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EDITORIAL

Australasian Gastrointestinal Pathology Society (AGPS) consensus guidelines for universal defective mismatch repair testing in colorectal carcinoma

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Summary

Lynch syndrome is the most common hereditary form of colorectal carcinoma caused by a constitutional pathogenic mutation in a DNA mismatch repair gene. Identifying Lynch syndrome is essential to initiate intensive surveillance program for the patient and affected relatives. On behalf of the Australasian Gastrointestinal Pathology Society (AGPS), we present in this manuscript consensus guidelines for Lynch syndrome screening in patients with colorectal carcinoma. The goal of this consensus document is to provide recommendations to pathologists for diagnosis of Lynch syndrome with discussion of the benefits and limitations of each test. Universal screening for defective mismatch repair is recommended, in agreement with the recent endorsement of universal testing by the National Health and Medical Research Council in Australia and the New Zealand Ministry of Health. The value of evaluating defective mismatch repair is acknowledged not only for Lynch syndrome screening but also for therapeutic decision information in patient management. AGPS advocates appropriate government funding for the molecular tests necessary for Lynch syndrome screening (*BRAF* mutation, *MLH1* methylation testing).

Key words: Lynch syndrome; colorectal cancer; mismatch repair protein; immunohistochemistry; microsatellite instability.

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INTRODUCTION

Lynch syndrome is an autosomal dominant disorder caused by constitutional pathogenic mutations in one of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, *PMS2* or mutations involving adjacent genes which affect the function or expression of these genes, for example *EPCAM* (*TACSTD1*) and *MSH2*, and *LRRFIP2* and *MLH1*.[1–3] Lynch syndrome is the most common form of hereditary colorectal carcinoma (CRC), accounting for 3% of all incident cases.[4] Two to six percent of all endometrial carcinomas are caused by Lynch syndrome.[5,6] The risk of cancer for affected individuals depends on age, gender, organs and which MMR gene is altered. The cumulative incidence of CRC at age 75 years is 46% in *MLH1*-mutation carriers, 43% in *MSH2*-mutation carriers, 15% in *MSH6*-mutation carriers, and 13% in *PMS2*-mutation carriers.[7,8] Lynch syndrome individuals with a mutation in *MLH1*, *MSH2* and *MSH6* also have an increased risk of cancer of the urinary tract, pancreas, hepatobiliary tract, stomach, small intestine, ovaries, and possibly breast and prostate.

The diagnosis of Lynch syndrome is currently a multistep process which requires close cooperation across multiple specialists. Pathologists play a crucial role by screening tumours for defective MMR using either immunohistochemistry (IHC) for MMR proteins and/or microsatellite instability (MSI) testing. Patients diagnosed with a defective MMR (dMMR) tumour that is not caused by sporadic alteration in MMR genes will be offered genetic counselling and germline mutation testing. Clear communication between pathologists, clinicians and genetic counsellors is important to ensure appropriate management of patients with a suspected diagnosis of Lynch syndrome. In this consensus document, we provide a list of guidelines on behalf of the Australasian Gastrointestinal Pathology Society (AGPS) to endorse universal testing in CRC. We also highlight urge for public funding of necessary molecular tests by the government. The consensus statements have been previously presented during two AGPS annual meetings, in 2015 and 2017.

TERMINOLOGY

1. Hereditary non-polyposis colorectal cancer (HNPCC) and Lynch syndrome

HNPCC is a clinical term, initially coined to separate familial predisposition to CRC (patients fulfilling Amsterdam criteria)

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from the polyposis syndromes (familial adenomatous polyposis and hamartomatous polyposis syndromes) before MMR genes were discovered.[9] HNPCC should not be used as a synonym of Lynch syndrome, which is genetically defined by the identification of a constitutional pathogenic mutation in a gene affecting the DNA MMR function. Not all HNPCC individuals have Lynch syndrome and not all Lynch syndrome individuals have HNPCC. Another confusing issue with the term HNPCC is that colorectal polyps can occur in Lynch syndrome individuals. The term HNPCC should no longer be used.

