



## Review Article

# Surgical Aspect of Microsatellite instability in Colorectal Cancer

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## Abstract

Approach to microsatellite instability associated colorectal cancer is a challenge faced by the surgeons. Various guidelines and implementation have been introduced to identify the microsatellite instability and MMR deficient status. Preoperative identification of the MSI status of the colorectal cancer by endoscopic, radiological guided and post operative surgical biopsy are important aspects for a surgeon and oncologist to make a strategic plan for the surgical intervention. Multiple factors need to be considered during the surgical decision keeping in view of the oncological response to the Microsatellite instability CRC treatment towards the chemotherapy and the new modulating immunotherapy. In this review we will briefly discuss the biological mechanism of the occurrence of MSI, the MSI testing results and its implementation on surgical aspects, and the current research on the immunotherapy response to the MSI in colorectal cancer.

## Introduction

Colorectal Cancer (CRC) is the fourth most common cancer in the UK and being the third most in males and females [1]. CRC has a diverse pathogenicity involving two recognized molecular pathways-chromosomal instability, majority of the cancers arises from this pathway and microsatellite instability accounts for approximately 15% of the cancer [2]. Microsatellite instability is further subdivided with one third of tumour arising as Hereditary Nonpolyposis Colorectal Cancer Syndrome (HNPCC), where there is constitutive mutation to one of the MMR genes and remaining cases as sporadic, with the silencing of the epigenome of MLH1 [3]. Lynch syndrome accounts for about 3.3 % of colorectal tumours and with annual estimate to be over 1100 in UK. It is estimated that 175,000 people in UK has Lynch syndrome, and a large proportion of the population are unaware that they have the condition and fewer than 5 % are diagnosed with this syndrome [4]. Apart from colorectal cancers, lynch syndrome predisposes to endometrial cancer being most frequent [5]. Biologists has been focused on decoding the biomolecular process of the microsatellite instability in colorectal cancer for over a decade to

guide the therapeutic decision making and the outcome of the CRC [6]. Various implication and guidelines have been introduced to testing and screening tool available to help diagnose the MMR and MSI status. The MSI testing provides valuable information of prognosis and therapeutic approach [7] It has been associated with good prognosis on patients with localised disease [8]. Although the genetic testing helps in deciphering the MSI status on patients who have developed cancer, identifying patients who are high risk is the challenge that is faced by many clinicians. In literature, more emphasis has been made on testing and surveillance to identify the high-risk candidates [9]. Extensive research has focused on the oncological and biological treatment with MSI status and still controversies exist [10]. The real challenge faced by the clinician are the surgical approach and the preoperative MSI testing prior to surgery which has a major impact on the outcome. The risk of metachronous colorectal cancer following segmental colectomy in lynch syndrome is increased over four-fold compared to extended colectomy [11]. In this review, we have focused on the surgical aspects of MSI in colorectal cancer. We have reviewed the current knowledge on the molecular background, the genetic testing, and the therapeutic approach in terms of surgical implication and the

oncological aspects.

### **Molecular Biology Mechanism**

Microsatellites also called as short tandem repeats which constitute of repetitive sequence of 1-6 nucleotides distributed throughout the human genome. The distribution features are different from 15 to 65 nucleotides tandem repeat of small satellite DNA, mainly located near the end of the chromosomes [12]. They are widely distributed and mostly located near the coding area and may be in region like intron or non-coding area. Each microsatellite has specific site composed of a central core and peripheral flanks. The specificity is mainly due to the change in the region in the number of core repeating units. It is believed that the sequence patterns are predisposed to accumulation of mutations when the DNA polymerase cannot bind DNA efficiently during the synthesis [13]. The frequent errors occur with microsatellite are at the base- base mismatches and the DNA slippage in the process of replication. These errors escapes the intrinsic proofreading activity of DNA polymerase and insertion-deletion loops of extrahelical nucleotide when the first nucleotide and template strand dissociate incorrectly in a microsatellite [14]. This insertion or deletions in microsatellites located in DNA coding regions create frameshift mutations that lead to protein truncated or non-functional.

The mismatch repair system which is the normal tissue DNA repair system is responsible for surveillance and correction of the DNA replication errors introduced in the microsatellites. The main proteins MLH1, MSH2, MSH3, MSH6 and PMS2 involved in the system, interact as heterodimers when a mismatch takes place. Various steps take place in the mismatch where MSH2 associates with MSH6 or MSH3 and MLH1 binds with PMS1, PMS2 or MLH3 and form complexes. It is to identify the mismatches and insertion- deletion loops are carried out by these complexes which interacts with the replication factor C. Excision of the mismatches is performed by proteins such as exonuclease 1 and proliferating cell nuclear antigen. Finally resynthesises and realignment of the DNA strands is carried out by the DNA polymerase  $\delta$  and ligase [15,16]. Mutation in these genes is responsible for accumulation of errors in DNA which results in Microsatellites instability and hence an important factor in development of tumours.

