Adherence to national Lynch syndrome testing guidelines for colorectal cancer in an Aotearoa New Zealand hospital-based population

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ABSTRACT

AIM: Lynch syndrome (LS) is estimated to affect 1–3.9% of patients with colorectal cancer (CRC). Testing for LS is important in determining management and establishing surveillance for "Lynch families". Previous studies have identified poor rates of testing for LS in CRC patients. This study aimed to describe adherence to guidelines for testing of newly diagnosed CRC for LS.

METHODS: A single institution cohort study of patients over 18 years with colorectal adenocarcinoma from 2018–2022 in Te Tai Tokerau, Aotearoa New Zealand was conducted. Rates of baseline immunohistochemistry (IHC) testing for mismatch repair (MMR) deficiency, further testing for MLH1-deficient cases and rates of germline mutational analysis were audited to determine adherence to national guidelines. The rate of LS in newly diagnosed CRC was estimated.

RESULTS: Six hundred and sixty patients were eligible for universal testing for LS, of which 84% (n=553) completed initial IHC testing. MMR deficiency was reported in 20% (n=114) cases. Eighty-nine percent (n=101) was attributable to MLH1 deficiency, of which 99% (n=100) were appropriately tested for BRAF-V600E mutation. Sixty-four percent (4/11) patients indicated for hypermethylation testing were appropriately tested. Seventeen patients had an indication for germline mutational analysis, of which only 29% (n=5) were tested. The estimated incidence of LS in newly diagnosed CRC was 0.7–3.8%.

CONCLUSION: Compliance with initial IHC testing was good. However, there is a need to improve rates of confirmation genetic testing. The incidence of confirmed LS in this study is 0.7%, however this may be as high as 3.9%.

The lifetime risk of colorectal cancer (CRC) for individuals with pathogenic mutations of mismatch repair (MMR) genes (Lynch syndrome) varies from 28–100% in males and 25–83% in females in the literature.[1–7] Despite considerable risk of CRC and other malignancies, it is estimated that in the United Kingdom (UK), 95% of individuals with Lynch syndrome (LS) are not aware of their condition.[8,9]

Testing patients diagnosed with CRC is an opportunity to identify individuals with LS. This has implications for the patient and their families, as other family members may be carriers of the same pathogenic gene variant. This may allow targeted risk-reducing interventions, including endoscopic surveillance, preventative surgery and chemoprophylaxis. Identification of LS preoperatively also influences management.[10] Clear guidelines for testing all patients with newly diagnosed CRC (universal testing) have been outlined by both the Aotearoa New Zealand Ministry of Health – Manatū Hauora and the National Institute For Health and Care Excellence (NICE) in the UK.[11,12]

In response to evidence of poor implementation of guidelines for LS testing in CRC, a recent national quality improvement project was commenced in the UK.[11,13] This group oversaw an increase in MMR deficiency testing from just 43% of CRC cases in 2019 to 91% in 2022. To our knowledge, there has been no published data on adherence to CRC screening guidelines recommended by the Ministry of Health – Manatū Hauora in Aotearoa New Zealand.

This retrospective cohort study aimed to identify adherence to guidelines published by the Ministry of Health – Manatū Hauora for LS testing among patients with CRC. Furthermore, we aimed to obtain the most accurate estimate of the incidence of LS in newly diagnosed CRC in Aotearoa New Zealand based on the currently available data.

Methods

Patient selection

All consecutive adult patients (>18 years) with newly diagnosed colorectal adenocarcinoma referred to Te Tai Tokerau locality between 1 January 2018 and 31 December 2022 were eligible. The Whangārei Hospital data warehouse was searched for all patients diagnosed with colorectal cancer. Patients with histology codes for neuroendocrine tumours, gastrointestinal stromal tumours, lymphomas, squamous cell carcinomas, leiomyosarcomas and melanomas were excluded. Patients with previously diagnosed LS were also excluded.

Variables

Patient data collected included: age at first treatment, sex (classified as male or female) and ethnicity (self-identified). Tumour, node and metastasis (TNM) staging and tumour grade were extracted for patients having resection of their cancer. It was not possible to provide stages of cancer given the lack of M staging available.