2. Defective mismatch repair (dMMR) and microsatellite instability (MSI)

dMMR within a tumour cell results in the loss of proofreading and repairing the nucleotide sequence from insertions and deletions that normally occur during DNA replication. Highly repetitive sequences (microsatellites) are particularly susceptible to DNA replication errors, which result in differing numbers of mononucleotide and dinucleotide repeats within microsatellites, referred to as MSI. Another consequence of dMMR is the loss of expression of MMR protein(s) by IHC. MSI testing and MMR protein IHC are two different methods to evaluate tumour for dMMR; these two terms should not be used as synonyms.

3. Lynch-like syndrome

Patients with a dMMR CRC suggestive of Lynch syndrome, with no constitutional pathogenic mutation detected in MMR genes, are referred to as Lynch-like syndrome. Defective MMR suggestive of Lynch syndrome include CRCs with loss of MLH1 and PMS2 by IHC that demonstrate absence of the somatic *BRAF* V600E mutation and absence of somatic *MLH1* promoter methylation, CRC with loss of MSH2 and MSH6, isolated loss of PMS2 and isolated loss of MSH6. These patients and their first-degree relatives are usually managed clinically as if they had Lynch syndrome until further somatic or germline testing might confirm or exclude Lynch syndrome.

CONSENSUS RECOMMENDATIONS AND SUMMARY OF SUPPORTING EVIDENCE

Statement 1. All newly diagnosed colorectal carcinomas should be tested for defective MMR

Regardless of patient age, clinical presentation, family history or tumour histological features, all new CRC should be tested for dMMR using MMR protein IHC and/or MSI analysis. This approach is commonly named universal testing.

Summary of supporting evidence and discussion

Defective MMR is usually the first step in screening for Lynch syndrome. A diagnosis of Lynch syndrome is important as it may impact clinical management, including more extensive surgical resection and intensive long term surveillance. Once the diagnosis of Lynch syndrome is made in a proband, cascade testing is offered to relatives, followed by intensive surveillance for mutation carriers. This approach has been shown to reduce the incidence and mortality of Lynch syndrome-associated cancers.[10,11] Testing CRC for dMMR also provides important therapeutic-decision information. Defective MMR is associated with better overall and disease-free survival in early stage CRC.[12] In addition, dMMR provides predictive information for non-response to 5-fluorouracil-based adjuvant chemotherapy in stage II/III CRC,[13] although this is still a subject of controversy.[14] Finally, dMMR predicts the clinical benefit of immune checkpoint blockade (PD-1 and PD-L1 inhibitors) in patients with metastatic CRC who have failed conventional therapy.[15]

Until recently, revised Bethesda Guidelines have been used in many centres as criteria for dMMR testing in CRC. However, selected approaches such as the revised Bethesda Guidelines have lower sensitivity of identifying Lynch syndrome compared with universal testing.[16] This is due to using age as one of the main criteria that excludes routine screening for patients >60 years. The clinical presentation of Lynch syndrome patients is variable and depends on sex and the gene involved.[4] The prevalence of CRC is higher in males, and higher in patients with *MLH1* or *MSH2* constitutional pathogenic mutation, compared to those with *PMS2* or *MSH6* mutation. Also, the average age at diagnosis is older for patients with *PMS2* or *MSH6* mutation, compared to those with *MLH1* or *MSH2* mutation. For better sensitivity and specificity of identifying Lynch syndrome, several computational prediction models have been developed to calculate the risk of individuals having Lynch syndrome.[17,18] However, these models are difficult to implement in routine clinical practice due to frequent lack of complete clinical information for pathologists at the time of pathology reporting.

A number of international jurisdictions have recommended the testing for dMMR to screen for Lynch syndrome in all CRC patients or in patients diagnosed with CRC <70 years. This includes the Evaluation of Genomic Applications in Practice and Prevention Working Group in 2009,[19] a group of European experts in 2013,[1] the United States Multi-Society Task Force on colorectal cancer in 2014,[4] the European Society for Medical Oncology endorsed by the American Society of Clinical Oncology in 2015[20] and the American College of Gastroenterology in 2015.[21] A growing number of institutions have also started to implement universal testing for endometrial cancer,[22] with emerging evidence for cost-effectiveness of this approach.[23] In Australia, there is no national policy on screening for Lynch syndrome. However, the National Health and Medical Research Council[24] and the New Zealand Ministry of Health[25] recently recommended universal testing for dMMR in all CRCs.

