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ANATOMICAL PATHOLOGY

Lessons learnt from implementation of a Lynch syndrome screening program for patients with gynaecological malignancy

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Summary

Despite a trend towards universal testing, best practice to screen patients presenting with gynaecological malignancy for Lynch syndrome (LS) is uncertain. We report our institutional experience of a co-ordinated gynaecological LS screening program.

All patients with endometrial carcinoma or carcinosarcoma, or gynaecological endometrioid or clear cell carcinomas undergo reflex four panel immunohistochemistry (IHC) for MLH1, PMS2, MSH2 and MSH6 followed by cascade somatic hypermethylation analysis of the *MLH1* promoter locus for dual MLH1/PMS2 negative tumours. On the basis of these results, genetic counselling and targeted germline mutation testing is then offered to patients considered at high risk of LS.

From 1 August 2013 to 31 December 2015, 124 patients were screened (mean age 64.6 years). Thirty-six (29.0%) demonstrated abnormal MMR IHC: 26 (72.2%) showed dual loss of MLH1/PMS2, five (13.9%) dual loss of MSH2/MSH6, three (8.3%) isolated loss of MSH6, and two (5.6%) isolated loss of PMS2. Twenty-five of 26 (96.1%) patients with dual MLH1/PMS2 loss demonstrated *MLH1* promoter methylation. Therefore, 11 (8.9%) patients screened were classified as high risk for LS, of whom nine (81.8%) accepted germline mutation testing. Three (2.4% of total screened) were confirmed to have LS, two with germline *PMS2* and one with germline *MSH2* mutation. Massive parallel sequencing of tumour tissue demonstrated somatic mutations which were concordant with the IHC results in the remainder. Interestingly, the one MLH1/PMS2 IHC negative but not hypermethylated tumour harboured only somatic *MLH1* mutations, indicating that universal cascade methylation testing in MLH1/PMS2 IHC negative tumours is very low yield and could be reconsidered in a resource-poor setting.

In conclusion, universal screening for LS in patients presenting with gynaecological malignancy using the algorithm described above identified LS in three of 124 (2.4%) of our population. Only three of nine (33.3%) patients considered at high risk for LS by combined IHC and hypermethylation analysis were proven to have LS. Only one of the LS patients was less than 50 years of age and none of these patients would have been identified had more restrictive Amsterdam or Bethesda criteria been applied.

Key words: Lynch syndrome; mismatch repair; Lynch-like syndrome.

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INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant cancer susceptibility syndrome associated with germline variants in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2,* which function to bind to and repair small replicative DNA sequence errors.[1–6] LS is characterised by a significantly increased risk of several malignancies, particularly colorectal, endometrial/gynaecological and urothelial, but also tumours arising at other sites including stomach, brain and pancreas.[1–7] More recently, deletions at the *EPCAM* locus, which subsequently lead to hypermethylation of *MSH2*, have been implicated in a group of patients with LS.[8]

Second only to colorectal carcinoma, endometrial cancer is the next most prevalent malignancy in patients with LS.[9] LS has been reported to account for between 2% and 6% of all endometrial cancers, and approximately 50% of female index patients with LS will present with a gynaecologic

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malignancy.[9] The identification of LS in patients presenting with gynaecological cancer facilitates risk reduction screening programs for other malignancies in these patients and, following cascade genetic testing, their relatives.[9–11]

Guidelines for screening patients with colorectal or endometrial carcinoma for LS vary. However there is a general trend away from targeting only patients who are considered at high clinical risk (for example with young age of onset, strong family history or multifocal tumours) towards universal screening.[5,6,9,12,13] This approach is supported by emerging evidence that a selective screening strategy has the potential to miss many high-risk patients and families.[10] For example, *MSH6* variants more commonly present with endometrial cancer in older patients, who may lack a strong family history and *PMS2* variants appear to have a lower penetrance than *MLH1* and *MSH2* variants.[14] For these reasons, many institutions have recently endorsed and adopted a universal approach to screening all patients with both colorectal and endometrial carcinoma.[5,6,10,14,15] However, the precise screening algorithms and therefore the relative costs, sensitivities and specificities endorsed by different institutions and professional bodies vary.

Our institution prospectively implemented a LS screening program for patients presenting with gynaecological malignancies in August 2013. We now report our experience with this program so that the data we have generated will help to further refine and optimise LS screening programs.

