CLINICAL AT

Risk of Cancer in Cases of Suspected Lynch Syndrome Without Germline Mutation

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This article has an accompanying continuing medical education activity on page e13. Learning Objective: Upon completion of this CME activity, successful learners will be able to assess the different diagnostic tests to establish a diagnosis of Lynch syndrome.

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BACKGROUND & AIMS: Colorectal cancers (CRCs) with microsatellite instability (MSI) and a mismatch repair (MMR) immunohistochemical deficit without hypermethylation of the *MLH1* promoter are likely to be caused by Lynch syndrome. Some patients with these cancers have not been found to have pathogenic germline mutations and are considered to have Lynch-like syndrome (LLS). The aim of this study was to determine the risk of cancer in families of patients with LLS. **METHODS:** We studied a population-based cohort of 1705 consecutive patients, performing MSI tests and immunohistochemical analyses of MMR proteins. Patients were diagnosed with Lynch syndrome when they were found to have pathogenic germline mutations. Patients with MSI and loss of MSH2 and/or MSH6 expression, isolated loss of PMS2 or loss of MLH1 without *MLH1* promoter hypermethylation, and no pathogenic mutation were considered to have LLS. The clinical characteristics of patients and the age- and sex-adjusted standardized incidence ratios (SIRs) of cancer in families were compared between groups. **RESULTS:** The incidence of CRC was significantly lower in families of patients with LLS than in families with confirmed cases of Lynch syndrome (SIR for Lynch syndrome, 6.04; 95% confidence interval [CI], 3.58–9.54; SIR for LLS, 2.12; 95% CI, 1.16–3.56; $P < .001$). However, the incidence of CRC was higher in families of patients with LLS than in families with sporadic CRC (SIR for sporadic CRC, 0.48; 95% CI, 0.27–0.79; $P < .001$). **CONCLUSIONS: The risk of cancer in families with LLS is lower that of families with Lynch syndrome but higher than that of families with sporadic CRC. These results confirm the need for special screening and surveillance strategies for these patients and their relatives.**

Keywords: Inherited Colon Cancer; Cancer Risk; Genetic Testing; Immunohistochemistry.

Lynch syndrome (LS) is the most common inherited colon cancer susceptibility syndrome and is caused by germline mutations in one of several DNA mismatch repair (MMR) genes, mainly *MLH1* and *MSH2* but also *MSH6* and *PMS2*.[1–3] Patients with LS have an increased risk of colorectal cancer (CRC), endometrial cancer, and several other cancers, including ovarian, upper urinary tract, gastric, small bowel, biliary/pancreatic, skin, and brain cancers. The molecular signature of LS is microsatellite instability (MSI), which is found in more than 95% of LS-associated CRCs.[4] However, MSI is also present in up to 15% of sporadic CRCs due to hypermethylation of the promoter region of *MLH1* in tumor cells. Immunohistochemical (IHC) studies of MMR proteins have been shown to be equivalent to MSI in detecting MMR-defective CRC.[5] CRC with MSI and a lack of staining of MSH2, MSH6, or MLH1 without promoter hypermethylation is a strong indicator of *MSH2*, *MSH6*, or *MLH1* germline mu-

Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; IHC, immunohistochemical; LLS, Lynch-like syndrome; LS, Lynch syndrome; LSRC, Lynch syndrome–related cancer; MMR, mismatch repair; MSI, microsatellite instability; SIR, standardized incidence ratio.

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tations.[6] However, some of these cases of CRC do not have pathogenic mutations in MMR genes. These cases are suspected to be nonsporadic because no mechanism of inactivation is known for these genes other than germline mutations in the context of LS. These patients are considered to have "probably nonsporadic" CRC or Lynch-like syndrome (LLS), and decisions about their management are not simple because of unconfirmed suspicions of hereditary cancer. These cases must be distinguished from familial CRC type X, in which tumors do not show MMR deficiency. No studies have characterized these patients with CRC, and the risk of cancer in this group of families is not known. Therefore, the surveillance strategy for these patients and their relatives is unclear.

We analyzed the clinical and familial characteristics of patients diagnosed with LLS, LS, or sporadic CRC. The main aim of this study was to determine the risk of cancer in families of patients with LLS.

Patients and Methods

Patients and Data Collection

This population-based, observational, cohort study included 1705 patients with CRC from 2 nationwide multicenter studies: EPICOLON I and EPICOLON II. EPICOLON I included consecutive patients with a new diagnosis of CRC between November 2000 and October 2001 with the main goal of estimating the incidence of LS in Spain.[7] EPICOLON II also included consecutive patients with newly diagnosed CRC between March 2006 and December 2007 with the aim of investigating different aspects related to the diagnosis of hereditary CRC.[8] All of the patients provided written informed consent. Both studies were approved by the institutional review boards of the participating hospitals.