Microsatellite instability has been classified as MSI-H, MSI-L and MSS phenotype. In MSI-H phenotype, the development of colorectal cancer can occur either sporadic because of MMR gene promotor methylation or HNPCC mutation. Germline mutation due to HNPCC affects the DNA MMR gene. The phenotype MSI-H is observed in more than 90% of HNPCC associated colorectal cancer and 15 % in sporadic [17,18]. To enhance the knowledge of the microsatellite instability and MMR deficiency in colorectal cancer for the optimal management, guidelines have been established to detect MSI testing and IHS for MMR deficiency.

### **MSI Detection**

In current literature, different laboratory approaches have been used to detect the MSI and MMR deficiency in colorectal cancer. It is not only focusing on the detection of lynch syndrome but also on sporadic colorectal cancer to aim for better management outcomes. The common approach of MSI testing is performed either directly through the PCR based assay for detecting the instability or indirectly through immunohistochemistry staining (IHC) by assessing the MMR protein expression [19,20]. The next generation sequencing based detection test is performed for comprehensive directed genotype to test the MSI in the tumour tissue [12,21]. The PCR based assay used for MSI testing is performed on fresh frozen or paraffin embedded tumour tissue for detecting the instability. The tumour caused by MMR deficiency protein exhibits inconsistent number of microsatellite nucleotide repeats compared to the normal tissue [22,23].

The molecular DNA of microsatellite instability of the tumour is evaluated through the PCR based MSI testing. It is designed to compare the 5 specific panel of microsatellite markers between the normal tissue and the tumour. It identifies the instability in the loci. The specific panel of markers include 3 dinucleotide and 2 mononucleotides, which are called Bethesda panel, are used in the MSI testing. The difference in the sequence of repeats in the normal tissue and tumour tissue is considered as MSI. If there are similar repeats in normal and tumour tissue it is considered as MSS. Based on the MSI testing, MSI-H is deemed if more than 30 % of the markers shows instability while lesser than 30 % is considered as MSI-L [15]. The immunohistochemistry assesses the loss of MMR gene that is the MLH1, MSH2, MSH6 and PMS2 staining on the tumour tissue. This leads to the DNA sequencing for germline mutation to that particular gene to detect Lynch syndrome. Further analysis is recommended if any of the approaches detect MSI or MMR deficiency, to identify the germline MMR gene mutations. The MSI testing and IHC test carried out simultaneously has a high concordant rate with a sensitivity of 92% and excellent specificity for detecting MSI [24,20].

BRAF V600E mutation and MLH1 promoter methylation differentiate the sporadic tumours from Lynch syndrome. It is a cost effective method for LS screening [25,26]. Isolated loss of MLH1 protein expression directs towards sporadic. However, loss of MLH1 and PMS2 protein indicates loss of MLH1 functions due to epigenetic silencing or underlying germline PMS2 mutation. Loss of MSH2 or MSH6 protein on IHC has a high specificity leading to the diagnosis of LS [27]. Research has shown that next generation tumour sequencing is simpler and superior sensitivity to multi-test approach. It may replace the current standard of tests performed for colorectal cancer [28]. The NICE guidelines 2017 has established a pathway for all colorectal cancer and has recommended that all colorectal cancers should be tested for LS

using IHC or MSI testing. After the initial tumour testing, germline testing is a part of mainstream approach. If the germline test is negative but a strong family history, then referral to clinical genetic service for consideration of chemoprevention or endoscopic surveillance is recommended[4].

### **Surgical Implication and Chemotherapy**

IHC testing can be performed with either endoscopic biopsy tissue or surgical resection specimen or radiological tissue sampling. Literature shows that biopsy retrieved through endoscopy shows strong staining and are as good as the resection specimen on those patients who did not undergo neoadjuvant chemotherapy. The resection specimen has proved to have weak focal staining in some of the studies and endoscopic biopsy tissue superior to it [29-31]. IHC staining is crucial on endoscopic biopsy especially with rectal cancer who undergo neoadjuvant therapy. The IHC staining post neoadjuvant therapy resection specimen shows decreased intensity scores on the stains and increased focal points or results inconclusive stains [32]. The ability to diagnose a patient with Lynch syndrome prior to surgery is one of the greater importance as the current surgical guidelines recommend patient who develop colorectal cancer to be offered total colectomy. Extended surgery is recommended for patients with MLH1 and MSH2 carriers at the time of diagnosis. Additional benefit of IHC staining of endoscopic biopsy helps in MSI testing, appropriate germline testing prior to surgery. A detail discussion of additional prophylactic surgery such as total hysterectomy and salphingo-oophorectomy should be considered as patients with Lynch syndrome as it carries a high risk of endometrial and ovarian cancers [33,24].

