CDI-00844-2018.R1

Original Article

An Experience of Implementation and Outcomes of Universal Testing for Lynch Syndrome in the United Kingdom

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We have no conflict of interest.

Word Count (excluding abstract, references, tables, figures): 2739

Ethics approval was granted for your study by Chelsea & Westminster Hospital NHS Trust.

Abstract

Introduction: Colorectal cancer (CRC) is diagnosed in approximately 45000 people annually in the UK, and it is estimated that Lynch Syndrome (LS) accounts for 3.1% of these cases. In February 2017 NICE guideline DG27 recommended universal testing of new cases of CRC for mismatch repair (MMR) status.

Methods: We prospectively collected data from Nov 2016 to August 2018 of consecutive newly diagnosed CRC patients at our centre, including evidence of MMR status performed by immunohistochemistry (IHC). We recorded clinicopathological data including age at diagnosis, stage, tumour site, reported histological findings and MMR tumour-status. Statistical analysis was performed using chi-square test and 2-tailed T test for binary and continuous variables respectively.

Results: A cohort of 203 consecutive CRC patients were diagnosed with CRC during this period. Universal MMR testing was performed of the 198 CRCs where a diagnosis of adenocarcinoma was confirmed, with colonoscopic biopsies used as the source material in 68.6% of cases. Twenty three CRCs (11.6%) were MMR deficient (dMMR). Most dMMR CRCs (21/23) were early stage tumours (Dukes' A or B, p=0.002). In 39 Dukes' B CRCs in patients under 70 years of age, the result of MMR testing influenced decision making about personalised treatment with 5-FU based chemotherapy.

Conclusion: Our results demonstrate that universal testing of all new cases of colorectal cancer for features suggestrive of Lynch Syndrome is feasible and effective in the UK. Our data also indicates the importance of genetic testing and personalised oncological care.

"What does this paper add to the literature":

We feel it is important to demonstrate that universal testing for Lynch Syndrome is feasible, effective and impacts on patient care diagnosed with colorectal cancer. (words 25).

Introduction

Lynch syndrome (LS), formerly known as hereditary non-polyposis colorectal cancer (HNPCC), is a common inherited cause of colorectal cancer (CRC) accounting for around 3% of newly diagnosed cases[1]. The lifetime risk for developing CRC in people with LS is up to70%, and LS patients are also at increased risk of other malignancies, notably endometrial, ovarian, as well as other cancers including stomach, hepatobiliary, urinary, brain and skin[2]. Thus LS accounts for approximately 2500 cases of cancer in the UK annually.

The syndrome is caused by mutations in one of four mismatch repair genes (MMR): MLH1, MSH2, MSH6 and PMS2, or in the EPCAM gene which is upstream of MSH2. Loss of heterozygosity in tumours is characterised by defective mismatch repair (dMMR) as demonstrated by DNA microsatellite instability (MSI) which is a PCR based assay, or loss of protein expression on immunohistochemistry. It remains unclear when the MMR-deficient phenotype is acquired during tumourigenesis although it is likely to be an early event[3].

Until very recently in the UK, universal testing of all new diagnosed CRC patients was considered to be impracticable, and so most centres tested for dMMR amongst phenotypically selected high risk patients - most commonly identified by either the Amsterdam[4] or revised Bethesda criteria[5]. The Amsterdam Criteria identify suspected LS families by the presence of 3 first degree relatives (FDRs) diagnosed with CRC, of whom one is a FDR of the other two, across at least 2 generations, with one affected individual being diagnosed with CRC under age 50 years. The revised Bethesda criteria incorporates patients diagnosed with CRC under age 50 years, those tumours with mucinous, signet ring, lymphocytic infiltration and other histological features, and some patients with a family history of CRC. However both these criteria are complex, creating variability in practice, and were originally designed as tools to select patients for research studies rather than in clinical practice.

However, the specificity and sensitivity of Bethesda criteria is poor, and many high risk patients do not receive testing[6]. A 2004 meta-analysis of the sensitivity and specificity of these

criteria concluded that the Amsterdam criteria was insufficient for predicting LS, while the Bethesda criteria was useful in the group of patients suspected of having familial CRC, but sensitivity was very low in the total group of newly diagnosed CRC patients[7].

In 2014 the UK Royal College of Pathology recommended as part of the minimal reporting dataset that all patients diagnosed with CRC under 50 years be tested for tumour features suggestive of LS[8]. A 2014 study of UK experience of testing for LS demonstrated wide variability in the application of the Bethesda criteria even in major cancer centres, with only 1 in 4 eligible patients receiving tumour testing for LS[6].