Patients were divided into 3 groups based on genetic data: (1) the LS group, in which patients had a confirmed pathogenic mutation in *MLH1*, *MSH2*, *MSH6*, or *PMS2*; (2) the LLS group, in which patients had MSI and loss of MSH2/MSH6 expression, isolated loss of PMS2, or loss of expression of MLH1 without *MLH1* promoter hypermethylation in which no germline mutation was found; and (3) the sporadic group, in which patients with CRC and microsatellite stable tumors had normal expression of MMR genes or a loss of MLH1 expression with *MLH1* promoter hypermethylation.

Demographic, clinical, and pathologic data were collected at the time of diagnosis. Cancer pedigrees were built at diagnosis for cases of CRC in the EPICOLON I and II studies. The pedigrees were traced backward and laterally as far as possible. This information was verified by reviewing medical records when available. Standardized incidence ratios (SIRs) for cancer were calculated as the ratio of the observed to expected number of cases diagnosed in the families at the time of inclusion in the EPICOLON I or II cohorts. To avoid recall bias, only cases of cancer in first-degree relatives were included in the calculation of SIR. We considered tumors in the endometrium, ovaries, upper urinary tract, stomach, small intestine, and hepatobiliary system as noncolorectal LS-related cancers (LSRCs). The index case was excluded for the analysis of family history at the time of diagnosis. Calculation of the SIR was only possible in families with complete pedigrees and information about the ages of all family members, including relatives without cancer.

In 2011, the pedigrees were updated by asking patients and/or relatives about new cases of cancer after diagnosis of the index case. We include the index case for this analysis, and the appearance of metachronous CRC or a new case of noncolorectal LSRC in the index case was considered a new case in the family.

MSI, Immunohistochemical Staining, and Detection of Germline Mutations

MSI analysis was performed in all patients. We ascertained MSI status using BAT26 and NR24 quasi-monomorphic markers as described previously.[9] MSI was present when one of the 2 markers was unstable. IHC analysis of the 4 MMR proteins MLH1, MSH2, MSH6, and PMS2 in tumor tissue was performed in all patients using tissue microarrays as described previously.[10] In patients with a loss of MLH1, methylation of *MLH1* and *BRAF* mutation status was analyzed. *MLH1* methylation analysis was performed using methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) according to the manufacturer's protocol using SALSA MS-MLPA Kit ME011 Mismatch Repair Genes (MRC-Holland, Amsterdam, The Netherlands).[11] The V600E *BRAF* mutation was detected using specific TaqMan probes in real-time polymerase chain reaction (ABI Prism 7500; Applied Biosystems, Foster City, CA) and allelic discrimination software as described previously.[12]

Germline mutation analysis was performed in accordance with the results of IHC analysis as described previously.[10] Patients with loss of MSH2 expression with no detected mutation were analyzed for *EPCAM* rearrangements using MLPA (MRC-Holland) according to the manufacturer's recommended protocol. DNA sequencing was performed to characterize the deletion breakpoints.[13] Large rearrangements (deletions and insertions) were tested using MLPA according to the manufacturer's protocol. The results of genetic analysis were interpreted based on the ACMG Recommendations for Standards for Interpretation of Sequence Variations (2000) and the InSIGHT database.[14]

Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median and 25th and 75th percentiles for non-normally distributed data. Categorical variables are reported as frequencies or percentages. Significant differences between groups were analyzed using the χ^2 test for categorical data and the non-parametric Mann–Whitney *U* test for quantitative data.

The SIR of each cancer was calculated as the ratio of the observed to expected number of cases among relatives. Person-years were calculated from 20 years of age to the earliest cancer diagnosis or death. The expected number of cases was calculated as the sum of the products of the number of person-years for each 5-year age/sex group and the corresponding age/sex-specific incidence rates in Spanish regional registers.[15] The confidence limits were based on Byar's approximation of the exact Poisson distribution, which is accurate even with small numbers.[16] All reported *P* values are 2 sided, and $P < .05$ was considered significant. All calculations were performed using SPSS 19.0 software (Chicago, IL).

Results

A total of 1705 patients with CRC were included in the study. The median age was 71 years (range, 27–101 years), and 59% of patients were male. Sixteen patients were excluded because of discrepancies between the IHC and MSI analyses; no mutation was found in these pa-