A systematic review was conducted on the risk of metachronous tumour in lynch syndrome following colectomy. Despite post operative endoscopic surveillance after segmental resection, risk of metachronous tumour were increased by four times when compared with extended colectomy. The risk of metachronous tumour need to be considered when deciding for the initial surgery and appropriate surgical decision making and functional consequences should be discussed with the patients [11,34]. A study conducted in ACS 98<sup>th</sup> Annual clinical congress by Dr Ryan and her colleague analysed the patient opting for prophylactic surgery has increase to more than 2 folds with the increase in genetic awareness and diagnosis of genetic susceptibility. They have shown concerns that although surgery can prevent cancer occurrence and recurrences, it can also cause potential harm to patient and health systems and a better understanding of the variations related to prophylactic surgery might aid in clinical practice and patient counselling [35].

The clinicopathological factors of MSI-H tumours are distinctive from the MSS CRC being early tumour, in younger age, mostly in the proximal colon, with poorly differentiated mucinous histopathology, and prominent lymphocytic infiltration.

The clinical picture of MSI- H CRC in metastatic cases are more pronounced in elderly and diagnosed in women with synchronous tumour involving the lymphatics and the peritoneal lining rather than the solid organ such as liver or lung. The MSI-H CRC tumour exhibits more localized recurrence than the MSI-L/ MSS CRC [8]. Many studies reported the association of MSI-H phenotype with improved prognosis with localised disease. It is thought the relation of the immunological response to the MSI-H tumours reaction is similar to Crohn's disease that increase the host immunity by peritumoral lymphoid nodule and a dense lymphatic infiltration in the tumour which are activated and cytotoxic [36]. The improved disease-free survival rate is associated with high total number of lymph node harvested. It is recommended in the guidelines to have adequate harvest lymph nodes of approximately more than 12 [37-39]. However, Kang et al study showed a new perception of MSI status with low LNR being a strong predictor to better outcomes [40]. In addition, recent studies proved that MSI-H CRC with stage 2 does not respond to 5 FU treatment [18]. This highlights the importance of MSI testing in early stages where they are managed with curative intention with the surgery approach only or combined with chemotherapy.

In stage 2 tumour CRC MSI-H has shown better overall survival rate with disease free survival with surgical treatment alone compared to CRC MSI-L. Studies has shown MSI-H recurrence survival rate has no significant difference if they receive adjuvant chemotherapy or not. It is now indicated that in MSI -H, chemotherapy is not indicated in stage 2 due to its non-responsive nature of 5 FU based chemotherapy and can be harmful at this stage as concluded by Sargent, et al. [41] In stage 3 CRC adjuvant 5 FU combined based oxaliplatin chemotherapy is still the standard of care, although the survival rate has not shown any improvement with MSI-H cases but has shown a better survival rate with MSI-L/ MSS [42]. Interestingly, MOSAIC trial of Oxaliplatin/5 FU/ Leucovorin based chemotherapy has shown improved response to the treatment and are independent prognostic factor to the MSI/ MMR status. The MOSAIC trial of 10-year follow-up shows the better survival rate with FOLFOX-4 treatment than 5FU and Leucovorin [43]. However, the National surgical adjuvant breast and bowel project has predicted that oxaliplatin has no benefits on the MSI-H/MMR d but has improved prognostic values in recurrence [10]. However, Benson et al, in his study shown that MMR status of the patient with stage 3 and 4 CRC responds to 5 FU and has a curative response [44].

In recurrence disease, one of the studies demonstrated that treatment strategy is affected in recurrent pattern. In recurrent MSI-H CRC, survival rate was worse than MSI-L/MSS and less curable. MSI-L/MSS status patient experienced twice more recurrent rate than MSI-H. CAIRO study and retrospective analysis has shown poor outcome for metastatic MSI-H patients with decrease survival rate [45]. One of the studies showed the

chemotherapy treatment was better among MSI-L/MSS compared to MSI-H which shows chemoresistance features in the colorectal cancer. Recently a clinical trial on advanced rectal tumour on MSS and MSI-H was conducted, preoperative chemoradiotherapy combined with Nivolumab showed promising result with complete pathological response in MSS and almost 60% in MSI-H, although the incidence of tumour with MSI-H status is low in distal colon [46]. Moreover, recurrent MSI-H tumour has been associated with immune response and developed mechanism to overcome the immunosurveillance.

