in sample preparation (such as formalin fixation times) have been demonstrated to influence the success of genomic analyses [7-9].

Optimal sample collection and preparation is crucial to the success of precision medicine and the use of targeted therapies. We therefore conducted an audit of patients who were diagnosed with OG cancer by an endoscopic biopsy to investigate the quality of tissue collection and preparation.

Methods

After approval from our institutional audit committee, patients who were diagnosed with OG cancer (localised or metastatic) between April 2013 and April 2015 were identified from medical records at West Middlesex University Hospital (WMUH), United Kingdom (UK). Patients from WMUH diagnosed with OG cancer were referred to the Royal Marsden for treatment. Demographic, clinical and sample preparation data were retrospectively collected from patients' endoscopic and pathology records. Diagnostic tissue samples were reviewed by a histopathologist, who documented the number of samples received and the number of tumour-containing samples. Data on the quantity of DNA extracted from biopsy samples were collected from study records for patients who were also participating in a molecular profiling feasibility study (the FOrMAT study, ClinicalTrials.gov identifier NCT02112357).

The primary endpoint was the proportion of patients who had at least 6 biopsy samples taken. Secondary endpoints included the time taken for each step of the sample preparation pathway. The statistical analysis is descriptive, with percentages being reported.

Results

Between April 2013 and April 2015, 60 patients were diagnosed with OG cancer at WMUH. Three patients were excluded from the audit because the biopsy samples were not available for review. The median age of the 57 patients included in the audit was 73 years (range 38 – 90 years) and 63% of patients were male. Thirty-four endoscopies (60%) were requested as "urgent", 19 (33%) as a two week referral for suspected cancer and 4 (7%) as routine. The main lesion types were ulcerated (40%), exophytic (35%) and strictures (7%).

Forty patients (70%) had ≥ 6 biopsy samples taken, 9 patients (16%) had < 6 biopsy samples and in 8 patients (14%) the number of samples taken was not recorded. The median number of samples taken was 8 (range 4-12) and the median number of samples received by histopathology was 9 (range 4-21) as some samples fragmented. Sixty-seven percent of the samples received contained tumour tissue. The median number of tumour-containing samples was 3 for patients with < 6 biopsy samples, compared to 6 for patients with ≥ 6 biopsy samples. Three patients had DNA extracted from their biopsy samples as part of the FOrMAT study, obtaining 469ng, 748ng and 3900ng of DNA respectively.

Fourteen endoscopies (25%) were recorded as being potentially challenging due to bleeding (7 patients), friable tumour (3 patients), poor patient tolerance (3 patients) or being technically difficult (1 patient). Of these 14 patients, 5 (36%) had < 6 biopsy samples collected, compared to 4 (9%) of the 43 patients with no reported endoscopic difficulties.

The median time the tissue samples spent in formalin was 1 day (range 0-4 days) and 11 samples (19%) spent ≥ 2 days in formalin. The median time from the sample being placed in ethanol to being embedded in paraffin was 1 day (range 0-6 days) and the median overall time from biopsy to paraffin embedding was 2 days (range 0-7 days). The median time from biopsy to paraffin embedding was influenced by the day of the week the biopsy was performed, as biopsies taken on a Friday spent a longer time in formalin (Table 1).

Discussion

There has been huge interest in using molecular profiling to transform the care of patients. For example, the UK Government aims to sequence 100,000 genomes from approximately 70,000 people (patients with a rare disease, their families and patients with cancer) and the United States Precision Medicine Initiative includes US$70 million in increased funding for precision medicine in oncology [10,11].

In order for precision medicine and molecular profiling to be feasible in routine clinical practice, it is crucial that diagnostic samples are of sufficient quality and quantity for analysis. As many patients undergo diagnostic procedures at their local hospital prior to referral to a specialist cancer centre, it is important for all endoscopists and pathologists to be aware of the importance of suitable sample collection and preparation.

Tissue samples that are inadequate for molecular analysis can result in patients either being ineligible for targeted therapies/clinical trials or having to undergo further biopsies to obtain more tissue for analysis. Additional biopsies have cost and resource implications as well as the risk of biopsy-related com-

plications and/or delays in starting treatment. In addition, clinical trials often mandate the submission of a tissue block to a central laboratory. Usually only one tissue block is created from the biopsy samples, so delays can be incurred whilst the block is being retrieved (particularly if a patient had previously participated in a different clinical trial) and this could be minimised by splitting the biopsy samples into multiple blocks.

