Abstract

Familial cancer clinics strive to identify at-risk individuals with an inherited predisposition to cancer. Familial predisposition to colorectal cancer includes Familial Adenomatous Polyposis and Lynch Syndrome. The latter condition has no clear phenotype, leading to difficulties in its recognition. While family history remains an important tool in diagnosing inherited predisposition to cancer, many cases of Lynch Syndrome are diagnosed in the absence of a clear-cut family history. Therefore identification of Lynch Syndrome cases has moved in the direction of tumour-based testing, initially on cases selected for family history, young age of onset and tumour histological features, but now it has been suggested that Lynch Syndrome be screened for more widely via tissue testing of all newly diagnosed colorectal cancers under a certain age (e.g. < 60 years).

Familial cancer clinics are staffed by a multi-disciplinary team, comprising clinical geneticists, genetic counsellors, medical oncologists and other relevant specialists with expertise in colorectal cancer (CRC), such as gastroenterologists or colorectal surgeons. Familial cancer clinics aim to reduce the morbidity and mortality associated with CRC by identifying at-risk individuals with an inherited predisposition.

This is achieved firstly by working with the person referred to the clinic, usually on the basis of family history of cancer. The family history (pedigree) is collected and confirmed where possible, through obtaining histological reports, hospital notes or death certificates. The pedigree is then analysed for possible, familial cancer syndromes that fit the spectrum of cancers seen. For the individual, their cancer risk is estimated based on the family history and the presence of other established risk factors.

Communication is of utmost importance in the clinic. Discussion with the individual about risk, inheritance and testing of cancer predisposition genes (where appropriate) is undertaken. Individuals at high genetic risk and their managing doctors are advised about strategies for cancer screening, early detection and prevention. Individuals seeking genetic testing are counselled about the uncertainties, risks and benefits associated with positive and negative test results. The results of any genetic testing performed are given and carefully explained in terms of the impact on the individual and on their family members. Follow-up and review is provided where necessary. For the individual, follow-up may be provided via the provision of a registry-based reminder service for surveillance and screening programs.

Any individual attending a familial cancer clinic is also regarded as being part of a wider family and part of the clinic’s role is to identify other high-risk relatives. Privacy legislation prevents direct contact from the clinic with such relatives, but strategies to spread the information within families are discussed with individuals and information regarding local services is provided. Upon receiving contact from relatives, the cycle of communication with the individual and their family is re-commenced.

Finally, as inherited cancer and genetics is a rapidly evolving discipline in terms of knowledge and practice, familial cancer clinics serve as a rich resource for research and education.

High-risk familial CRC syndromes

Approximately 15% of all CRCs demonstrate familial clustering and 1-5% are caused by specific germline genetic mutations. The two most common hereditary colon cancer syndromes are familial adenomatous polyposis (FAP), caused by germline mutations in the APC gene, and Lynch Syndrome, caused by germline mutations in the mismatch repair (MMR) genes. FAP is relatively easy to identify because of the distinctive phenotypic feature of hundreds to thousands of polyps present within the colonic wall. In contrast, Lynch Syndrome does not present with easily recognisable clinical features that distinguish it from sporadic colon cancer, thus many cases of Lynch Syndrome remain undiagnosed.

Lynch Syndrome accounts for approximately 1-2% of all CRCs and is also associated with an increased risk of extra-colonic cancers, including gastric, endometrial and urinary tract tumours. This syndrome is caused by inherited mutations in one of the DNA MMR genes MLH1, MSH2, MSH6 or PMS2. Carriers of a MMR gene mutation have a 45-90% lifetime risk of developing CRC. Compared with the general population, the cancer risk in mutation carriers is also increased for uterine, ovarian, gastric, biliary tract uro-epithelial and kidney cancers, and central nervous system tumours. The identification of MMR gene mutation carriers is of great benefit for the management of their individual and family risk of cancer. Early and regular colonoscopic surveillance can potentially prevent the development of CRC by detecting tumours at a pre-cancerous and thus more easily treatable stage.

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Furthermore, the ability to exclude MMR mutation negative individuals in Lynch Syndrome families reduces the burden of participating in unnecessary high-risk surveillance and prevention programs.