Both IHC for MMR protein expression and MSI analysis can be used for dMMR testing of CRC. A recent comprehensive review in adults with CRC showed the pooled sensitivity (95% confidence interval) for finding cases of Lynch syndrome by IHC, MSI analysis and both methods were 0.91 (0.85–0.95), 0.93 (0.87–0.96) and 0.97 (0.90–0.99), respectively.[26] There is no preference for one approach over another, but because of cost, availability and ability to direct further mutation testing, MMR protein IHC is preferred by most centres.

Cost effectiveness analysis of strategies for Lynch syndrome screening has been assessed in the health care system of various countries, including the USA, the Netherlands and Australia.[27–32] Most studies reported a reasonable trade-off between cost and yield of Lynch syndrome diagnosis for screening strategies testing CRC patients <70 years. The

study from Australia used empirical data from two large population-based studies, one with a 5.2% prevalence of Lynch syndrome from a population aged <60 years and one with a 0.8% of Lynch syndrome from a population aged 41–86 years.[33] The authors concluded that screening CRC cases diagnosed <70 years will identify Lynch syndrome at a reasonable cost.[28] With the used cost assumptions for the analysis (AU$175 for MMR IHC, AU$231 for *BRAF* V600E testing and AU$314 for *MLH1* methylation testing), testing CRC cases with loss of MLH1/PMS2 expression by *MLH1* methylation was more cost-effective than by *BRAF* V600E mutation, partially due to the decreased efficiency of *BRAF* V600E mutation testing to exclude sporadic MLH1-deficient cancers. The data from this study could not accurately evaluate the cost effectiveness of universal testing for Lynch syndrome as patients >70 years were under-represented in the studied population and none of them had been diagnosed with Lynch syndrome. However, Lynch syndrome diagnosed in CRC patients >70 years is well documented.[34,35] Furthermore, subsequent costs and cost savings from cascade screening and surveillance of at-risk relatives, as well as the benefit in identifying Lynch syndrome to gains of life, have not been taken into account in this analysis. Finally, none of the published cost effectiveness studies considered the clinical benefit of dMMR for prognostic stratification and predictive information for response to 5-fluorouracil and PD1 blockade therapy.

Statement 2. Colorectal carcinoma with loss of MLH1 and PMS2 immunohistochemical expression should be tested for the somatic *BRAF* V600E mutation and/or *MLH1* promoter methylation

The majority of dMMR CRCs demonstrate loss of expression of MLH1 and PMS2 and are not caused by constitutional pathogenic mutation but by somatic epigenetic silencing of *MLH1*. To reduce the number of patients unnecessarily referred to genetic counselling, a follow-up test is required to identify sporadic MLH1-deficient patients. An algorithm for Lynch syndrome screening in CRC is presented in Fig. 1.

Summary of supporting evidence and discussion

About 15–20% of all CRCs are dMMR secondary to somatic methylation of the promoter of *MLH1* (sporadic MLH1-deficient CRC).[36,37] Sporadic MLH1-deficient CRC develop from serrated polyps via the serrated neoplasia pathway. Most of the precursor serrated polyps harbour the *BRAF* V600E mutation and acquire *MLH1* methylation as they progress to dysplasia and cancer.[38] On the other hand, CRC in Lynch syndrome patients usually arise from conventional adenomas, which are not associated with *BRAF* mutation. *BRAF* V600E mutation testing plays no role in further triaging genetic testing in tumours other than CRC (endometrial cancers, small bowel cancers and gastric cancers) with IHC loss of MLH1 and PMS2. Contrary to CRC,

