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Original Research

# Immunohistochemistry testing for mismatch repair deficiency in Stage 2 colon cancer: A cohort study of two cancer centres

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ABSTRACT

*Background/Objectives:* Adjuvant chemotherapy for Stage II colon cancer offers a small (2-3%) overall survival benefit and is not universally recommended. Mismatch repair deficiency (dMMR) confers an improved prognosis identifying patients unlikely to benefit from adjuvant chemotherapy. The aim of this study was to investigate the use of dMMR immunohistochemistry in two major cancer treatment centres.

*Methods:* Prospective data were collected on all patients with resected Stage II colon cancer between 2010 and 2015 across two large Australian hospitals. Data collected included patient demographics, tumour histology, dMMR immunohistochemistry, chemotherapy use, and outcomes.

*Results:* All 355 patients (56.1% female, median age 81) with resected Stage 2 Colon cancer entered on to the surgical database were included in this analysis. MMR testing was performed on 167 patient samples (47%), most occurred post-2013 (73.1% vs. 26.9% patients). dMMR rates were 34.1%. 25 (7.3%) received adjuvant chemotherapy, with no patient > 80 years receiving treatment. Presence of ≥2 high-risk feature increased the likelihood of adjuvant chemotherapy. Only 3.6% dMMR patients received chemotherapy; both were young with high-risk features. 27/288 (7.6%) patients (with follow up) relapsed, with 7 disease-free post-resection of metastatic disease, 9 are alive with metastatic disease, and 11 deceased.

*Conclusions:* Unlike clinical trial populations, Stage 2 colon cancer patients are often elderly, have high rates of dMMR tumours, are rarely offered chemotherapy, yet still have excellent outcomes. dMMR immunohistochemistry is being increasingly used to identify Stage 2 patients who do not require chemotherapy.

## 1. Introduction

Colorectal cancer is a leading cause of morbidity and mortality worldwide, with approximately 17,500 new cases annually in Australia [1]. While mortality rates are improving, incidence rates continue to increase, especially in developing countries. Stage II colon cancer is associated with a good prognosis, with a 5-year survival in the 75-80% range, with recent improvements to 85% [2,3]. Whilst adjuvant chemotherapy has a clear survival benefit in node positive patients with increased benefit for oxaliplatin based regimens, the incremental benefit is far less in Stage II cancer. There is only a 2-4% increment in 5-year survival with fluoropyrimidine based adjuvant chemotherapy and no additional benefit observed with the addition of oxaliplatin [3,4]. As a result, the routine use of adjuvant chemotherapy for all patients with Stage II cancer is not recommended, although is often considered for those at increased risk of recurrence and younger age [5].

Higher risk patients are currently identified by clinical and histological features such as age, tumour pathological grade, obstruction or perforation, and low lymph node yields [6]. Whilst there is substantial research interest in the development of more accurate and

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individualised assessment for the likelihood of recurrence such as gene expression profiling platforms (e.g. Oncotype DX), these remain expensive, unvalidated, may not provide predictive benefit, and so are not routinely used in clinical practice [7]. The roles for CDX2 and the presence of circulating tumour DNA as potential markers for defining a sub-group of Stage II cancers with a high risk of relapse also continue to be explored but remain experimental [8,9].

One simple and useful prognostic and predictive indicator is whether a tumour is proficient (pMMR) or deficient (dMMR) for mismatch repair (MMR) with a number of studies confirming the more favourable prognosis of the dMMR sub-group [10]. Immunohistochemical testing for protein expression of the four common MMR genes (MLH1, MSH2, MSH6 and PMS2) is readily available and can be carried out at the time of routine pathological assessment of the resected tumour specimen. Approximately 20% of patients with Stage II resected colon cancer are deficient for mismatch repair (dMMR). Further retrospective analyses of trials have demonstrated that this sub-group do not benefit from adjuvant fluoropyrimidine chemotherapy [11,12].

