Incidence of Microsatellite Instability in Synchronous Tumors of the Ovary and Endometrium

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ABSTRACT

Purpose: Families with hereditary nonpolyposis colorectal cancer (HNPCC) have an increased lifetime risk of endometrial (40%) and ovarian (10%) carcinomas. Endometrial and ovarian carcinomas from members of these families frequently display a mutator phenotype as manifest by high levels of microsatellite instability (MSI-H). Microsatellite instability (MSI) occurs in 17–32% of sporadic endometrial carcinomas and 3–17% of sporadic ovarian carcinomas. We hypothesized that there might be a higher rate of MSI in tumors from women with synchronous primary carcinomas of the ovary and endometrium.

Experimental Design: We identified 52 cases of synchronous tumors of the ovary and endometrium from the databases of four gynecological oncology units. Archival material and clinical data were available on 45 of these patients. We examined DNA extracted from ovarian and endometrial tumor tissue for MSI using DNA extracted from normal tissue of that patient as a germline DNA control. MSI was assessed using a panel of five standard microsatellite markers: D2S123, D5S346, D17S250, BAT25, and BAT26. MSI-H was defined by more than two markers being positive. Low-level MSI (MSI-L) was defined as one or two markers positive and microsatellite stable (MSS) was defined as no markers positive.

Results: The 45 patients had a median age at diagnosis of 53 years. Of a total of 134 samples analyzed, only three samples (3.3%) were MSI-H. No patient had high levels of MSI in both ovarian and endometrial tumors. One ovarian carcinoma had five of five markers positive with the corresponding endometrial carcinoma being MSI-L. Two endometrial carcinomas were MSI-H, and the corresponding ovarian carcinomas were MSI-L and MSS, respectively. Seven ovarian tumors and seven endometrial tumors were MSI-L. The majority of patients had early-stage ovarian carcinoma [International Federation of Gynecology and Obstetrics (FIGO) stage I, 44.4%; stage II, 26.7%; and stage III, 26.6%]. Eighty-two% of the endometrial primaries were FIGO stage I. Progression-free survival was significantly better for patients with synchronous primaries than those presenting with ovarian carcinoma alone [adjusted hazards ratio, 1.94; P = 0.023; 95% confidence interval, 1.096–3.44].

Conclusion: Synchronous primary carcinomas of the ovary and endometrium are unlikely to be part of the HNPCC syndrome unless the family history is in keeping with the modified Amsterdam criteria.

INTRODUCTION

MSI was first described in colorectal tumors from patients with HNPCC (1). In this syndrome, germline defects in DNA mismatch repair genes result in a tumor replication error repair (RER) phenotype that is detectable as generalized instability of short, tandemly repeated DNA sequences, known as microsatellites, which occur ubiquitously throughout the genome (2). MSI is caused by a failure of the DNA mismatch repair system to repair errors that occur during replication of DNA and is characterized by the accelerated accumulation of single nucleotide mutations and alteration in the length of microsatellite sequences (3). Families with HNPCC have an increased lifetime risk of colorectal cancer (80%; Ref. 4). In addition, family members have an increased lifetime risk of endometrial (40–60%), ovarian (10–12%), and other cancers (5). Individuals from families with HNPCC are more likely to have synchronous or metachronous second primary tumors and are more likely to develop tumors under the age of 50 years. MSI occurs in 90% of colorectal tumors from patients with HNPCC compared with 10–15% of sporadic colorectal tumors (2). MSI has also been demonstrated in 75% of endometrial and up to 100% of ovarian cancers from members of HNPCC families (6, 7).

MSI can be found in 17–32% of sporadic endometrial carcinomas, and several studies have found that it is more common among endometrial carcinomas with endometrioid histology (7–19). Previous authors found that MSI-H tended to occur in endometrial tumors from younger patients with poorer prognostic features (8, 9). Several authors had not found any

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2 The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; MSI-H, high levels of MSI; MSI-L, low levels of MSI; DFS, disease-free survival; MSS, microsatellite stable; HR, hazard ratio; FIGO, International Federation of Gynecology and Obstetrics; CI, confidence interval.
difference in prognosis based on tumor MSI status but recently Fiumicino et al. (8) found a significant association between the presence of tumor MSI and shorter DFS in stage I and II endometrial carcinoma. This is in contrast to the finding in patients with colorectal carcinoma in which the presence of MSI-H has been associated with prolonged survival independent of classic prognostic factors (2).