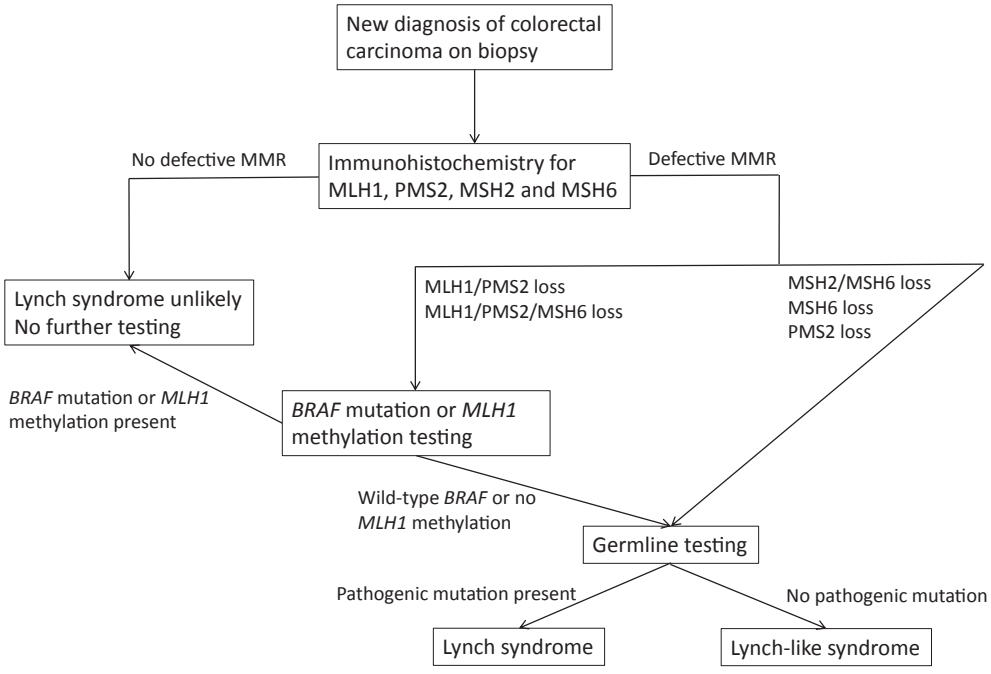

Fig. 1 Screening algorithm for Lynch syndrome in colorectal carcinoma using MMR protein immunohistochemistry as the initial step. Every newly diagnosed colorectal carcinoma should be tested for immunohistochemical expression of all four MMR proteins (MLH1, PMS2, MSH2, MSH6), preferentially from the initial diagnostic biopsy. If the tumour shows normal expression of all four MMR proteins, no further testing is required. For tumours demonstrating loss of MLH1 and PMS2, or loss of MLH1, PMS2 and MSH6, reflex *BRAF* mutation testing is recommended. If the tumour is positive for *BRAF* mutation, no further testing is required for low to average risk patients. If no *BRAF* mutation is identified in the tumour (wild-type *BRAF*), genetic counselling with germline mutation testing is recommended. Alternatively, *MLH1* promoter methylation testing can be performed as an additional step in *BRAF* wild-type tumours. If the tumour is *BRAF* wild-type and positive for *MLH1* promoter methylation, no further testing is recommended for low to average risk patients (constitutional *MLH1* methylation analysis is indicated for patients with suggestive history of Lynch syndrome). Another approach for tumours with loss of MLH1 and PMS2 is to perform *MLH1* promoter methylation as a single reflex test. Genetic counselling with germline mutation testing is recommended for tumours demonstrating other patterns of MMR loss of expression (MSH2/MSH6 loss, MSH6 loss, PMS2 loss). If MSI testing is used as the initial step for screening, all tumours with MSI present should be tested for *BRAF* mutation or *MLH1* methylation as described above. A diagnosis of Lynch syndrome is established if a constitutional pathogenic mutation is identified in an MMR gene (or *EPCAM*). If no pathogenic mutation is identified, the patient has Lynch-like syndrome.

sporadic MLH1-deficient tumours from other organs do not develop through a *BRAF*-mutated pathway. *MLH1* promoter methylation is the only test for the triaging of these cases.