A small proportion of dMMR tumours will be due to an underlying germline mutation in one of the mismatch repair genes (Lynch syndrome) while the remaining are related to acquired methylation (e.g. MLH1) or somatic mutation. While the implications of a dMMR tumour are significant for the individual in terms of treatment planning, the identification of dMMR tumours in the context of Lynch Syndrome have broader ramifications for both future cancer risk for the individual and for other family members. Thus, the identification of a dMMR phenotype requires a closer examination of family history, potential further molecular evaluation (e.g. somatic BRAF mutation or MLH1 methylation testing) and consideration of referral to a family cancer clinic (FCC) [13].

This study examines consecutive patients with resected Stage 2 colon cancer over a five-year period at two large cancer centres, with the primary aim to evaluate the rationale, use and frequency of chemotherapy, and whether dMMR immunohistochemistry (IHC) testing was incorporated into the clinical management algorithm.

## 2. Materials and methods

A retrospective study was carried out on prospectively collected data from two large public and private hospitals in Melbourne, Australia. Data were collected on all patients with resected Stage II colon cancer between February 2010 and February 2015, prospectively entered onto the Department of Surgery colorectal neoplasia database [14]. Follow-up data for these patients was collected until 20th December 2015. Rectal cancers were excluded due to the uncertainty regarding true clinical stage in patients who are routinely treated with neoadjuvant chemoradiation. High risk features were defined as; poor/undifferentiated histology, < 12 nodes collected, T4 tumour, obstruction, perforation [6,15]. Human research ethics committee approval was granted across both private and public sites (CHREC Reference # 09-22-06-15).

Immunohistochemistry was carried out in hospital pathology departments using Dako antibodies for PMS2, MLH1, MSH2 and MSH6 (Agilent Technologies Inc., Santa Clara, CA, USA), and the Leica Polymer Refine Detection kit on a Leica Bond-III Automated immunohistochemistry stainer (Leica Microsystems Pty Ltd, Mt. Waverley, VIC, Australia).

Data was cross-referenced through manual searching of source records as a check for accuracy. Statistical analyses were performed on SPSS (version 22.0. Armonk, New York, NY, USA) and GraphPad (GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA). Fisher's exact tests and $\chi$-squared tests were used to compare variables and a $P$ value $< 0.05$ was considered significant.

This study the work has been reported in line with the STROCSS criteria [16].

**Table 1**
Patient demographics, clinical features and pathology.

| | N (%) |
|---|---|
| Patients | 355 |
| Male | 156 (43.9) |
| Female | 199 (56.1) |
| Private Hospital | 271 (76.3) |
| Public Hospital | 84 (23.7) |
| Age | |
| Mean (+/− SD) | 77.9 years (11.4) |
| Median (range) | 81.0 years (34-101) |
| Age spread | |
| < 60 years | 22 (6.2) |
| 60-80 years | 146 (41.1) |
| > 80 years | 187 (52.7) |
| High risk features | |
| T Stage | |
| 3 | 329 (92.6) |
| 4 | 26 (7.4) |
| Histology | |
| Moderate/Well differentiated | 265 (74.6) |
| Poor/Undifferentiated | 90 (25.4) |
| Number of nodes collected | |
| < 12 | 52 (14.6) |
| 12 or greater | 303 (85.4) |
| Perforation | 9 (2.5) |
| Obstruction | 33 (9.3) |
| Number of high risk features | |
| 0 | 182 (51.3) |
| 1 | 130 (36.6) |
| 2 or more | 43 (12.1) |

SD, standard deviation.

## 3. Results

### 3.1. Demographics

From 1st February 2010 to 1st February 2015, 355 patients had bowel resection for Stage II colon cancer at a private hospital (271) and a public hospital (84) and were entered into the colorectal neoplasia database. The mean age was 77.9 years (median 81, range 34–101), with 56.1% female. This cohort was predominantly elderly with 187 (52.7%) over 80 years, and only 22 (6.2%) under 60 years (Table 1). Tumour histological features are described in Table 1. 182 (51.3%) patients had no high-risk features (poor/undifferentiated histology; < 12 nodes collected; T4 tumour; obstruction; perforation), 130 (36.6%) with one feature, and 43 (12.1%) with two or more.