**Challenges in the identification of Lynch Syndrome cases**

Due to the high cost and technical difficulties associated with testing for germline mutations in MMR genes, the means of identifying likely mutation carriers among CRC patients has been a work in progress. In 1990, the International Collaborative Group on hereditary non-polyposis colorectal cancer was formed to develop clinical criteria that could help to identify patients with this hereditary condition. The specific aims were to establish a common nomenclature and to permit uniform identification of families for research and clinical purposes. The resulting criteria, known as Amsterdam 1 criteria (AC1), were quite rigid and focused entirely on a dense family history of early onset CRC. Although there was widespread acceptance and use of these criteria within the expert community and among some clinicians, many classic Lynch Syndrome families were missed because the AC1 were not fulfilled and hence families were not investigated further for possible MMR gene mutations. The Amsterdam II criteria (ACII) were subsequently developed and included a spectrum of extra-colonic Lynch Syndrome cancers. These new criteria were later modified again to take account of small families with insufficient members to fulfil the generational criteria. Although the Amsterdam criteria were highly specific for the detection of Lynch Syndrome families, their sensitivity was quite low.

The Bethesda guidelines were subsequently formulated in 1996 at a meeting held at the National Cancer Institute. These guidelines were proposed as a cost-effective measure to improve the identification of Lynch Syndrome-like families who did not meet Amsterdam criteria but in whom pre-screening of their CRC tissue using the microsatellite instability (MSI) test was recommended. The guidelines were revised in 2004 to include tumour features and a less stringent family history of cancer. Compared with the Amsterdam criteria, the Bethesda guidelines demonstrated a higher sensitivity, but lower specificity for the detection of Lynch Syndrome families. Approximately 20% of all diagnosed CRC cases meet the revised Bethesda guidelines, for which molecular evaluation of MSI and/or loss of MMR protein expression via immunohistochemistry (IHC) testing is recommended.

MSI was recognised as a feature of hereditary colon cancer in the early 1990s. The MSI phenotype is characterised by ubiquitous changes in the length of nucleotide repeat sequences in DNA, with mononucleotide repeat tracts (e.g. AAAn) being particularly susceptible to deletions. Testing for MSI is performed in the laboratory using polymerase chain reaction to amplify specific microsatellite sequences, followed by gel electrophoresis to identify changes in microsatellite length. Approximately 10% of sporadic CRCs also exhibit MSI. These tumours occur almost exclusively in the proximal colon and more often in older women. The large majority of sporadic MSI+ CRCs arise because of acquired, methylation-induced transcriptional silencing of MLH1 gene expression. The MSI phenotype alone cannot therefore be used as a specific marker for Lynch Syndrome. However, the presence of a hot-spot point mutation (V600E) in the BRAF oncogene occurs in sporadic, but not familial cases of MSI+ CRC and can therefore be used to exclude sporadic cases that arise in the setting of Lynch Syndrome. Methylation of the MLH1 gene promoter region can also be used as a marker to discriminate between sporadic and Lynch Syndrome CRC cases, with methylation being present in the former but not the latter.

Bi-allelic mutations in MMR genes almost always lead to the loss of protein expression, as evidenced pathologically on IHC analysis. The MMR proteins exist as heterodimers, thus loss of expression often occurs in pairs, with the loss of MLH1/PMS2 or MSH2/MSH6 pairs, most common, although other rarer patterns of loss have also been reported.

There has been considerable debate as to whether MSI or IHC is the superior technical approach as the initial test for Lynch Syndrome screening. IHC is a relatively rapid test that can be undertaken in most general pathology laboratories, is cheaper and can be used to ascertain which gene should begin the germline mutation search. However, IHC interpretation is subjective and highly dependent on the quality of the tissue, staining methods and reporting pathologist, thus there is much inter-observer variability in the evaluation of results. In Australia, technical protocols differ between laboratories and there is a lack of quality control measures at a national level. MSI does not require subjective interpretation, but is more expensive, labour intensive and requires involvement of a molecular genetics laboratory. There is, however, a high correlation in the results from both methods when used by experienced laboratories.

**Moving towards population-based screening for Lynch Syndrome**

Because of the complexities involved in applying the Amsterdam and Bethesda guidelines, there were concerns that many MMR gene mutation carriers in the population remained undetected. The main reason for not utilising the proposed criteria and guidelines was that the primary onus was placed on clinicians to carefully document family history and to subsequently refer patients for genetic evaluation. The ongoing challenges relating to assessment, referral and follow-up were highlighted in a prospective study which found that of 228 CRC patients who may have benefited by attending a familial cancer clinic, only 22% were referred and just 14% actually attended.