BRAF V600E somatic mutation analysis therefore can be used as a surrogate marker for *MLH1* promoter methylation in CRC only. If the *BRAF* V600E mutation is detected, Lynch syndrome is very unlikely and no further testing is required in low or average risk patients. The absence of *BRAF* V600E mutation (wild-type *BRAF*) indicates that Lynch syndrome is a possible diagnosis. Genetic counselling with germline testing for MMR genes is recommended for these patients. Another option is to test *BRAF*-wildtype CRC for *MLH1* promoter methylation before giving recommendation for genetic counselling. The reason for this is that *BRAF* mutation testing is not as efficient as *MLH1* methylation testing to identify sporadic MLH1-deficient CRC. Only 65–75% of CRC with *MLH1* promoter methylation harbour the *BRAF* V600E mutation. Depending on the environment and available resources, *MLH1* promoter methylation can be performed as an additional step to reduce the referral rate for genetic testing.[39] If *MLH1* methylation is detected, no further testing is required in low to average risk patients. If *MLH1* methylation is not detected, genetic counselling with germline testing is recommended. *BRAF* V600E mutation analysis and *MLH1* promoter methylation analysis should be performed using appropriately validated assays subject to ongoing quality assurance.

In rare patients with strong family history and/or young age at CRC diagnosis, Lynch syndrome may not be excluded even if all tests suggest sporadic CRC. Some Lynch syndrome patients with a constitutional pathogenic *MSH6* mutation can be missed as the tumour may not show MSI and MSH6 IHC expression may be normal or weak.[40] In rare Lynch syndrome patients, *MLH1* methylation can be present in the tumour due to constitutional *MLH1* methylation.[41] Finally, up to 1% of CRCs arising in Lynch syndrome may harbour the *BRAF* V600E mutation.[41]

BRAF V600E mutation specific IHC (VE1 clone) has been proposed as an alternative method to detect *BRAF* V600E mutation. While some groups have reported high sensitivity and specificity for BRAF V600E specific IHC, others have found IHC to be unreliable.[42–47] Tumours that are wild-type for *BRAF* can rarely demonstrate weak staining with VE1 IHC, resulting in false positive results. Some pathology departments have used VE1 IHC in routine clinical care for triaging of CRC with loss of MLH1 and PMS2 expression. However, pathologists need enough experience to be aware of the specific issues related to the use of BRAF V600E IHC (poor performance due differences in fixation, antibody concentration, antibody retrieval solution, type of IHC platform used).[47] Using the VE1 IHC to determine *BRAF* mutation status requires solid validation of the antibody performance and ongoing quality assurance. If these conditions are not met, the VE1 IHC is not recommended for triaging Lynch syndrome CRC.

Statement 3. Defective MMR testing should preferentially be performed on the initial biopsy specimen

Summary of supporting evidence and discussion

Defective MMR testing can be performed either on the initial diagnostic biopsy specimen or on the surgical resection specimen. Studies comparing MMR protein IHC results from both types of specimen demonstrated complete agreement in the final MMR status.[48,49] Advantages of testing biopsy specimens are the possible change in patient management (more extensive surgery) if Lynch syndrome is suspected and better tissue fixation with less staining artefacts. Most of the difficulties in interpreting MMR IHC (see below) are seen in surgical resection specimens and result from fixation issues. In addition, further tissue specimens may not be available for patients who will not have surgery and for rectal cancer patients with complete response to pre-operative chemo-radiation therapy.

Testing the surgical resection specimen for MMR IHC is an acceptable alternative option if the biopsy is not available, if it does not include sufficient tumour for assessment or if the result of MMR IHC is equivocal in the biopsy. For MSI analysis, a biopsy specimen may not be adequate if normal tissue is required.