### 3.2. Mismatch repair testing by immunohistochemistry

MMR immunohistochemistry was performed in 167 of 355 patients (Table 2) with far fewer tests performed 2010-2012 (50 of 195, 25.4%), compared to 2013-2015 (117 of 160, 73.1%). Of those tested, 57 (34.1%) were found to have loss of at least one of the MMR proteins and were therefore classed as dMMR. dMMR rates varied significantly by age: less than 60 yrs (37.5%), 60-80 yrs (24.4%), and over 80 yrs (44.9%, $p = 0.028$). There was no difference in dMMR immunohistochemistry rate between genders. Patient tumours were divided between right (proximal to hepatic flexure) and left (distal to splenic flexure) sided pathology. Transverse colon tumours and multiple primaries were excluded from this analysis only. Higher rates of dMMR were observed in right (n = 38, 46.9%) versus left sided tumours (n = 5, 9.6%, $p < 0.0001$). Between hospital differences were not significant.

**Table 2**
Characteristics of MMR immunohistochemistry.

| MMR immunohistochemistry | N | | % | |
|---|---|---|---|---|
| Not tested | 188 | | 53 (all patients) | |
| Tested | 167 | | 47 (all patients) | |
| MMR stable | 110 | | 62.9 | |
| MMR deficient | 57 | | 34.1 | |
| Year of diagnosis | MMR not tested N (%) | | MMR tested N (%) | |
| 2010–2012 | 145 (74.6) | | 50 (25.4) | |
| 2013–2015 | 43 (26.9) | | 117 (73.1) | |
| Age | Not tested | pMMR N (%) | dMMR N (%) | *P* |
| < 60 years | 6 | 10 (62.5) | 6 (37.5) | 0.028 [a] |
| 60-80 years | 64 | 62 (75.6) | 20 (24.4) | |
| > 80 years | 118 | 38 (55.1) | 31 (44.9) | |
| Sex | | | | |
| Male | 91 | 44 (67.7) | 21 (32.3) | 0.74 [b] |
| Female | 97 | 66 (64.7) | 36 (35.3) | |
| Tumour side | | | | |
| Left | 78 | 47 (90.4) | 5 (9.6) | < 0.0001 [b] |
| Right | 96 | 43 (53.1) | 38 (46.9) | |

[a] $\chi^2$ test.
[b] Fisher's Exact test. MMR, mismatch repair

### 3.3. Chemotherapy

Twenty-five patients (7.3%) received adjuvant chemotherapy. Twenty-one of these were treated with fluorouracil (5-FU), one with capecitabine, and three received oxaliplatin-based chemotherapy. Younger patients were more likely to receive chemotherapy, with 9 of those 22 patients less than 60 years (41%), compared with none of the 187 patients over the age of 80 years ($p < 0.001$; Table 3). Patients with high-risk features were also more likely to receive adjuvant chemotherapy – with 6.9% of those with one risk factor, and 18.6% with two or more features versus only eight of 182 (4.4%) patients with no high-risk features were administered chemotherapy ($p = 0.0046$). Of the 56 patients with documented MMR deficient tumours, only two patients with MMR deficient tumours received adjuvant chemotherapy. Both of these patients were young (aged 34 and 54), had high-risk features, and were diagnosed in 2011 and 2012 respectively. One of these patients was treated with oral capcitabine and the other FOLFOX.