LS has a population prevalence of up to 1:279 based on extrapolated data from population studies published in Denmark and the USA [9](private communication Ian Frayling). However it is estimated that only 5-10% of LS patients are known in the UK, thus many opportunities for colorectal and other cancer prevention are being lost.

Studies in Europe and the US have consistently shown that universal testing of all CRC patients for LS is desirable, given the scope not only for reducing morbidity and mortality in family members but also for preventing secondary cancers in affected individuals[10, 11, 12, 13]. For every new case of LS identified, and further 3-4 FDRs are also diagnosed with LS gene mutations pre-symptomatically[14]. Therefore, patients identified with LS are advised to inform their relatives about the options of DNA testing and preventive measures as surveillance colonoscopies with removal of premalignant lesions, the use of aspirin, and other interventions which reduce the mortality and incidence of CRC in patients with LS.

Given the superiority of universal testing over selective targeting of high risk groups, and evidence suggesting its cost effectiveness, NICE issued guidance last year recommending it be offered to all people with CRC when first diagnosed in the UK, regardless of age, either by immunohistochemistry or microsatellite instability testing[14]. It is estimated that with universal testing for Lynch Syndrome 300 lives will be saved in the UK annually. As such we decided to implement these guidelines at our centre in November 2016.

Methods

Study Population

West Middlesex University Hospital is a district general hospital in West London with a catchment population of approximately 400,000 persons. Between 110-130 new CRC cases are diagnosed within the hospital every year. It is a UK bowel cancer screening programme centre via which approximately 14-18 new cases are diagnosed annually. All new CRC cases are discussed at a colorectal cancer multidisciplinary meeting (MDM) with data recorded using Somerset Cancer Registry software®, and data is submitted to the national bowel cancer audit (NBOCA https://www.nboca.org.uk/). Data was prospectively collected of all colorectal cancer cases between November 2016 and August 2018.

Assessment of LS status

The LS status of CRC patients at our centre is determined following the NICE DG27 guideline testing algorithm, initailly by MMR testing of all new CRC at the time of diagnosis. Universal testing commenced following the publication of the draft NICE guideline in November 2016. Prior to universal testing CRCs were selected for MMR testing using the revised Bethesda Criteria [5], with approximately 20-25% of all patients tested prior to November 2016.

MMR status was defined as deficient (dMMR) and proficient (pMMR), as determined by either microsatellite instability testing and/or IHC for MMR proteins. MMR IHC is used a the standard initial testing method in our centre. Systematic further reflex somatic tumour testing is undertaken by our pathologists, including *BRAF* V600E mutation testing, and MLH1 promoter methylation studies, as per the NICE DG27 algorthim.

Pathology laboratory testing for MMR status in our laboratory is linked to a UK national quality assurance process (www.gov.uk/government/publications/pathology-quality-assurance-review). The source tissue used for MMR testing was recorded i.e. colonoscopic biopsies or surgical resection specimens.

Clinicopathological data recorded included age at diagnosis of CRC, gender, and tumour stage and site.

Histological review was retrospectively performed of 'real world' pathology reports for features of mucinous tumours, signet ring cells, lymphocytic infiltrate, Crohn's-like reaction, or medullary pattern. Where surgical resection specimens were available these histology reports were reviewed for these classically associated LS-tumour features, in the absence of resection specimens endoscopic biopsies were reviewed.

Patients tumour testing results are reviewed at the colorectal MDM. MDM minutes were reviewed to assess the impact of MMR testing on oncological decision making.

Patients with loss expression of MSH2 or MSH6 proteins, or with loss of MLH1 or PMS2 proteins but without evidence of MLH1 promoter methylation, are subsequently offered in-house genetic counselling and germline testing where clinically indicated. Germline testing occurs in our hospital in the 'Family History of Bowel Cancer Clinic', a joint service performed with a gastroenterologist and a genetic counsellor, and linked to the North West Thames regional genetic service. We use the Illumina Trusight next generation sequencing panel for germline testing, with Sanger sequencing of the PMS2 gene performed because of associated pseudogenes. Cascade testing of such new LS patients is offered to their relatives.

Statistical analysis

Data for continuous variables are reported as median and range. Analysis was performed using chi-square test (or Fisher's Exact test for lower number tests) for binary variables and 2 tailed T test for continuous variables using Stata software v15.1.

Results

Patient Demographics

A total of 203 patients were consecutively diagnosed with CRC between November 2016 and August 2018 at West Middlesex University Hospital. The median age at diagnosis of CRC was 66 years (range 22-95), and 51.7% (103 patients) were male. Twenty nine patients were diagnosed under the age of 50 years. Tumour tissue was available for 198/203 patients, with 5 diagnoses made radiologically during this time excluded from further analysis. A histological diagnosis of colorectal adenocarcinoma was confirmed in all 198 patients where tumour tissue was available, including 3 patients with synchronous colorectal adenocarcinomas.