Outcomes

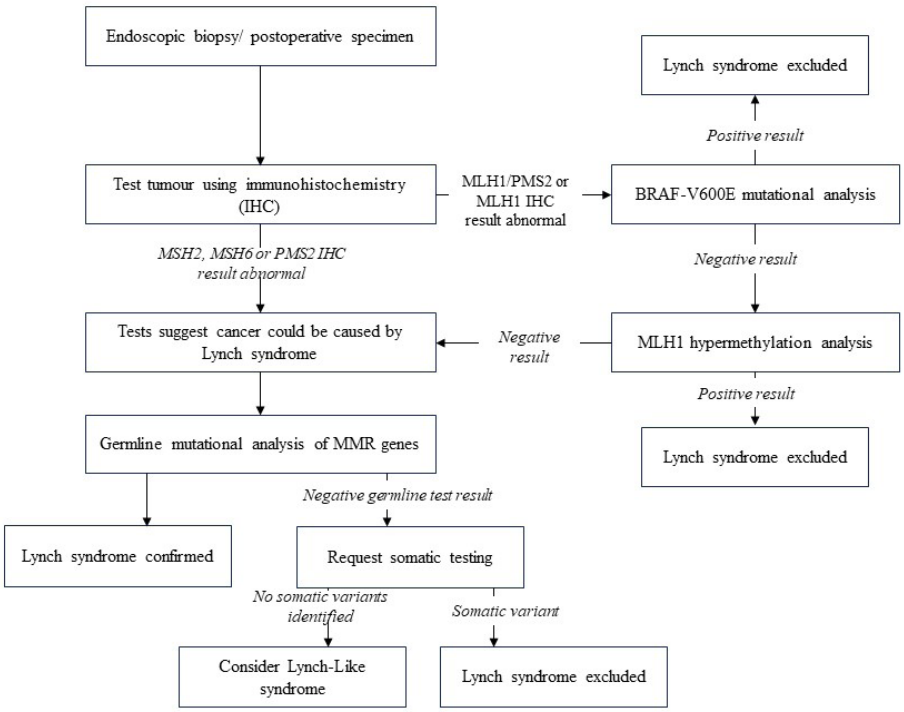
The primary outcome was adherence to testing protocols as established by the Ministry of Health – Manatū Hauora (**Figure 1**); this was established by i) proportion of patients with CRC receiving immunohistochemistry (IHC) testing, ii) proportion of patients with an MLH1 deficient proceeding to mutational analysis of the BRAF-V600E gene and hypermethylation testing of the promoter region respectively, and finally iii) proportion of patients identified to have a high likelihood of LS completing genetic testing.

Secondary outcomes included patterns of MMR deficiency and the proportion of patients with newly diagnosed CRC and completed IHC testing, with LS or Lynch-like syndrome (LLS—MMR deficiency in the absence of a proven germline mutation that cannot be explained by BRAF and MLH1 hypermethylation[14]) in Te Tai Tokerau, Aotearoa New Zealand.

IHC for MMR proteins

IHC was performed for four MMR proteins (MLH1, MSH2, MSH6 and PMS2) using a Staining Automat according to the manufacturer's protocol, in a 4-µm-thick formalin-fixed paraffin-embedded (FFPE) sections. The primary antibodies used for detecting MMR proteins were the anti-hMLH1 antibody (clone ES05), anti-MSH2 antibody (clone 25D12), anti-hMSH-6 (clone PU29) and anti-hPMS2 antibody (clone M0R4G). The normal staining

Figure 1: Aotearoa New Zealand Ministry of Health – Manatū Hauora guidelines for the testing of Lynch syndrome in newly diagnosed patients with colorectal cancer.



patterns for MLH1, MSH2, MSH6 and PMS2 are nuclear. The absence of nuclear staining in tumour cells and the presence of nuclear staining of non-neoplastic cells were considered to represent an abnormal pattern.

Methylation analysis of MLH1 gene promoter C region

Methylation status of the promoter region of the MLH1 gene was established by means of Agena Bioscience's EpiTYPER MassARRAY System, using base-specific cleavage and MALDI-TOF Mass Spectrometry. This was performed following DNA extraction from paraffin-embedded tissue and treatment with bisulfite. Hypermethylation of the MLH1 promoter region leads to transcriptional slicing of the MLH1 gene. The following thresholds were used: non-hypermethylated (0–10%); equivocal (11–20%); hypermethylated (>20%).

