# Early Screening and Risk Stratification of Lynch Syndrome-Associated Multiple Cancers: From Colorectal Cancer to Endometrial Cancer and Pancreatic Cancer

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**Abstract:** Lynch syndrome (LS), an autosomal dominant hereditary disorder, represents the most prevalent form of hereditary colorectal cancer. LS patients are prone to developing multiple types of cancer, and the risk of different cancers caused by different pathogenic genes varies. The characteristic feature of LS is early damage to colorectal tumors, which mainly depends on the type of pathogenic mutation in mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2, and EPCAM). Although colorectal cancer (CRC) is the most common cancer type in LS families, patients also have an increased lifetime risk of other types of tumors such as endometrial cancer (EC) and pancreatic cancer (PC). This study reviews the early diagnosis of multi-cancer in LS patients, explores approaches to reduce missed diagnoses and high mortality rates in LS-associated hereditary multi-cancers, and for the first time proposed the concept of multicancer screening, which encompasses several closely related and common cancers in LS syndromes and guided clinicians in early identifying and diagnosing at-risk individuals.

***Keywords*:** lynch syndrome, hereditary colorectal cancer, endometrial cancer, pancreatic cancer, early screening, mismatch repair deficiency.

## 1. Introduction

Lynch syndrome (LS) is the most common hereditary form of colorectal cancer (CRC), with a population incidence of approximately 1 in 280 and accounting for 3%-5% of all CRC cases[1, 2]. LS arises from germline mutations in mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2, and EPCAM), inherited in an autosomal dominant pattern[3, 4]. Individuals with LS have significantly elevated risks for multiple malignancies, with risk profiles varying by specific pathogenic mutation[5, 6].

Early-onset colorectal tumors characterize LS, with risk determined by both the specific MMR gene mutation and demographic factors[7, 8].Early identification of LS patients is essential due to their increased risk for developing multiple cancers throughout life[3]. Recent studies highlight differences in genetic and epigenetic alterations between LS-associated and sporadic CRC, suggesting that identifying methylated DNA markers (MDMs) through methylome sequencing may improve diagnostic accuracy and reduce missed diagnoses.[9]. Thus, there is a pressing need for novel tests to supplement current screening methods and enhance detection of LS-related CRC. In addition to CRC, LS patients face increased risks for other malignancies, particularly endometrial cancer (EC) and pancreatic cancer (PC). The lifetime risk of EC in women with LS ranges from 16% to 60%[10], while the cumulative lifetime risk of PC is approximately 3.7%, with an 8.6-fold higher risk than the general population[11, 12]. Despite established screening protocols for CRC, effective risk stratification and early detection strategies for other LS-associated cancers remain limited[13].

This review examines early screening methods and risk stratification approaches for CRC, EC, and PC, evaluates current diagnostic criteria, explores emerging molecular screening technologies, and proposes integrated strategies to enhance early detection across multiple cancer types, potentially reducing mortality associated with these hereditary malignancies.

## 2. Diagnosis and Early Screening for CRC in Lynch Syndrome

LS is diagnosed by germline mutations in MMR genes. These proteins form functional heterodimers, and mutations in one gene can affect both the corresponding protein and its partner[3]. MLH1 and MSH2 mutations are most common (30% each), while MSH6 and PMS2 mutations are less frequent (around 5% each). EPCAM mutations cause MSH2 promoter methylation. Approximately 30% of LS cases remain genetically undefined, complicating genetic counseling and risk assessment.

Current LS screening criteria include the Amsterdam I and II criteria, the Bethesda criteria, and prediction models like PREMM5 and MMRpro. These tools identify individuals who should be tested for deficient mismatch repair (dMMR) or microsatellite instability (MSI)[7]. The Amsterdam criteria have high specificity but low sensitivity, while the revised Bethesda criteria offer higher sensitivity but lower specificity. Prediction models improve diagnostic accuracy but require extensive family history, limiting their clinical use. As a result, LS screening has evolved from criteria-based assessment to tumor-based screening with germline confirmation (Figure 1).