**Table 3**
Use of chemotherapy.

| | Chemotherapy | | *P* |
|---|---|---|---|
| | Not Given N (%) | Given N (%) | |
| Private Hospital | 248 (91.9) | 22 (8.1) | 0.22 [b] |
| Public Hospital | 81 (96.4) | 3 (3.6) | |
| Total | 329 (93.0) | 25 (7.0) | |
| Year of resection | | | |
| 2010–2012 | 178 (91.3) | 17 (8.7) | 0.21 [b] |
| 2013–2015 | 152 (95.0) | 8 (5.0) | |
| Age | | | |
| < 60 years | 13 (59.1) | 9 (40.9) | < 0.001 [b] |
| 60-80 years | 129 (89.0) | 16 (11.0) | |
| > 80 years | 187 (0.0) | 0 (0) | |
| Number of high risk features | | | |
| 0 | 174 (95.6) | 8 (4.4) | 0.0046 [a] |
| 1 | 121 (93.1) | 9 (6.9) | |
| 2 or more | 35 (81.4) | 8 (18.6) | |
| MMR immunohistochemistry | | | |
| Not done | 177 (94.2) | 11 (5.8) | 0.146 [a] |
| MMR stable | 99 (89.2) | 12 (10.8) | |
| MMR deficient | 54 (96.4) | 2 (3.6) | |

[a] $\chi^2$ test.
[b] Fisher's Exact test. MMR, mismatch repair.

**Table 4**
Factors affecting patient relapse.

| | Relapse N (%) | No relapse N (%) | *P* |
|---|---|---|---|
| MMR immunohistochemistry | | | |
| Not done | 17 (62.9) | 144 (55.2) | 0.717 [a] |
| MMR stable | 7 (25.9) | 77 (29.5) | |
| MMR deficient | 3 (11.1) | 40 (15.3) | |
| Chemotherapy | | | |
| Chemotherapy given | 4 (14.8) | 18 (6.9) | 0.138 [b] |
| No chemotherapy | 23 (85.2) | 243 (93.1) | |
| Number of high risk features | | | |
| 0 | 12 (44.4) | 159 (60.9) | 0.003 [a] |
| 1 | 8 (29.6) | 84 (32.2) | |
| 2 or more | 7 (26.0) | 18 (6.9) | |

[a] $\chi^2$ test.
[b] Fisher's Exact test. MMR, mismatch repair.

### 3.4. Relapse

Follow up data was available for 288 (81%) patients with several patients being followed up by local general practitioners. Some patients with multiple medical comorbidities or advancing age declined surveillance. Median follow-up period was 20.5 months and during this period 12 patients died of non-cancer related causes. There were 27 relapses, 9 at the public site, 18 at the private (Table 4). Ten patients underwent curative intent resection of metastatic disease, with six remaining disease free. Of the remaining 17 patients, eleven were deceased. There were no differences in the testing for dMMR between the relapse and non-relapse patient groups ($p = 0.688$; Table 4). Additionally, there was no difference in the rates of chemotherapy given to the relapse or non-relapse groups (14.8% v. 6.9%, $p = 0.138$). Patients with 2 or more high risk features had significantly higher rates of relapse than patients in the no relapse group (26.0% vs. 6.9%, $p = 0.003$). Between hospital differences were not significant.

## 4. Discussion

This retrospective study of patients who have undergone surgery with Stage II colon cancer revealed a number of unexpected findings in a 'real world' unselected Australian population; a relatively old cohort with very low rates of administration of adjuvant chemotherapy, and high rates of dMMR. Our finding of the age distribution of patients with Stage II disease is noteworthy, not the least because the inclusion of elderly patients in the seminal trials of adjuvant chemotherapy for colon cancer was restricted either by entry criteria or by referrers' bias not to enrol [17]. For example, the median age of Stage II patients in the QUASAR trial was 63 years; and in the seminal adjuvant oxaliplatin based trials was 59 years (NSABPc07 trial) and 60.5 years (MOSAIC trial) [18,19], whereas the median patient age in this study was 81 years.