Ten to 17% of sporadic ovarian carcinomas display MSI but if the analysis is limited to the subset of endometrioid ovarian cancers (15–25% of ovarian cancers), then MSI is found in 30–50% (10, 21–24). Synchronous tumors of the ovary and endometrium account for 0.7% of gynecological malignancies (25). In studies of synchronous tumors of the ovary and endometrium, patients tend to be younger at diagnosis and have lower-grade and earlier-stage tumors than patients diagnosed with a single primary (25–27). Detection of these tumors at an earlier stage may be facilitated by the early symptoms from the endometrial tumor. Several studies have suggested that synchronous tumors have a better prognosis, but this may relate to the early detection and low grade of the malignancies (26–29).

There have been several papers investigating the use of genetic markers, such as loss of heterozygosity, for definitively diagnosing tumors as synchronous primaries rather than one being a metastasis of the other, but none of these has specifically addressed whether there is a common predisposing genetic event leading to the development of synchronous neoplasms (30, 31). Shenson et al. (31), in a previous study, found that two of seven synchronous primary tumors of the ovary and endometrium had MSI in both tumors and hypothesized a common predisposing event for both tumors. The synchronicity and young age at diagnosis make it plausible to consider that there may be some common (and possibly germ-line) predisposing genetic event. Families with HNPCC have a predisposition to synchronous tumors, tumors developing at a younger age and in the bowel, endometrium, and ovary, but little is known about whether the tumors of patients who develop synchronous ovarian and endometrial tumors have any of the hallmark molecular features of HNPCC. Lynch and Watson (32) have previously described a series of 80 patients with ovarian cancer from HNPCC families and found that 21.5% of them had a synchronous endometrial carcinoma at diagnosis. There are no published data on the frequency of MSI in patients who present with synchronous tumors of the endometrium and ovary.

The aim of our study was to determine whether MSI occurred more frequently in synchronous tumors of the ovary and endometrium than has been reported for either site as a single primary. We also sought to correlate the presence of MSI with the clinical features of these tumors. If DNA mismatch repair were indeed a predisposing event for these synchronous tumors, this might have implications for screening at other sites and for screening these patients for germ-line mutations in DNA mismatch repair genes.

MATERIALS AND METHODS

The databases of four gynecological oncology units were searched for patients who had been diagnosed with synchronous tumors of the ovary and endometrium. Fifty-two cases were found. Paraffin-embedded tumor tissue and clinical follow-up was available for 45 of these patients. Clinical data were collected for the 45 patients and entered into a database in a de-identified manner. All of the paraffin-embedded tumor material was de-identified before microsatellite testing, and laboratory personnel were blinded to the clinical details of the cases tested. The Western Sydney Area Health Service Research Ethics Committee approved the study.

Personal history of malignancy and family history of malignancy in first- and second-degree relatives was ascertained from patient records. No patient had a family history that met the modified Amsterdam criteria for the diagnosis of HNPPC (33), and no patient had a documented germ-line mutation predisposing them to malignancy. The median age of the patients at diagnosis was 53 years. The characteristics of the patients are listed in Table 1. Ovarian and endometrial tumors were staged according to International Federation of Gynecology and Obstetrics (FIGO) classification and were graded as: well- (G1), moderately (G2), or poorly (G3) differentiated; histological subtype was recorded. Staging and histological characteristics of the tumors are listed in Table 2. Information was collected on residual disease after primary surgery and any adjuvant radiotherapy or chemotherapy. DFS was calculated from the day of surgery until the date of clinical recurrence or the date of last follow-up. Overall survival was calculated from the date of surgery to the date of death or last follow-up. The median follow-up was 30 months (range, 4–107 months). Progression-free and overall survival was compared with that of a database of 345 patients with ovarian cancer diagnosed in the last 10 years.