### Immunomodulation and Biological Treatment

Immunotherapy has become the focus of the cancer therapeutic treatment. Clinical use of immunotherapy is limited by poor response of some tumours. The immunobiology of CRC has important therapeutic implications as microsatellite status appears to predict response to immunotherapy. It improves the efficacy of treatment and survival of individuals in various cancers. Clinical efficacy is predominantly limited to MSI tumours whilst MSS tumours are largely refractory [47]. Anti-PD-1/PD-L1 immunotherapies have led to tremendous success in treating certain cancers. Mismatch repair deficiency/microsatellite instability-high represents a good prognosis in early colorectal cancer settings without adjuvant treatment and a poor prognosis in patients with metastasis. Several clinical trials have demonstrated that mismatch repair deficiency or microsatellite instability high is significantly associated with long-term immunotherapy-related responses and better prognosis in colorectal and non-colorectal malignancies treated with immune checkpoint inhibitors. Impressive results of pembrolizumab (Anti-Programmed cell death-1 inhibitor) in patients with dMMR or MSI-H tumours after progression from prior chemotherapies have been shown in the KEYNOTE-016, 164, 012, 028, and 158 trials [48]. In these trials 149 patients from 15 different trials were enrolled. 61 patients with CRC were enrolled in KEYNOTE 164 trial, 28 in 016 and 6, 5, 9 patients enrolled in KEYNOTE-012, 028, and 158 trials, respectively. Different doses of pembrolizumab were given in these trials for 2-year periods. Results showed Overall Response Rate (ORR) of more than 39.6% with 78% responder sustained response for more than 6 months. O'Neil, et al. in his study, also demonstrated that Pembrolizumab is safe and less adverse events for patients with advanced PD-L1 positive CRC [49]. In 2017, Based on above response and sustainable result FDA approved the use of pembrolizumab as second- or higher-line choice for the treatment of patients with unresectable or metastatic dMMR/MSI-H solid tumours, irrespective of tumour type or site.

Nivolumab is another biological agent showed significant improvement in treatment of patients over age of 12 years with dMMR/MSI-H for advanced metastatic colorectal tumour post chemotherapy. Efficacy of Nivolumab in patients with dMMR/

MSI-H tumors that progressed during or after one-line conventional chemotherapy was checked in CheckMate 142 trial. It was Phase II, multicenter study. 69% of patients had disease control for more than 12 weeks overall survival rates (OR) at 12 months were 73% [50]. Another study by Overman et al. demonstrated that Nivolumab is also effective for those with poor prognosis of BRAF mutation in CRC [50]. Nivolumab is now approved by FDA and is a new treatment option for mCRC patients over age of 12 years with MSI-H or dMMR who had disease progression after chemotherapy with fluoropyrimidine, oxaliplatin, and irinotecan. Combined use of nivolumab with ipilimumab was assessed in nivolumab plus ipilimumab cohort of the CheckMate 142 trial. Low dose ipilimumab was combined with nivolumab to reduce the toxic side effects. The results showed that the ORR was 55% and OR was 85% [51]. Another Phase II trial by Chalabi investigated nivolumab plus ipilimumab as a neoadjuvant therapy in resectable, early-stage colon cancers with dMMR and pMMR in a small sample. 100% response observed in dMMR patients and no response in pMMR patients. (52)

There are significant numbers of ongoing clinical trials to check efficacy of immunotherapy at different stages of cancer. (53) The phase III COMMIT trial is currently evaluating efficacy of atezolizumab (anti-PD-L1) and bevacizumab as first-line therapy in patients with MSI metastatic CRC. Combine therapy may give promising results for MSS tumours when used with therapy of target agent with checkpoint blockade.

### Conclusion

As discussed in the review the unique biological features of the MSI carries a high risk of colorectal cancer with sporadic and lynch syndrome. The need to incorporate the MSI testing and the IHS testing in the clinical practice helps to distinguish patients to have appropriate surgical intervention plan preoperatively. Due to their distinctive features of how they respond to the FU based chemotherapy the need to identify them is of utmost importance. With the expansion of immunotherapy for in MSI-H CRC/ MMR d and its good response to the treatment, MSI testing in all stages of the CRC is crucial to guide the surgical and oncological management.

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