The low rate of referrals to familial cancer clinics led to calls for the introduction of population-based screening for Lynch Syndrome based upon molecular analysis of MSI and/or IHC loss of expression of MMR proteins in the tumour. Universal screening of all CRC patients for these markers is unlikely to be cost-effective because the majority of Lynch Syndrome cases occur in younger patients. A further difficulty is that the incidence of sporadic CRC with MSI+ tumours increases markedly after the age of 55 years, although these can be distinguished from
familial cases by additional testing for the presence of a specific BRAF oncogene mutation.\(^1\)\(^3\)

A large retrospective study was carried out in the state of Western Australia to detect Lynch Syndrome among CRC patients aged <60 years at diagnosis and in the absence of any information on family cancer history.\(^2\)\(^3\) This work established that MSI screening followed by testing for the BRAF mutation in the MSI+ cases was an effective strategy for the identification of previously unrecognised Lynch Syndrome mutation carriers in the Western Australia population. Based on these earlier findings, starting in 2008, routine MSI and/or IHC testing was recommended for all CRC patients (<60 years) in Western Australia, regardless of their family history of cancer. A recent analysis of the population-based screening program for Lynch Syndrome in Western Australia has shown a significant increase in the number of new Lynch Syndrome cases identified each year.\(^2\)\(^4\)

Although the laboratory tests used to screen for MSI, MMR protein loss, BRAF mutation and MLH1 methylation are not technically difficult or prohibitively expensive, their systematic introduction at a population level for the identification of Lynch Syndrome has proven challenging. This is because of the need for cooperation and effective communication between multiple disciplines, including gastroenterology, pathology, surgery, oncology and medical genetics.\(^2\)\(^5\) Even greater diligence is required when the service providers are located at different sites or work for different organisations.

Based on the Western Australia experience with population-based screening, three key elements have been identified that are likely to be important for successful implementation of Lynch Syndrome screening in other states or regions. Firstly, reflex IHC testing should be carried out in accredited pathology services with ongoing quality control systems. This should be performed for all younger (<60 years) CRC patients, those with an individual and/or family history of cancer suggestive of Lynch Syndrome, and patients whose tumours have histological characteristics suggestive of Lynch Syndrome. Second, a state or region-wide reference laboratory for MSI testing is required to confirm all abnormal MSI/IHC abnormalities by a genetic counsellor was an effective means of ensuring attendance at a familial cancer clinic.\(^2\)\(^7\) At present in Western Australia, only a small number of potential germline mutation cases are not being referred by clinicians to Geological Survey Western Australia (approximately 5/35 per year, 15%).

Finally, with regards to screening for Lynch Syndrome, several issues require further investigation. Firstly, what is the cost-effectiveness of laboratory-based screening for Lynch Syndrome? This has been investigated for single institutes,\(^2\)\(^8\)\(^9\) but not for population-wide screening. Secondly, do Lynch Syndrome individuals and families identified by population-based laboratory screening have a different cancer penetrance to those identified in familial cancer clinics using the Amsterdam and Bethesda criteria? Finally, would routine IHC and/or MSI screening of endometrial cancers and other Lynch Syndrome-related cancers, particularly in younger patients, identify previously unrecognised cases of Lynch Syndrome?

This allows monitoring of both referrals and attendance at familial cancer clinics. A recent study by the Cleveland Clinic demonstrated that direct contact of patients with MSI/IHC abnormalities by a genetic counsellor was an efficient means of ensuring attendance at a familial cancer clinic.\(^2\)\(^7\) At present in Western Australia, only a small number of potential germline mutation cases are not being referred by clinicians to Geological Survey Western Australia (approximately 5/35 per year, 15%).

**Recommendations**

Clinicians need to be aware about the possibility of an underlying inherited predisposition when managing a patient with CRC. The implications of such a diagnosis apply not only to that individual, but to the wider family as well. A three generation family history remains the most important tool to alert clinicians towards a possible inherited predisposition, but turn-out-based testing via IHC and/or microsatellite testing should also be considered in all patients diagnosed with colorectal cancer under the age of 60 years.

**References**

Risk Profiling and Surveillance: Previous Adenomas and Colorectal Cancer

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Abstract

The brief of this issue of Cancer Forum is to review information available since the 2005 publication of the National Health and Medical Research Council relating to risk management of individuals with previous adenomas or colorectal cancer. However, this can be abbreviated to the last three years, as Cancer Council Australia commissioned a review of colonoscopy in surveillance for colorectal cancer, which included adenoma and cancer follow-up. This has subsequently been endorsed by the National Health and Medical Research Council. Since then, there have been advances in some areas, although many questions remain and clinical judgement comes into play. In the current era of accountability, economic hardship and increasing demand, surveillance strategies should be proven effective and individualised, based on issues such as fitness, quality of life and personal preferences. International guidelines have aligned, although the simpler strategies specified in European guidelines are noted with interest. Despite clear recommendations, the lack of guideline use in routine practice is concerning and widespread promulgation of simple ‘aid-memoirs’ could help, along with incentives. Information supports risk related to multiplicity, size and histopathology of adenoma and cancer findings at the index colonoscopy. Quality issues relating to colonoscopy and pathology reporting are being driven through professional fora and training. The paradox of multiplicity and...