DNA Preparation. Formalin-fixed paraffin-embedded archival specimens of ovarian tumor, endometrial tumor, and normal tissue (usually lymph node) were used for DNA extraction. DNA was extracted from representative 10-μm paraffin sections of each endometrial and ovarian carcinoma and from matched normal tissue from each subject.

Microsatellite Analysis. Microsatellite status was assessed by examining five independent genomic sites, including three dinucleotide repeat microsatellites and two mononucleotide repeat microsatellites (D2S123, DSS346, D17S250, BAT25, and BAT26) as recommended by the National Cancer Institute workshop on MSI (3). Microsatellite analysis was performed on 134 specimens from 45 patients. One patient did not have a normal tissue block available for a germ-line control.
Each reaction mixture consisted of H$_2$O (7.5 µl), 10X PCR buffer (1.25 µl), 25 mM MgCl$_2$ (1.25 µl), 2.5 mM dNTPs (0.5 µl), 1 µl of [γ-32P]-dATP end labeled forward primer and 0.25 µl reverse primer (20 µM) and AmpliTaq DNA polymerase (0.1 µl).

The PCR consisted of an initial denaturation step at 94°C for 4 min, followed by 25–35 cycles of denaturation at 94°C for 30–45 s, annealing at 54–64°C for 15–45 s, and elongation at 72°C for 30–45 s. Amplicons were separated on 5% denaturing gels.

Samples were considered to show MSI-H if more than two markers were positive. MSI-L was defined as one or two markers positive; tumors were MSS if no markers were positive. An example is shown in Fig. 1.

**Statistical Analysis.** Statistical analysis was performed using SPPS version 10.0 statistical software package. Multivariate analysis was performed by the Cox proportional hazards model (34). Survival of patients was compared with that of a database of 345 ovarian cancer patients adjusted for age and stage. Cox regression analysis was used to calculate the adjusted HR for mortality and progression of the 45 patients with synchronous primary tumors compared with the ovarian carcinoma patients.

**RESULTS**

Overall, one ovarian carcinoma and two endometrial carcinomas were MSI-H. One case showed instability at all five loci in the ovarian tumor and instability at two loci (BAT25, BAT26) in the corresponding endometrial tumor with no instability in the normal DNA control (see Table 3). One case showed instability at three of five loci (D2S123, D5S346, D17S250) in the endometrial tumor and was MSI-L (D17S250) in the ovarian tumor and MSS in the germ-line control. The third case showed instability at three loci (D2S123, D5S346, D17S250) in the endometrial tumor, with the ovarian tumor and the normal control being MSS. No patient had MSI-H in both ovarian and endometrial tumors. Only 2.2% of all ovarian tumors and 4.4% of all endometrial tumors studied had MSI-H. All of the normal DNA controls were MSS.

MSI-L were detected in 7 (15.6%) of 45 ovarian cancers and in 7 (15.6%) of 45 endometrial carcinomas (see Table 4). Two patients were MSI-L with both their ovarian and endometrial tumors being positive for the same markers suggesting a possibility that these tumors may have arisen from a single primary or a common genetic pathway. In four cases, the ovarian tumor had MSI-L, and the endometrial tumor was MSS. In four patients, the endometrial tumor had MSI-L, with the corresponding ovarian tumor having no MSI.

Median age at diagnosis for the 45 patients with synchronous tumors of the ovary and endometrium was 53 years. Four (8.8%) patients had a personal past or present history of malignancy (See Table 1). One patient was diagnosed with a synchronous bowel neoplasm at the time of gynecological surgery, although interestingly this patient had MSI-L in her endometrial tumor. Two patients were found to have colonic tubulovillous adenomas 2 months and 5 years after their surgery respectively and one of these patients had a single positive microsatellite marker (BAT26) in both the ovarian and endometrial tumor. One patient developed breast cancer 3 years after diagnosis. None of these patients had MSI-H in their ovarian or endometrial neoplasm.