The use of dMMR immunochemical testing to document protein expression of the four MMR genes was not widespread in Australia until approximately 2013 and is still not universally available despite international guidelines [5]. The clinical relevance of detecting dMMR is well recognised now to extend beyond that of identifying potential

familial colon cancer syndromes to forming a contraindication to the use of adjuvant single agent fluoropyrimidine chemotherapy [11,12]. Our observed rate of dMMR (34.1%) is higher than the generally quoted rate of 15–25%, however these studies have a younger median age, and importantly also include rectal cancer which has much lower positivity [10–12]. Our finding of a 34.1% overall rate mirrors at least one previous series where rectal cancer was excluded; our rate of 45% in patients aged over 80 is consistent with data reporting dMMR in up to 50% of tumours in patients over 90 years [18,20]. Much higher rates of dMMR were observed in right sided tumours in this study consistent with previously described colorectal molecular subtypes where 31% of subtype 1 (microsatellite instability immune) were right-sided tumours compared with 7% of left-sided tumours [21].

Published rates of use of adjuvant chemotherapy for Stage II colon cancer range from 20 to 59% in clinical populations, with patients at higher-risk and younger age being most likely to receive treatment [10,20]. In our study, only 7.0% of patients received treatment and patients with 2 or more high risk features present were more likely to receive chemotherapy. Even when excluding the cohort over 80 years (of whom none received chemotherapy) only 17.6% received chemotherapy, despite the fact that almost half these patients had risk features present. The rate of chemotherapy usage decreased in the latter half of the study time period. This is likely a reflection of a greater understanding in recent years of the lack of benefit of adjuvant treatment in Stage II disease [5,20]. This is of particular relevance to this cohort with a large proportion of patients over 80 years, where chemotherapy may be of diminished benefit and increased risks due to comorbidities.

The distribution of age in our dataset and other studies supports specific trials of adjuvant chemotherapy in the elderly, as are now being reported in patients with metastatic disease [22]. The use of chemotherapy and whether a patient was dMMR were not factors in patient relapse however, relapse was associated with patients with 2 or more high risk features. This is in line with a study of Stage 2 colorectal cancer patients with 5 year follow up periods showing that high risk patients with < 12 lymph nodes, perforations and obstructions had lower disease free survival [23].

A limitation of this study was incomplete follow-up, this reflects the elderly cohort of patients presented who either decline ongoing surveillance or are not considered suitable for resection of solitary metastatic recurrences and hence are usually not offered intensive follow up. Decision making around chemotherapy was reviewed retrospectively through case notes, and in many cases was not articulated. Future prospective studies may explore how dMMR testing influences clinician and patient decision making.

## 5. Conclusions

In conclusion, this study reports a large cohort of Australian patients with Stage II colon cancer and highlights issues of clinical importance in contemporary practice including the high frequency of elderly patients - a notable finding as they are often excluded from clinical trials - the low utilisation of adjuvant chemotherapy and the high rate of patients whose tumours demonstrate deficient DNA mismatch repair. Given the utility of this simple immunochemical test as both a prognostic and potentially a predictive biomarker, we recommend it be utilised in all Stage 2 patients being considered for chemotherapy.

## Ethical approval

The study was approved. The Alfred and Cabrini Hospitals - CHREC #09-22-06-15.

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## Author contribution

1 Study design
2 Prospective data collection
3 Retrospective data collection
4 Data analysis
5 Manuscript preparation

Matthew Grant (1,3,4,5), Andrew Haydon (1,2,3,4,5), Lewis Au (1,3,4,5), Karen Oliva (1,2,4,5), Simon Wilkins (1,2,4,5), Eva Segelov (4,5), Yoland Antill (2,4,5), Peter Carne (2,3,5), Pravin Ranchod (2,3,5), Adrian Polglase (2,3,5), Chip Farmer (2,3,5), Martin Chin (2,3,5), Roger Wale (2,3,5), Paul Simpson (2,3,5), Stephen Bell (2,3,5), Stewart Skinner (2,3,5), Paul McMurrick (1,2,3,4,5), Jeremy Shapiro (1,2,3,4,5).

## Conflicts of interest

Nil.

## Trial registry number

Researchregistry3162.

## Guarantor

Dr Matthew Grant.
A/Prof Jeremy Shapiro.

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