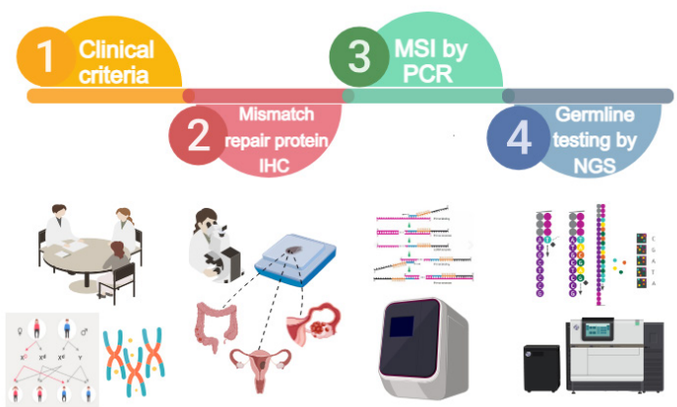


**Figure 1. Evolution of Lynch syndrome screening (created with image.medpeer.cn)**

IHC, immunohistochemistry; MSI, microsatellite instability; PCR, polymerase chain reaction; NGS, next-generation sequencing

The diagnostic process for LS involves assessing personal and family history, testing for dMMR or MSI, and confirming germline mutations. Given the limited sensitivity of dMMR/MSI testing alone, germline mutation testing is recommended for CRC patients under 50 or with a family history, regardless of initial test results. LS diagnosis is confirmed when a clinically significant MMR gene mutation is identified (Figure 2).

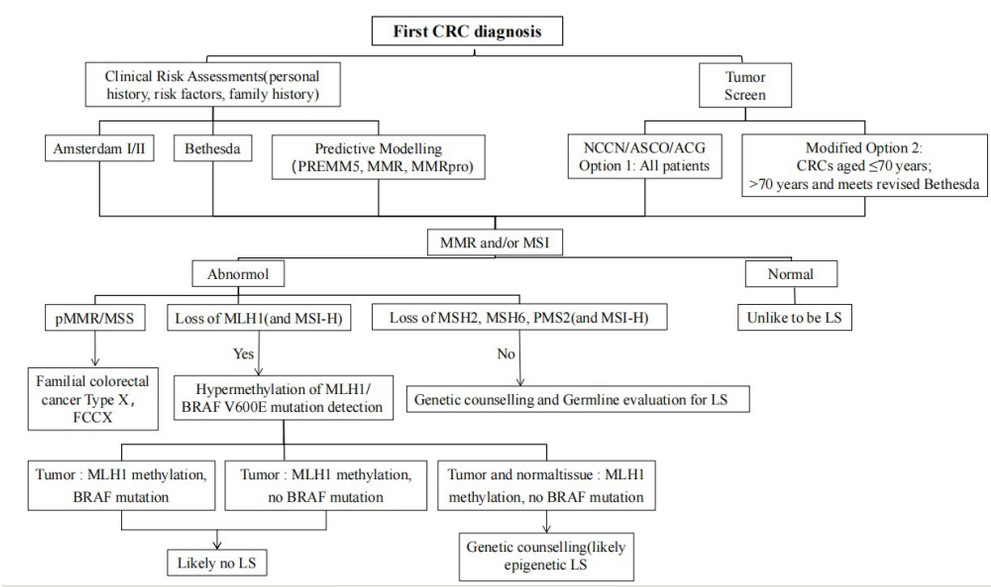


**Figure 2. Flowchart of Lynch syndrome screening and diagnosis using CRC as an example**

LS is the most common hereditary cause of CRC, occurring in approximately 1 in 400 individuals and accounting for at least 3% of all CRC cases and 10%-20% of early-onset CRC[14]. CRC ranks among the five most common cancers in China, with an increasing incidence. GLOBOCAN data reported over 20 million new cancer cases worldwide in 2022, including more than 1.9 million CRC-related deaths[15]. Data from the U.S. National Cancer Institute indicate a five-year survival rate of 60% for CRC patients diagnosed between 2013 and 2022, though screening has helped reduce both incidence and mortality[16]. Current CRC screening guidelines recommend fecal occult blood test (FOBT) and colonoscopy. While colonoscopy is effective, its adoption in China remains low (20%) due to its invasive nature, preparation requirements,

limited insurance coverage, and lack of awareness. FOBT provides non-invasive detection but has inadequate sensitivity (73.8%), particularly for early CRC, leading to missed diagnoses. These limitations highlight the need for alternative non-invasive screening methods.

Recent research indicates that epigenetic processes play a role in the initiation and progression of CRC [17]. DNA methylation changes can detect tumors up to four years earlier than traditional methods[18, 19]. Fecal DNA testing is a promising non-invasive approach, with the SDC2 gene methylation test approved for CRC detection in China[20]. Identifying methylation-derived markers (MDMs) using methylome sequencing could improve early detection strategies for CRC in LS, enabling more accurate identification of tumors in both LS and sporadic CRC tissues.