Twelve patients (27%) had a documented family history of malignancy. No patient had a family history that met modified
Amsterdam criteria for HNPCC. Five patients had a first-degree relative who had breast cancer, with two of these occurring before the age of 45 years. Two patients had second-degree relatives with breast cancer, and one patient had both a first- and second-degree relative with breast cancer. One patient had a first-degree relative with bowel cancer and another had an affected second-degree relative. Interestingly, the patient who had MSI-H in her ovarian tumor and MSI-L in the endometrial tumor had a family history of both her mother and maternal grandmother developing endometrial carcinoma in their 50s. Aside from this, no other patient who had MSI-H or MSI-L in her tumors had a significant family history of malignancy.

The FIGO stage, histological type, and grade of the tumors are shown in Table 2. The stage distribution of ovarian tumors (stage I, 44.4%; stage II, 26.7%; stage III, 26.7%; and stage IV, 2.2%) shows a preponderance of early-stage tumors compared with population-based series. Eighty-two % of the endometrial tumors were stage I. Twenty-eight (62.2%) of the ovarian tumors and 39 (86.7%) of the endometrial tumors were of endometrioid histology. Fifty-eight % of cases had the same histological pattern in both the ovarian and endometrial tumors. The majority of the endometrial tumors (53.3%) and one-third of the ovarian tumors were well differentiated. Nineteen cases (42.2%) had the same histological grade of differentiation at both sites. Of the two endometrial tumors that were MSI-H, both were stage I, of endometrioid histology, and moderately well differentiated. The ovarian tumor with MSI-H was stage I, endometrioid, and moderately differentiated. Three of the four endometrial tumors with MSI-L were endometrioid and stage I, and the remaining one was serous papillary. Of the four ovarian tumors having MSI-L, three were low-grade endometrioid and one was mucinous. The two patients who had identical markers positive in both the ovarian and endometrial tumors had similar histology at both sites, one with mixed endometrioid/clear cell and the other with undifferentiated adenocarcinoma.

Thirty-two (71%) of the patients had platinum-based chemotherapy after their primary surgery, despite most of these tumors being stage I. Nineteen (42.2%) patients had adjuvant radiotherapy after their surgery. The median follow-up for the 45 patients is 30 months. Thirty-six (80%) of the patients are still alive and 32 are disease-free. The median disease-free and overall survival has not yet been reached. Of the patients who had MSI-H tumors, two are alive and disease-free at 4 and 6 1/2 years after diagnosis, respectively; one patient relapsed 12 months after diagnosis and died 6 months later.

We compared the survival of the 45 patients who had synchronous primary tumors with a database of 345 patients with ovarian carcinoma diagnosed over the last decade in our institution. Progression-free survival was significantly longer for patients with synchronous primaries than with ovarian cancer alone (HR, 3.19; 95% CI, 1.78–5.46; P < 0.001). This effect persisted when adjusted for the earlier-stage distribution of the patients with synchronous primaries (adjusted HR, 1.94; P = 0.023; 95% CI, 1.096–3.44). There was a trend toward better overall survival in the patients with synchronous primary tumors (HR, 3.19; P = 0.001; 95% CI, 1.63–6.23). This trend did not reach statistical significance when adjusted for stage (adjusted HR = 1.89; P = 0.69; 95% CI, 0.95–3.74).

**DISCUSSION**

In our series of 45 patients with synchronous tumors of the ovary and endometrium only one (2.2%) of the ovarian carcinomas and two (4.4%) of the endometrial carcinomas were MSI-H. All of the normal controls were MSS. This is in contrast to the 10–17% of sporadic ovarian carcinomas and 17–32% of sporadic endometrial carcinomas that are reported to display MSI (7–24). Compared with historical controls there is lower frequency of MSI in patients who develop synchronous tumors of the female genital tract.

Shenson et al. (31) studied seven cases of synchronous ovarian and endometrial carcinoma and found that two of the cases had MSI-H in both the ovarian and endometrial cancer. Shenson et al. hypothesized a possible common genetic predisposing event relating to DNA mismatch repair. In our study, we have found that the incidence of MSI is, in fact, lower than expected for sporadic primary carcinomas of the ovary and endometrium. This suggests that, if there is a common genetic pathway for the development of these synchronous tumors, it is
unrelated to the DNA mismatch repair system. The present study suggests that patients who develop synchronous tumors of the ovary and endometrium are unlikely to be part of a HNPCC syndrome in the absence of a family history that meets the modified Amsterdam criteria.