MMR testing

Universal MMR testing was performed in all 198 newly diagnosed CRC patients (including synchronous tumour specimens). Colonoscopic biopsies were used as the source material for MMR IHC in 136 patients (68.6%), with MMR testing of the surgical resection specimen performed in the remaining 62 CRCs.

Twenty-three CRCs (11.3%) were MMR deficient, as evidenced by loss of expression on IHC of MMR proteins. Eleven of these 23 dMMR CRCs (47.8%) would have not been selected for MMR tumour testing using the revised Bethesda criteria. The median age in patients with normal or abnormal MMR IHC was 64.6 years and 68.3 years respectively (p= 0.41). There was not a higher frequency of younger patients with dMMR tumours compared to those with pMMR tumours in this cohort - of the 23 dMMR CRCs, 13% were under 50 years of age, equivalent to the 14.9% of pMMR CRCs diagnosed under the age of 50 years (p=0.8 chi squared).

dMMR CRCs were predominantly early stage tumours, with 21/23 being Dukes' A or B, p=0.002).

Histological features of dMMR CRC tumours were also assessed (see table 2). The presence poorly differentiated histological features were predictive of dMMR CRC (p 0.023). Specifically mucinous tumours more frequently manifested dMMR, with 8/23 dMMR CRCs showing this feature compared with 18 of 175 non-dMMR CRCs tested ($p < 0.001$). The presence of signet ring cells and lymphocytic response was found in 1 and 2 dMMR CRCs respectively.

In 20 of 22 patients with dMMR the cancer was located in the right colon ($p < 0.001$).

In 39 Dukes' B CRCs in patients diagnosed under 70 years of age (20.5% of the total number), the MMR result was considered relevant to decision making about personalised treatment with 5-FU based chemotherapy as per ESMO guidelines (p=0.002).

Germline testing of MMR genes

All 23 patients with dMMR CRC underwent genetic counselling and were offered germline testing, with universal patient uptake of this germline testing. MMR germline mutations were found in a total of 7 patients (3.5%) of which 3 had pathogenic mutation of *MLH1*, in 2 cases of *MSH2* and in 1 case each with *MSH6* or *PMS2* mutations (see table 3). As we followed the NICE DG27 algorithm strictly we could not exclude the possibility of a BRAF somatically mutated tumour being due to dMMR germline mutations.

Discussion

A 'diffusion of responsibility' amongst physicians has been characterised with regard to testing for Lynch Syndrome, with uncertainty over who to test and variability in patient care [15]. Further to this, in the UK following the ublication of the NICE DG27 guideline, an expert letter called for a coordinated approach with a 'genomics champion' within colorectal MDTs, and linked up clinical pathways for the management of patients with LS [16]. We describe our

experience with universal and systematic testing for LS in a continuous cohort of CRC patients , and this paper is the first report of universal testing in a district general hospital in the UK.

Half of our patients would not have been selected for MMR testing via the revised Bethesda Criteria[15]. In an previously published experience of universal testing implementation in Houston, Texas the authors concurred with our findings that modified Bethesda criteria were inferior to universal MMR testing as per our data [17]. Several groups internationally have implemented universal testing for LS, as per national recommendations[19-21,] and published their experience [22-23].

Our view is that the application of a selective method such as the revised Bethesda criteria, rather than a universal method for patient selection, results in an ad hoc approach to MMR testing in CRC patients [6]. In the UK national health service (NHS) we have an advantage that universal testing may be incorporated in to national guidelines, systematically applied, and monitored via national quality assurance structures [18].

Nevertheless significant bariers to implementation of these guidelines remain. Prior to the publication of NICE guideline DG27 Snowsill et al demonstrated the cost-effectiveness of mismatch repair gene tumour testing for patients diagnosed with colorectal cancer under the age of 7 years [24]. Also, the Royal College of Pathologists recommended that all patients under the age of 50 diagnosed with CRC should be tested for Lynch syndrome. The adherence to these recommendations was poor, with 29% of UK hospitals still not testing this group by 2016, and of those who were, just 56% were doing so automatically leading to crucial delays in diagnosis[25]. It is important to Identify LS as it can lead to the early detection of cancer [26,27].

Cohen and co-authors described their experience on how informed molecular oncology interventions can improve patients' outcomes [28]. Preventative methods are available and there are also options for surgical prophylaxis as colectomy and bilateral salpingo-oophorectomy in patients with LS. The identification of dMMR, subsequent germline testing and personalised treatment based on molecular-targeting will help drive such testing forward [14].