# 3. Early Screening and Risk Stratification for EC in Lynch Syndrome

EC is the most common extraintestinal tumor in LS patients and one of the three most prevalent malignant neoplasms in the female reproductive system. About 5% of EC cases result from genetic factors, mainly LS. Female LS patients have an EC risk comparable to or even higher than their CRC risk, with a lifetime risk ranging from 16% to 71%, mostly occurring before age 50[21, 22]. Given the lack of established LS screening guidelines following gynecological cancer diagnosis, many female patients rely on healthcare professional guidance. Screening for LS in EC patients is crucial for early diagnosis and prevention of LS-related tumors in patients and their family members.

The Society of Gynecologic Oncology (SGO) has developed LS screening guidelines based on the Amsterdam criteria, recommending genetic counseling and evaluation for patients with risk assessments of 5%-10% and 20%-25%[23]. SGO recommends DNA MMR mutation testing on histological specimens for all diagnosed EC cases. Traditional screening methods using patient and family history are convenient but lack specificity. Prediction models like PREMM5 may improve identification, though research on its applicability to EC remains needed[24]. If PREMM5 is confirmed to have high sensitivity and specificity for LS-EC, it could become a routine clinical tool with significant benefits.

Immunohistochemistry (IHC) of MLH1, MSH2, MSH6, and PMS2 is commonly used for LS screening, showing 91% sensitivity and 83% specificity for EC-LS detection[25]. The National Comprehensive Cancer Network (NCCN) guidelines recommend MMR protein IHC testing as a universal LS screening method. However, MSH6 mutations occur more frequently in EC patients and often present with MSI-low or MSS status, making MSI testing alone inadequate for gynecologic cancers[26]. Risk stratification based on specific MMR mutations reveals critical differences: PMS2 carriers show minimal cancer risk increase before age 50, while MLH1, MSH2, and MSH6 carriers face sharply rising EC risk from age 40.Unlike CRC, MLH1 methylation rarely occurs in EC-LS, though exceptions exist. Therefore, when detecting MLH1 promoter methylation, clinicians should evaluate family history, age of onset, and personal tumor history before ordering additional testing.

A stepwise screening approach is recommended for optimal EC-LS diagnosis (Figure 3): Start with IHC testing for MMR proteins in tumor tissue, followed by MSI analysis for patients with normal MMR expression but strong clinical suspicion. If MLH1 and MLH1+PMS2 protein expression is lost, perform MLH1 methylation analysis to exclude sporadic EC, with next-generation sequencing as the confirmatory diagnostic method. Early EC-LS screening helps reduce cancer incidence while balancing treatment needs with fertility preservation options.

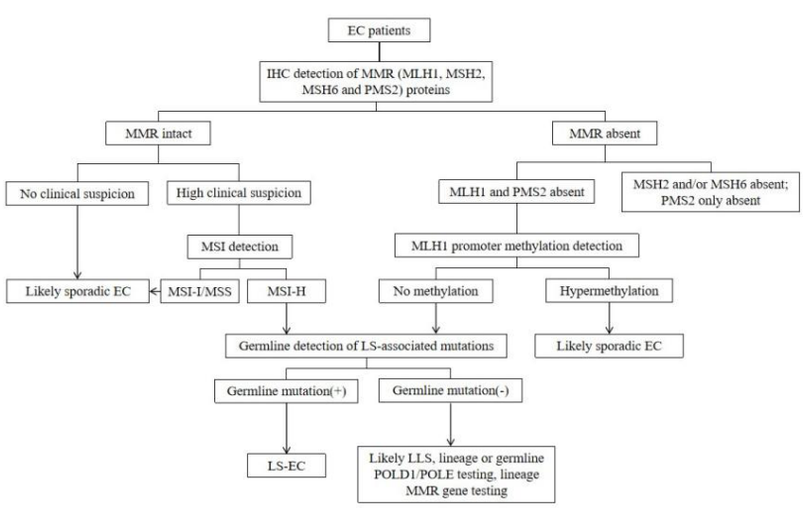


**Figure 3. Recommendations on the screening procedures for LS-EC patients**

## 4. Early Screening and Risk Stratification for PC in Lynch Syndrome

PC is a significant concern for Lynch syndrome (LS) patients, with a cumulative risk of approximately 3.7%, which is 8.6 times higher than in the general population[11]. PC is challenging due to its insidious onset, difficult early detection, and poor prognosis. Only 25% of diagnosed PC patients can undergo surgery, with most dying within a year without effective intervention. Despite research advances, survival rates remain low, with over 490,000 new PC cases and more than 460,000 deaths worldwide in 2020. The 5-year survival rate in the U.S. was only 12.5% from 2013 to 2019, and PC is predicted to become the second leading cause of cancer-related deaths by 2030[27].