A previous report found, in a series of synchronous tumors of the ovary and endometrium, that patients are likely to be younger at diagnosis, to have lower-stage and lower-grade tumors, and to have a better prognosis (25–29, 35). These findings were all confirmed in our study. Patients were diagnosed at a median age of 52 years, which is younger than usually reported for patients with ovarian or endometrial carcinoma as a single primary. The stage distribution for both endometrial carcinoma (82.2% stage I) and ovarian carcinoma (44.4% stage I) confirms a trend for synchronous tumors to be diagnosed at an earlier stage. The majority of both endometrial (84.4%) and ovarian (71%) carcinomas in this series were well or moderately differentiated. There were very few high-grade tumors in this series. This is in keeping with the finding that the majority of synchronous tumors in the female genital tract are low grade (25–27, 35). There is also another interesting implication of this finding. In studies of sporadic endometrial and ovarian carcinoma, a significant association has been found between the presence of MSI and poor histological grade (8, 9). Perhaps the paucity of high-grade tumors in this series provides an explanation for the lower frequency of MSI.

Endometrioid histology is significantly more common among synchronous ovarian and endometrial carcinomas (25, 26, 31, 35). In the largest reported series of synchronous ovarian and endometrial carcinomas, Zaino et al. (35) reported that 86% of patients had endometrioid carcinomas in both the ovary and endometrium. Eighty-seven percent of the endometrial and 62% of the ovarian tumors in the current series were of endometrioid histology. Twenty-six patients had endometrioid histology at both sites, which is similar to a previous series of synchronous tumors, in which up to 88% of patients had endometrioid histology at both sites (26, 28, 35). Studies of sporadic endometrial carcinoma have shown that MSI is more likely to be present if the tumor is of endometrioid histology. Fujita et al. (30) found that MSI occurred in 17% of all endometrial carcinomas but up to 50% of those with endometrioid histology. MSI is also more likely to occur in ovarian carcinoma of endometrioid histology (up to 33%; Ref. 24). The fact that this series with its preponderance of endometrioid histology shows lower frequency of MSI than expected lends further credence to the hypothesis that there is some independent molecular pathway involved in synchronous tumors.

Previous reports of series of synchronous tumors of the ovary and endometrium have observed better prognoses than expected, with 5-year survivals of up to 85% (25–29, 35). They suggest that survival is correlated with the stage of the individual tumors and that the presence of a second primary does not adversely affect prognosis. These findings are also confirmed in our series. Significantly better progression-free survival and a trend toward better overall survival after adjusting for stage would suggest that patients developing synchronous tumors might have a better prognosis than that of patients diagnosed with ovarian carcinoma alone. Seventy-one percent of the patients received adjuvant platinum-based chemotherapy. This would seem unusual in a series in which 44.4% of the ovarian tumors and 82.2% of the endometrial tumors were stage I. It is possible that some patients were treated more aggressively in the adjuvant setting because of their younger age and the presence of two primaries. This may be contributing to a survival advantage. Only three patients had MSI-H in either of their tumors, and no patient was MSI-H in both. Because of the small numbers it is difficult to correlate the presence of MSI with the features and clinical behavior of the tumors. Recent work with sporadic stage I and II endometrial carcinomas showed that the presence of MSI was a poor prognostic factor in multivariate analysis (8). Too few patients displayed MSI in this series to make any confident statements about the prognostic implications of MSI in synchronous primary carcinomas of the ovary and endometrium.

When patients are found to have two tumors, problems invariably arise as to whether these represent independently arising primaries or metastases of a single primary. A number of authors have sought to find molecular markers that would assist in making this distinction (30, 31). It had been suggested that MSI analysis might be a method of determining synchronicity of the tumors rather than metastases of a single primary. The very low frequency of MSI among this large series of synchronous tumors suggests that MSI testing will not be useful for this purpose.

Our study suggests that MSI is not a common feature among synchronous neoplasms of the ovary and endometrium. These tumors are unlikely to arise as part of a syndrome of defective DNA mismatch repair. There is likely to be an independent pathway of genetic evolution for synchronous tumors of the female genital tract.

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REFERENCES