Statement 4. Immunohistochemistry should be performed for all four MMR proteins MLH1, PMS2, MSH2 and MSH6

Summary of supporting evidence and discussion

MMR protein immunohistochemistry can be performed using either a two-antibody (PMS2 and MSH6) or a four-antibody (MLH1, PMS2, MSH2, MSH6) approach. MMR proteins bind to DNA as heterodimer complexes. Each heterodimer has a dominant protein, MLH1 for the MLH1/PMS2 pair and MSH2 for the MSH2/MSH6 pair. When the dominant MMR protein is altered by a pathogenic mutation or methylation, the corresponding non-dominant partner is degraded: PMS2 expression is lost if MLH1 is altered, and MSH6 expression is lost if MSH2 is altered. However, the opposite is not true due to the compensatory effects of other MMR proteins. MLH1 expression is retained if PMS2 is altered, and MSH2 expression is retained if MSH6 is altered.

These binding properties would therefore justify the use of PMS2 and MSH6 as a first antibody panel to test for dMMR. Using PMS2 and MSH6 as a two MMR protein panel has been reported to be as effective and cheaper than using an upfront four MMR protein panel for dMMR IHC testing.[50,51] If one of the two MMR proteins shows loss of expression, the corresponding partner is tested. However, using an upfront four-panel approach provides significant advantages. The workflow is improved by avoiding a second run of IHC for 15–20% of tested CRC demonstrating loss of PMS2 expression. For the prognostic and predictive information of dMMR, a comprehensive report listing all MMR protein expression results is preferred by treating clinicians, with clear mention of MLH1 expression. Furthermore, the two-panel protocol may fail to detect some cases with weak or patchy loss of MSH6 expression interpreted as normal expression in CRC from Lynch syndrome patients with a germline mutation in *MSH2*.[52]

Statement 5. The reporting of MMR protein immunohistochemistry result should use clear terminology

Effective communication between pathologists, treating clinicians and genetic counsellors is essential for the success of universal testing for Lynch syndrome. Pathologists need to

provide clear reports of MMR protein IHC results and explain the significance of abnormal results.

Summary of supporting evidence and discussion

MMR proteins are normally expressed in the nuclei of most human cells, including lymphocytes and stromal cells that are used as internal positive controls. When reporting results for each tested MMR protein by IHC, the descriptors 'positive' and 'negative' should be avoided due to the potential confusion of a 'negative' stain being abnormal. The preferred terminology is to use 'normal', 'retained' or 'preserved' expression, and loss of expression. A comment should be added to the results of MMR protein staining, including the potential utility of additional testing and a recommendation for genetic counselling if necessary.

Examples of report

Results of MMR protein immunohistochemical staining in colorectal cancer cells:

- Normal staining for all four MMR proteins (MLH1, PMS2, MSH2, MSH6). This is the normal pattern; no deficient MMR is identified. Lynch syndrome is very unlikely.
- Loss of staining for MLH1 and PMS2; normal staining for MSH2 and MSH6. This is an abnormal pattern indicative of defective MMR. *BRAF* V600E somatic mutation testing (or *MLH1* methylation analysis) is recommended to help distinguish sporadic CRC from possible Lynch syndrome-associated CRC.
  - o *BRAF* V600E somatic mutation is present (or *MLH1* promoter methylation is present). Lynch syndrome is very unlikely.
  - o *BRAF* V600E somatic mutation is absent. This result can be caused by Lynch syndrome. Either cascade referral for *MLH1* promoter methylation analysis or direct referral to Genetic Service is recommended.
  - o *BRAF* V600E somatic mutation is absent and *MLH1* promoter methylation is absent. This result can be caused by Lynch syndrome. Referral to Genetic Service is recommended.
- Loss of staining for MSH2 and MSH6; normal staining for MLH1 and PMS2. This is an abnormal pattern indicative of defective MMR that can be caused by Lynch syndrome. Referral to Genetic Service is recommended.