Detection of germline mutations and copy number variances

Targeted gene sequencing of coding regions and splice sites was performed on DNA extracted from blood. Libraries were prepared and enriched using SureSelectXT target enrichment (Agilent Design ID 0825941). Indexed libraries were pooled and sequenced to a targeted coverage of 700 reads/base (Illumina NextSeq500 2x75bp). Seqliner v0.8 was used to generate aligned reads and call variants against hg19 human reference genome. PathOS v1.5 was used to annotate and transform variants to standard nomenclature and filter for rare, non-synonymous variants within 20bp of coding exons. Copy number was analysed using Gaffa 3.0 Targeted. Variants are described according to HGVS Nomenclature version 19.01 with minor differences in accordance with Molecular Pathology policy.

Statistical analysis

Baseline categorical data were presented as number and percentage and analysed using the Fisher's exact probability test. Continuous data were presented as median and interquartile range (IQR) and analysed using the Wilcoxon signed-rank test. A significance level of $p < 0.05$ was used to indicate statistical significance.

Ethical consideration

This study was deemed "out of scope" by the Health and Disability Ethics Committee on 23 August 2023. Locality approval was obtained from the Te Tai Tokerau research group.

Results

Participants

In total, 718 patients were referred to Te Tai Tokerau in the 5-year time period with CRC.

Table 1: Demographic variables.

Characteristic	N	Overall, N=660	Patients not completing IHC testing, N=107	Patients completing IHC analysis, N=553	p-value
Gender	660				>0.9
F		303/660 (46%)	49/107 (46%)	254/553 (46%)	
M		357/660 (54%)	58/107 (54%)	299/553 (54%)	
Age	660	72.00 [64.00–80.00]	76.00 [65.00–85.50]	72.00 [64.00–79.00]	0.005
Ethnicity	660				0.14
NZ European		482/660 (73%)	80/107 (75%)	402/553 (73%)	
NZ Māori		91/660 (14%)	19/107 (18%)	72/553 (13%)	
Other		11/660 (1.7%)	1/107 (0.9%)	10/553 (1.8%)	
Other European		73/660 (11%)	6/107 (5.6%)	67/553 (12%)	
Pacific peoples		3/660 (0.5%)	1/107 (0.9%)	2/553 (0.4%)	

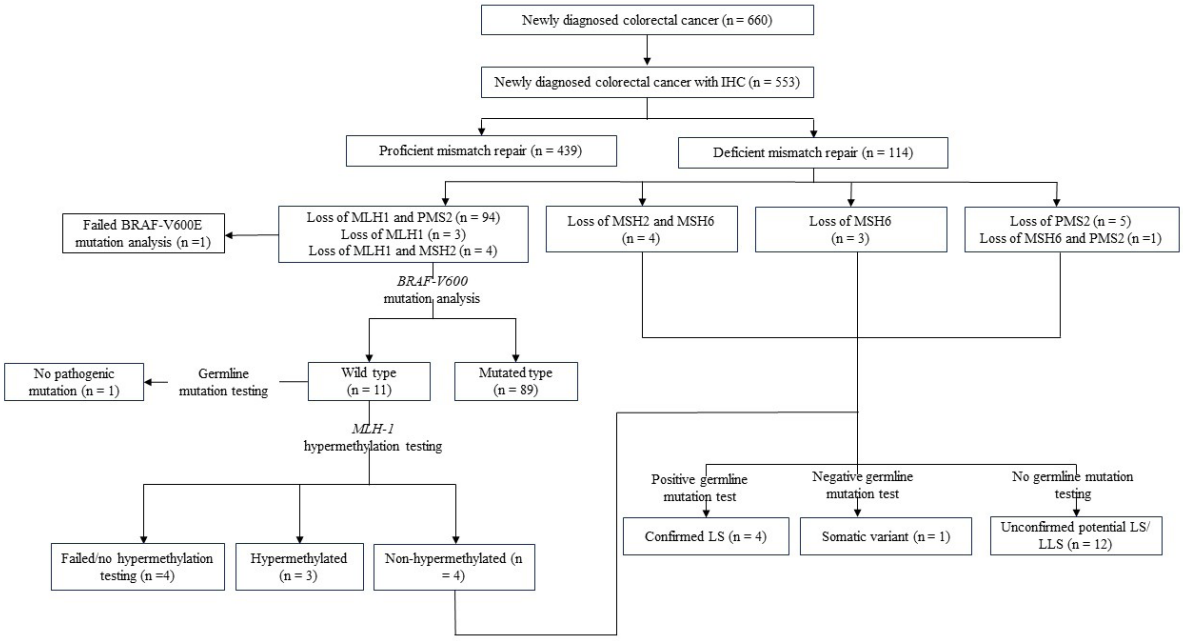
Table 2: Clinicopathological variables.