Risk stratification shows varying PC risks among LS patients based on specific mutations. Among individuals aged 37-40 diagnosed with PC, 0.2%-3.6% have LS. Cumulative PC incidence at age 75 varies by genotype: MLH1 (6.2%), MSH2 (0.5%), and MSH6 (1.4%) carriers show different risks, while PMS2 carriers may not have a significantly increased risk compared to the general population. Despite these differences, comprehensive data on risk stratification for LS patients at high risk for PC is limited.

Current monitoring guidelines recommend annual surveillance starting at age 50 or 10 years younger than the youngest diagnosis in the family, using endoscopic ultrasound or magnetic resonance cholangiopancreatography, or alternating between these methods. High-risk LS carriers include those with MLH1, MSH2, or MSH6 mutations, especially if they have one or more affected first- or second-degree relatives[28]. Early diagnosis of PC remains difficult, and combined detection of multiple PC-related genes and serum tumor markers is currently the most effective approach[17].

Similar to CRC, DNA methylation plays a significant role in PC development. Detection of hypermethylated genes may aid both diagnosis and targeted therapy[29]. Methylation markers such as BNC1, NPTX2, ppENK, and p16 show promise for PC diagnosis[27]. These epigenetic changes provide valuable insights into pancreatic carcinogenesis and could serve as diagnostic biomarkers. Further research into methylation patterns specific to LS-associated PC may enhance early detection, recurrence prediction, prognosis evaluation, and targeted therapies.

## 5. Integrated Multi-Cancer Risk Stratification in Lynch Syndrome

Research on stratifying LS patients to detect multi-cancer risks remains limited. CRC molecular profiling reveals that 2-16% of cases exhibit MSI-H status, while most are MSS, with some showing MSI-L features[30]. MSI-L tumors are characterized by increased KRAS mutations, reduced 5q loss, decreased Bcl2 expression, enhanced apoptosis, and greater lymphocyte infiltration. MGMT promoter methylation is linked to KRAS mutations in these tumors, suggesting KRAS plays a crucial role in early development, associated with serrated polyps. Kras mutations are also important in PC, with diagnostic markers including Kras gene codon 12, p53, and telomerase. Clinical data shows a 93.3% mutation rate of Kras codon 12 in PC patients, detectable via PCR in both precancerous lesions and metastases. This mutation appears early in PC (in tumors as small as 2 cm), making it a valuable early diagnostic marker.

Novel multi-cancer screening methods offer potential for integrated risk assessment. The SPOT-MAS method, which evaluates circulating tumor DNA, demonstrated 60% positive predictive value and 83.3% accuracy in detecting tumor sites in asymptomatic individuals[31]. Liquid biopsies, using markers like ctDNA, cfDNA, mRNA, and miRNA from peripheral blood, are emerging as promising tools for LS-associated cancers (CRC, EC, and PC). These techniques offer practical benefits, including simplicity, reproducibility, affordability, and patient comfort, and may enable early tumor detection and monitoring of treatment effectiveness[32]. These approaches could enhance comprehensive risk stratification for multiple LS-related cancers.

## 6. Conclusions

This review emphasizes the critical need for comprehensive early screening and risk stratification for LS-associated cancers. We advocate for universal screening in all diagnosed cases of CRC, EC, and PC, along with genetic counseling for individuals with a suspected LS family history. While screening strategies for CRC are well-established, these approaches must be adapted for other LS-related malignancies. The evidence supports implementing risk-stratified management guidelines based on specific MMR gene mutations (MLH1, MSH2, MSH6, and PMS2), which will enable tailored preventive measures and optimized treatment strategies. Personalized screening approaches are necessary for each cancer type due to the distinct risk profiles associated with different mutations. Large-scale population studies are required to collect comprehensive data on incidence, clinical features, and genetic alterations in LS-associated cancers, especially in diverse populations.

Early detection methods using molecular and epigenetic markers hold promise for improving screening efficacy across multiple LS-related cancers. Integrating these technologies with conventional methods may enhance sensitivity and specificity, while improving patient compliance. Further investigation into gene mutation methylation patterns and mechanisms across

LS-related cancers will be crucial for developing more effective screening protocols and reducing mortality rates associated with these hereditary malignancies.

## Authors Contributions

All authors contributed to the study conception and design. Conceptualization, X.L., and H.Y.; Methodology, N.W.; Software, X.L.; Validation, X.L., J.G.; Formal Analysis, X.L.; Investigation, J.G.; Resources, H.Y.; Data curation, X.L., and J.G.; Writing-Original Draft Preparation, X.L., and N.W.; Writing-Review & Editing, J.G., and H.Y.; Visualization, H.Y.; Supervision, J.G. and H.Y.; Project Administration, H.Y.; All authors have read and agreed to the published version of the manuscript.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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