COMMON PITFALLS IN MMR PROTEIN IHC INTERPRETATION

Staining heterogeneity

Variability in the proportion of stained cells and in the intensity of the immunostaining can occur in surgical resection specimens, more rarely in biopsy specimens. In most cases, this is due to tissue hypoxia and fixation issues (intratumoural heterogeneity). A cut-off of 5% or 10% of unequivocal nuclear staining in tumour cells is commonly used.[40,53] If this is not achieved, using another representative tumour tissue block for IHC should be considered. Weak or extensive loss of staining of MSH6 can be seen in rectal carcinoma after pre-operative chemoradiation therapy.[54] Decreased expression of PMS2, MLH1 and MSH2 has also been reported in these specimens.[55] Zonal heterogeneity in MLH1/PMS2 expression may also reflect differences in *MLH1* methylation within a tumour.[56] Zonal MSH6 heterogeneity staining can be seen in tumours with loss of MLH1 and PMS2 expression as clones with somatic *MSH6* mutation secondary to dMMR (the coding sequence of the *MSH6* gene contains a mononucleotide microsatellite).[57]

Unusual patterns

Loss of expression of all four MMR proteins (with positive internal control) can rarely occur and may be secondary to a combination of constitutional pathogenic mutation and sporadic alteration (methylation or mutation), or to sporadic alterations affecting both dominant MMR genes.[58] Loss of expression of MMR protein(s) in tumour cells and normal cells may be attritutable to technical difficultlies but can also be indicative of constitutional MMR deficiency syndrome in the appropriate clinical context.

Punctuate nuclear staining has been reported for MLH1 in endometrial and colorectal carcinomas.[59,60] In these two recent studies, the authors reviewed cases initially reported as isolated loss of PMS2. In 6 of 7 endometrial carcinomas and 9 of 12 colorectal carcinomas, a punctate pattern of nuclear staining for MLH1 was observed and incorrectly reported as normal MLH1 expression. The artefactual punctate staining was present in the carcinoma cells but not in the normal cells. Most endometrial carcinoma cases with this pattern were associated with *MLH1* promoter methylation. Pathologists need to be aware of this pitfall in misinterpreting normal MLH1 immunostaining, resulting in false reporting of isolated loss of PMS2 and unnecessary referral to genetic services.

CURRENT AND FUTURE MANAGEMENT OF LYNCH-LIKE SYNDROME

When no MMR gene germline mutation is identified in a patient with dMMR CRC suggestive of Lynch syndrome, a provisional diagnosis of Lynch-like syndrome is made. The first step in this situation is to re-evaluate the initial MMR IHC stains to exclude incorrect interpretation. Repeating the stains may be necessary. Up to 20% of discrepancy between germline mutation results and MMR IHC is attributable to incorrect IHC interpretation.[61] A proportion of Lynch-like cases are caused by bi-allelic somatic mutations in a MMR gene.[62] For other patients, a germline mutation in a MMR gene is possibly responsible for dMMR but the current DNA testing method failed to identify the mutation. Most Lynch-like syndrome patients and their first-degree relatives are currently followed up as if they had Lynch syndrome. To further stratify these patients, testing the tumour for somatic mutation in MMR genes may provide useful information and be more frequently requested to pathology departments. Constitutional pathogenic mutations in *POLE* and *POLD1* have been implicated in the aetiology of bi-allelic MMR gene somatic mutations.[63]

CONCLUSION

In this article, we provide consensus guidelines on behalf of the Australasian Gastrointestinal Pathology Society to support universal dMMR testing in CRC. Testing all newly diagnosed CRC is the only strategy with 100% sensitivity to detect Lynch syndrome, the most common hereditary cancer condition in the gastrointestinal tract.[16] Universal testing is now recommended by the National Health and Medical

Research Council in Australia and the New Zealand Ministry of Health. Moreover, dMMR provides useful clinical information for CRC patient management. Pathologists need to be familiar with the different steps in the triaging of patients for Lynch syndrome diagnosis. They need to understand the benefits and limitations of MMR protein IHC, MSI testing, *BRAF* mutation testing, *MLH1* methylation analysis and BRAF V600E IHC. Currently, *BRAF* V600E somatic mutation and *MLH1* promoter methylation tests are not funded by the Australian government. The Australasian Gastrointestinal Pathology Society strongly advocates public funding for these molecular tests by the government.

With advances in molecular diagnostics, the multistep approach for Lynch syndrome diagnosis is likely to be soon replaced by next generation sequencing (NGS)-based methodologies. MSI testing, BRAF/RAS mutation status, germline and somatic MMR gene testing can potentially be all determined by NGS with a more efficient allocation of resources.[64]

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