Characteristic	N	Overall, N=553	Non-LS associated CRC, N=534	LS associated CRC, N=4	p-value
T stage	450				0.20
T0		6/450 (1.3%)	6/447 (1.3%)	0/3 (0%)	
T1		48/450 (11%)	48/447 (11%)	0/3 (0%)	
T2		71/450 (16%)	69/447 (15%)	2/3 (67%)	
T3		238/450 (53%)	237/447 (53%)	1/3 (33%)	
T4		87/450 (19%)	87/447 (19%)	0/3 (0%)	
Unknown		103	102	1	
N stage	450				>0.9
N0		273/450 (61%)	271/448 (60%)	2/2 (100%)	
N1		114/450 (25%)	114/448 (25%)	0/2 (0%)	
N2		61/450 (14%)	61/448 (14%)	0/2 (0%)	
N3		2/450 (0.4%)	2/448 (0.4%)	0/2 (0%)	
Nx		103	101	2	
Unknown					
Acuity	535				>0.9
Acute		80/535 (15%)	80/531 (15%)	0/4 (0%)	
Elective		455/535 (85%)	451/531 (85%)	4/4 (100%)	
Unknown		18	18	0	
Tumour differentiation	460				0.11
G1		189/460 (41%)	186/456 (41%)	3/4 (75%)	
G2		213/460 (46%)	213/456 (47%)	0/4 (0%)	
G3/4		58/460 (13%)	57/456 (12%)	1/4 (25%)	
Unknown		93	93	0	

Fifty-six patients were excluded for the following reasons: neuroendocrine tumour (NET) (n=25), anal squamous cell carcinoma (SCC) (n=16), benign polyp (n=1), gastrointestinal stromal tumour (GIST) (n=2), mesothelioma (n=1), appendiceal mucinous neoplasm (n=2), lymphoma (n=1), goblet cell carcinoma (GCC) (n=2), known LS (n=2) and no documentation of CRC (n=7).

Six hundred and sixty patients were identified to have colorectal adenocarcinomas and were eligible for the current study. The median age of patients was 72 (IQR: 64–80) and 46% (n=303) were female. Seventy-three percent (n=482) were NZ European, 14% (n=94) were Māori and 11% (n=73) were "other European". 0.5% and 1.7% comprised Pacific peoples and other ethnicities respectively. There were no significant differences between patients completing and not

Figure 2: Flow chart of screening for Lynch syndrome (LS) by immunohistochemical staining for mismatch repair (MMR) proteins in patients with newly diagnosed colorectal cancer (CRC).



completing IHC testing on univariate analysis (**Table 1**). Table 2 shows the pathological tumour and nodal staging of the resected cancers—189 (41%), 213 (46%) and 58 (13%) of patients had well, moderately and poorly differentiated tumours, respectively (**Table 2**).

IHC testing

Eighty-four percent (n=553) of patients had initial IHC testing (**Figure 2**). The proportion of patients completing IHC testing in 2018–2020 and 2021–2022 were 77% (n=302) and 93% (n=251) respectively. Notably, in the years 2018–2020 all patients proceeding to surgery were tested for MMR deficiency using the postoperative specimen (n=249). Testing of endoscopic and distant metastatic (n=53) biopsies were also carried out, but irregularly and infrequently. In contrast to this, in the years 2021–2022 the endoscopic biopsies of primary tumour or biopsy of distant metastasis was performed routinely for all patients (n=203), except in acute CRC resections, where postoperative specimens were tested (n=48).