Two thirds of our patients had MMR testing performed from colonoscopic biopsies rather than the surgical resection specimen. Pre-treatment testing of colonoscopic biopsies has many potential advantages over testing of a tumour resection specimen. According to the national cancer intelligence network approximatley 1 in 3 CRC patients in the UK will not undergo surgical resection, and only 42% will have surgery as a sole treatment modality [29].

A number of studies have verified concordance in MMR testing between colonoscopic biopsy and colorectal resection specimens. In a study of 112 unselected CRC cases performing Immunostaining for MLH1, PMS2, MSH2 and MHS6 with both endoscopic biopsy and surgical resection samples. They showed complete agreement in MMR status between the endoscopic biopsy and corresponding surgical resection specimens in those cases that were conclusive [30]. An Australian group assessed CRC biopsy specimens pre-op matched with post-op specimens in germline MMR mutation patients. These patients were matched with Bethesda positive, but pMMR patients. The sensitivity of the endoscopic biopsies in predicting germline status was almost 95%. An Irish study assessed concordance of MMR staining between biopsy and resection specimens. They analysed 53 patients and showed complete agreement in MMR IHC status between the preoperative endoscopic biopsies and corresponding resection specimens in all CRC cases [31].

Conversely there is evidence that neoadjuvant chemoradiotherapy may alter the results of MMR, particularly, but not only, with regard to interpretation of MSH6 expression [32]. We believe this evidence underlines the importance and increases the utility of universal testing on colonoscopic biopsies where possible, woth surgical resection specimens undergoing MMR testing only in the absence of a tissue available from pre-treatment tumour tissues. With molecular advances in testing, in the near future germline testing of such colonoscopic biopsy specimens may lead to pre-operative germline diagnosis of Lynch Syndrome to help guide surgical and oncological decision making. However, as demonstrated in this study, testing can influence the choice of chemotherapeutic agensts such as the use of 5-fluoruracil based chemotherapy in the treatment of Dukes' B CRC as per current ESMO guidelines [33, 34].

The importance of genomics is growing in clinical practice. Tranformative aspects of the UK 100.000 genomes project in colorectal cancer has the aim to mainstream diagnosis and outcomes for patients with rare diseases and cancers, and testing for Lynch Syndrome will be feasible for members of the colorectal mutidisciplinary team who could not previously directly avail of this testing in their clinics.

Our study has a number of limitations. As a real world evaluation of implementation of a national guideline, we have not performed an economic evaluation of the impact of such testing on a colorectal service. The role of clinical commissioning groups in supporting and commisioning of this service has not been formalised, as demonstrated in a recent Bowel Cancer UK report to be a problem in many areas of the UK [35]. For non-mandatory 'non-core' reporting items in the Royal College of Pathology colorectal cancer dataset [8], may result in under-reporting of the presence of lymphocytic infiltratation as may be true in this study. Increasing elucidation of the role of the immune system in colorectal cancer may lead to the development of new immunotherapies and tumour vaccines [36] and is therefore likely to be of increasing value in the personalised care of our patients.

Conclusions

Our data demonstrate that universal testing, as recommended by NICE DG27, is both feasible and effective in order to improve outcomes for patients. This practice allows us to identify patients that may have not been selected for MMR testing by pre-existing revised Bethesda criteria. We believe it is relevant and provides an advance in the surgical and oncological treatment for these patients and progress towards more personalised care of CRC patients.

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Tables

Table 1: Comparison of age and gender of patients with defective mismatch repair (dMMR) versus proficient mismatch repair (pMMR).

MMR status	dMMR	pMMR
Male Gender	11/23 (47.8%)	92/175 (52.6%)
Median age (range in brackets) (years)	68.3 (46-92)	64.6 (22-95)
CRCs <50 years of age (%)	3/23 (13%)	26/175 (14.9 %)

Table 2: Histological features of CRCs with defective mismatch repair (dMMR, total number 23) versus proficient mismatch repair (pMMR, total number 175)

Histological feature	dMMR	pMMR	p value
Mucinous	8	18	<0.001
Signet ring cells	1	3	0.38
Lymphocytic response	2	11	0.63

Table 3: CRCs with defective mismatch repair (dMMR), somatic testing and corresponding diagnosis of germline MMR gene mutations

MMR protein	Loss of Expression	*BRAF* testing Mutant	*BRAF* testing Wt	MLH1 methylation	Germline mutation	No mutation identified
MLH1 alone	1	0	1	0	1	0
MLH1/PMS2	11	7	4	1	2	1
MSH2/MSH6	6	-	-	-	1	0
MSH2 alone	2	-	-	-	1	0
MSH6 alone	2	-	-	-	1	0
PMS2 alone	1	-	-	-	1	0