METHODS

The LS screening algorithm which we adopted is summarised in Fig. 1. Briefly, all patients with endometrial carcinoma of any histology (including carcinosarcomas) and all patients with endometrioid or gynaecological clear cell carcinoma of any site undergo reflex immunohistochemistry (IHC) with a panel of four mismatch repair markers: MLH1, MSH2, MSH6 and PMS2.

IHC was performed on a representative block on the definitive surgical resection specimen and interpreted by the primary surgical pathologist at the time of initial reporting. We have previously reported our IHC methods in detail.[5] Briefly, only slides with valid internal positive controls were considered informative and if internal positive controls were lacking, staining was routinely repeated on other blocks. The presence of nuclear staining in tumour cells for all four markers was interpreted as a positive result and taken to exclude mismatch repair deficiency and to make LS unlikely. The complete absence of nuclear staining in tumour cells for one or more markers in the presence of nuclear staining in adjacent non-neoplastic cells which serve as

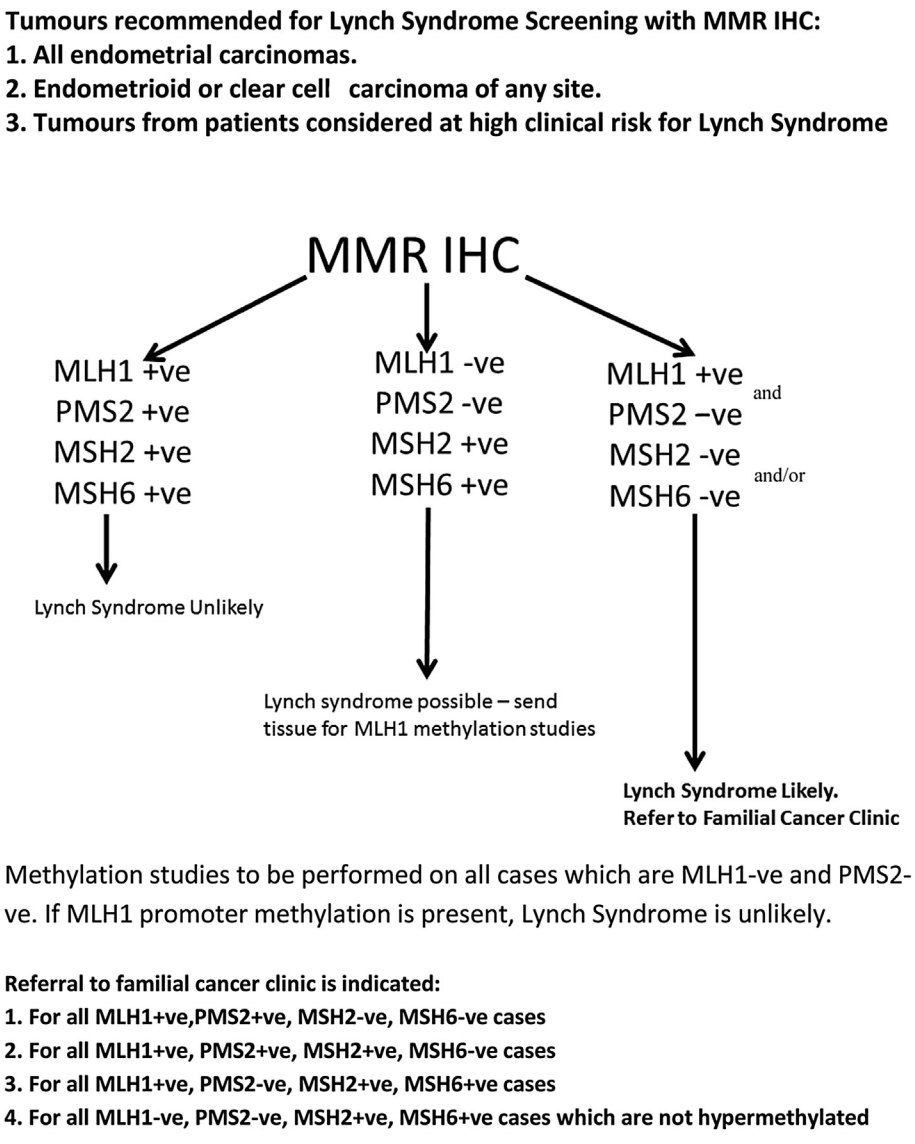

Fig. 1 Lynch syndrome screening protocol for gynaecological malignancies adopted at our institution.

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