One hundred and seven (16%) patients with colorectal adenocarcinoma did not undergo tumour IHC testing. From 2021–2022, 17 patients did not complete IHC testing for the following reasons: patients that proceeded directly to palliative care (n=6); endoscopic biopsies not being tested for patients not proceeding to surgery (n=8); MMR not processed (n=1); and missing results (n=2). Patients proceeding directly to palliative care were diagnosed on the basis of radiological imaging and therefore did not have biopsies taken. From 2018–2020, 90 patients with adenocarcinoma did not have results for IHC testing because studies were not performed for endoscopic/distant metastases biopsies of patients not proceeding to surgery (n=88) and due to missing results (n=2).

MMR results

In IHC evaluation, loss of any mismatch repair (MMR) protein expression occurred in 114 (20%) of all patients. The most frequent pattern of MMR deficiency was loss of expression of both MLH1 and PMS2 (n=94, 82%). Loss of expression of both MSH2 and MSH6 was observed in four (3.5%) patients and loss of only MSH6 expression in three (2.6%) patients. Other patterns of MMR deficiency were as follows: PMS2 only (n=5; 4.4%), MLH1 and MSH2 (n=4; 3.5%), MSH6 and PMS2 (n=1; 0.9%) and MLH1 only (n=3; 2.6%). Therefore, 13 patients had a deficiency of at least one of MSH2, MSH6 or PMS2 proteins in the absence of MLH1 deficiency and were eligible for germline mutational analysis.

MLH1 BRAF-V600E and hypermethylation analysis

One hundred and one MMR patients had MLH1 deficiency, of which 100 (99%) underwent analysis for mutation of the BRAF-V600E gene as outlined by Ministry of Health – Manatū Hauora guidelines. Eleven patients had tumours that were negative for the BRAF-V600E mutation and therefore identified to potentially have LS or LLS. Seven of these patients (64%) eligible for hypermethylation testing completed it. Of the seven that did proceed, two did not have hypermethylation of the promoter region of the MLH1 gene (**Figure 2**). Therefore, five patients with MLH1 deficiency were eligible for germline mutational analysis.

Genetic analysis

Five patients (29%) eligible of the eligible 17 were referred to the New Zealand Familial Gastrointestinal Cancer Service (NZFGCS) and proceeded to germline mutational analysis. Three of these patients had MLH1 and PMS2 deficiency on MMR analysis, one had a MSH6 deficiency and one had PMS2 deficiency only. Four patients were confirmed to have pathogenic mutations of MMR genes (LS). One patient did not have a pathogenic variant of the MMR protein on genetic testing (**Figure 2**).

Best estimate of proportion of CRC cases with LS

The rate of confirmed LS in the present study is 0.72% (4/554). Notably, there were 17 patients that did not complete the testing protocols (12 did not finish germline mutational analysis, four did not complete hypermethylation testing and one did not undergo BRAF-V600E mutation analysis). The upper limit of the estimate, or maximum number of patients that potentially have LS in this cohort, is therefore 4.0%.

Discussion

This study analysed adherence to established testing protocols for LS in patients diagnosed with CRC adenocarcinoma. There was a high level of IHC testing for MMR proficiency of newly diagnosed CRC; this significantly improved once colonoscopic and radiological biopsies were tested and was 93% of all eligible patients in the most recent 1-year. BRAF-V600E mutation and MLH1 promoter hypermethylation analysis was conducted appropriately in 99% of patients and 64% of patients respectively. Only 29% (5/17) of patients eligible for germline mutational analysis proceeded to undergo this test. The estimated incidence of LS/LLS in newly diagnosed CRC ranged from 0.7–4.0% in Te Tai Tokerau.

The Aotearoa New Zealand Ministry of Health – Manatū Hauora and NICE recommend microsatellite instability testing (MSI) or IHC testing to detect abnormalities that may indicate LS for all patients diagnosed with CRC (universal screening).[11,12] There is considerable variation in testing availability and adherence to established guidelines internationally and between institutions. In previous audits of universal screening, rates of IHC testing varied internationally 74–98%.[15–17] Notably, most previous institutional audits only reviewed 1 year of testing. In contrast to this, our study spanned a 5-year time period and reported an overall testing rate of 84% of all CRC cases. Testing rates noticeably improved in 2021–2022 (93%) from 2018–2020 (77%) due to a change in practice to reflexive testing of all tumour biopsies instead of postoperative specimens. Therefore, IHC testing rates have been optimised to include as many patients as clinically feasible.