Inadequate tissue sampling can be a significant issue. For example, 10-33% of patients in large molecular profiling trials were not successfully analysed [12-16], and in theUS$30 million MATCH trial, 13% of samples did not yield sufficient DNA for analysis [16]. Although some samples will have failed due to inherent tumour characteristics (such as low tumour content and cellularity), poor DNA/RNA quality, insufficient biopsy tissue and poor biopsy quality may also be contributing factors [8,17]. For example, in the MATCH trial, samples were supposed to contain four tissue cores, but often only one or two were received and these frequently had a low tumour content [18].

In our audit, 70% of patients had ≥ 6 biopsies taken (although this does not include the 14% of patients in whom the number of samples taken was not recorded). The reason for less than 6 biopsies being taken may, in some cases, have been due to difficulties during the endoscopy procedure. However, in other cases the explanation was not apparent from the endoscopy records. The clinical significance of taking less than 6 biopsies was unknown for our patients as not all patients subsequently had treatment and/or molecular analysis (e.g. due to poor performance status). Nevertheless, as a result of this audit, the importance of taking adequate numbers of samples has been highlighted to endoscopists at our institutions.

Formalin fixation can lead to fragmentation, deamination artefacts, cross-linking and false positives and DNA quality can be influenced by variations in the fixation process [19-23] . Lengthy fixation times result in highly fragmented, low molecular weight DNA which may be unsuitable for genomic analyses [23]. There is limited guidance currently available for OG cancers, but for lung cancer biopsies the best results are generally seen with fixation times of 6-12 hours, although fixation times of 6-48 hours should also be acceptable [24]. In addition, poor formalin fixation can influence immunohistochemical testing (e.g. for HER2) and fixation times of 8-48 hours are recommended [25].

In our audit, 19% of tissue samples had a formalin fixation time of ≥ 2 days and the median fixation time was 3 days for biopsies performed on a Friday. This has potential implications for the quality of these samples and their subsequent use for molecular diagnostics. Consideration should be given to ways to minimise the formalin fixation time, such as providing a pathology processing service on Saturdays (which might involve consolidating local services).

In the future, new techniques such as analysing circulating tumour DNA (ctDNA) in blood samples may supersede tissue-based analysis, as blood samples are less invasive, easier and cheaper than biopsies and may overcome the problems of low tumour content and tumour heterogeneity [26]. However, until ctDNA has been validated for clinical use it is important to optimise tissue sample collection and preparation in order to facilitate the use of targeted therapies and recruitment into clinical trials, thereby maximising patients' treatment options.

Contributors

IC and CC conceived the idea for the audit. SD and LTM reviewed the tissue samples, UM collected the pathology processing data, RB and IR assisted with sample collection and processing. CC identified the patients, SYM collected the clinical data, analysed the data and wrote the manuscript. All authors contributed to the editing of the manuscript.

Summary

What is already known about this subject?

Tissue samples are increasingly used for biomarker analysis to identify patients who are suitable for treatment with targeted anti-cancer drugs or to determine eligibility for clinical trials.

Variations in sample collection and preparation can adversely impact on the success of molecular analysis.

What are the new findings?

Clinicians are not always taking the recommended number of biopsy samples.

Tissue samples are frequently spending prolonged amounts of time in formalin, particularly if biopsies are performed on a Friday.

How might it impact on clinical practice in the foreseeable future?

Oesophagogastric cancer guidelines should be updated to include details on sample collection for molecular diagnostics, but in the meantime clinicians should take at least the recommended 6 biopsy samples.

When structuring a biopsy/endoscopy service, optimal sample preparation should be considered and prolonged formalin fixation times should be avoided.

Tables

Table 1: Impact of biopsy day on sample processing timelines.

Day of the week	Number of patients	Median number of days spent in formalin (range)	Median number of days from biopsy to paraffin embedding (range)
Monday	13	1 (0 – 2)	1 (1 – 3)
Tuesday	8	1 (0 – 1)	2 (1 – 3)
Wednesday	11	1 (0 – 1)	2 (1 – 2)
Thursday	8	1 (0 – 1)	4 (1 – 7)
Friday	15	3 (1 – 4)	4 (2 – 4)
Saturday	2	2 (2 – 2)	3 (3 – 3)
Sunday	0	N/A	N/A

Acknowledgement

SYM, RB, IR, LTM and IC receive support from the National Institute of Health Research's Royal Marsden/Institute of Cancer Research Biomedical Research Centre.

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