In the present study, only 29% of patients were referred to the NZFGCS (for genetic testing) when indicated as per the NICE guidelines. In a systematic review of universal LS testing in CRC, the pooled proportion of patients completing germline mutational analysis was 76.3%, which suggests that our institution may be performing below international standards.[18] The rate of testing ascertained in this study, however, is comparable to the 29% of eligible patients receiving germline mutational analysis in 2019 from the recent UK quality improvement study.[19] This statistic was obtained prior to the establishment of mainstreamed constitutional gene testing as part of the project. In the present study, both "major" DNA MMR proteins (most common pathogenic variants in LS occur in MLH1 and MSH2), as well as "minor" (MSH6 and PMS2) protein deficiencies failed to be referred to germline mutational analysis.[20] The absence of any discernible trend demonstrates systemic failure in reflexive referral germline mutational analysis. It should be noted, however, that this number may also represent some patients that were offered genetic testing but opted out. Poor rates of germline mutational analysis prevent the conclusive identification of individuals with LS or LLS. This can then be used to carry out familial "cascade" testing to identify other individuals with LS and reduce the proportion of people with LS unaware of their condition.

Identifying individuals and families then allows for appropriate genetic counselling and surgical follow-up. This is especially important in the Aotearoa New Zealand context, where the rate of early onset CRC (EOCRC) is rising.[21] Pathogenic genetic variants are responsible for up to one-third of all CRCs in patients below 35 years.[22] Screening and early detection are likely to curtail the morbidity and mortality in this sub-group, who have excellent outcomes with early intervention.[23]

The incidence of LS among unselected newly diagnosed CRC reported in the current literature ranges from 0.7–3.7%.[6,24–27] The rate reported in the current study (0.7%) lies within this range; however, a number of patients with MMR-deficiency failed to proceed to germline mutational testing when indicated, making this a likely under-estimation of the true rate. Variation in reported incidence may be due to geographic differences but may also likely be impacted by the proportion of elderly patients included in the study. Furthermore, some studies also exclusively test patients that have undergone surgery; this may also impact the reported rates of LS. When analysing proteins individually, the following percentages of germline variants were found: in non-hypermethylated MLH1 negative cases, 20.34% was explained by germline MLH1 variants; in MSH2 this was 44.70% (including EpCAM), in MSH6 this was 58.16% and in PMS2 this was 45.13%. In the current study, patients not receiving germline mutational analysis had MMR deficiency of MSH2, MSH6 and PMS2, which again increases the likelihood that the true rate of LS in our cohort is higher than the confirmed rate. It also underlines the importance of completing germline mutational analysis in this cohort.[18] The low rate of germline mutational analysis may be due to a lack of clinician awareness of specific gene profiles that warrant further investigation. This phenomenon may also be due to poor clinician cognisance of referral pathways for these patients. In the UK, improved awareness through the appointment of a local "Lynch champion", along with regional expert networks, showed improvements in all testing related to LS screening in patients diagnosed with CRC.[19]

The present study has several limitations, primarily its retrospective design and reliance on electronic records, which means that there may be incomplete and missing information. This is also a single-institutional review, and therefore the generalisability of the results to other institutions in Aotearoa New Zealand is not known. However, the objective of this study was not to make conclusive comments on national testing standards and instead to identify any existing gaps in testing which may then be subject to quality improvement. Missing data and incomplete testing also limit the accuracy of the estimate of LS in patients diagnosed with CRC. It does, however, include a large number of patients over a reasonable time period.

Conclusion

Rates of universal IHC testing in patients with colorectal cancer in Te Tai Tokerau are high and improved significantly once colonoscopic and radiological biopsies were included in assessment. Low rates of hypermethylation testing of the MLH1 promoter region and germline mutational analysis persisted across the study period and may mean that patients who have LS are not appropriately identified and followed up. Based on the results of this study, we would recommend similar audits be carried out across Aotearoa New Zealand. A future quality improvement study should also increase the awareness of LS testing through local "Lynch champions", as was proven successful in the UK.

COMPETING INTERESTS
None to declare.

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