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Risk Factors for Ovarian Cancers with and without Microsatellite Instability

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Abstract

Objective—In a population-based sample of epithelial ovarian cancers, to evaluate the association between microsatellite instability status and: 1) Ovarian cancer risk factors and 2) The distribution of the specific histologic subtypes.

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Patients and methods—Participants were drawn from three population-based studies of primary epithelial ovarian cancer: Tumor DNA was analyzed using five standardized microsatellite markers to assess microsatellite instability (MSI) status. Patients were divided into three groups (MSI-high, MSI-low and MSI-stable) according to NCI criteria. We compared the prevalence of specific known risk and protective factors among the three subgroups, including BMI, smoking history, parity, *BRCA1* and *BRCA2* mutation status, past oral contraceptive use, and tubal ligation. Similarly, we compared the distribution of the histologic subtypes among the three subgroups.

Results—A total of 917 ovarian cancer patients were included. One hundred twenty seven (13.8%) cancers were MSI-high. Subgroup analyses according to smoking, BMI, parity, past oral contraceptive use and past tubal ligation did not reveal any statistically significant differences among the groups. Among the 29 patients with *BRCA1* mutations, 20.7% had MSI-high cancers compared with 5.9% among 17 *BRCA2*-mutation patients. The proportions of different ovarian cancer histologies among the various microsatellite instability subgroups were similar.

Conclusions—The prevalence of risk and protective factors among ovarian cancer patients is similar for cancers with and without microsatellite instability. The distributions of microsatellite instability do not differ significantly among ovarian cancers with different histologies. Ovarian cancer patients with *BRCA1* mutations had a 21% rate of MSI-high tumors, compared to 6% among patients with *BRCA2* mutations, but this difference was not statistically significant.

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic malignancies. Worldwide in 2008, approximately 225,000 women were diagnosed with ovarian cancer and 140,000 died from it [1]. Approximately 15% of invasive ovarian cancers are caused by hereditary susceptibilities. Germline mutations in *BRCA1* or *BRCA2* account for 13% of all cases [2]. Lynch syndrome (hereditary non-polyposis colon cancer) is a third cause of hereditary ovarian cancer and germline mutations in the relevant genes (*MLH1*, *MSH2*, *MSH6*) account for about 1% of ovarian cancers [3]. The lifetime risk of ovarian cancer in the general population is about 1.4%, compared to 3-14% in Lynch syndrome families and up to 44% in *BRCA* carriers [2,3].

Germline mutations in the genes that underlie Lynch syndrome result in genetic instability as a consequence of deficiency in the mismatch repair (MMR) pathway. MMR deficiency is a cancer-initiating pathway for several sites, including colon, uterus and ovaries [4] and may arise from inherited or somatic mutations in the MMR genes. The classical expression of MMR is Micro-satellite Instability (MSI). Micro-satellites are widely distributed repetitive DNA sequences composed of short, tandem repeated nucleotide motifs. In some neoplasms, these sequences exhibit a form of genetic instability characterized by the gain or loss of repeat units at multiple independent loci (microsatellite instability, MSI). Such alterations have been observed to accumulate in cells defective for DNA repair activities [5,6] and occur with high frequency in association with Lynch syndrome. MSI has also been observed in cancers not involving inherited mutations, including those of the colon, endometrium, stomach, pancreas, and ovary [7]. Microsatellite instability occurs in 7-22% of sporadic ovarian carcinomas [8]. According to the NCI definition, tumors are classified as high-frequency MSI (MSI-high) if two or more of the five NCI markers show instability or if 30% or more of all markers tested demonstrate instability. Tumors are characterized as intermediate or low-frequency MSI (MSI-low) if only one of the five NCI markers show instability or if <30% of all of the markers do so. If no marker shows instability the tumor is considered to be microsatellite stable (MSI-stable) [9].

Similarities of the etiology of ovarian cancers across MSI status are unknown. A number of risk factors for ovarian cancer have been identified, including hormone replacement therapy (HRT), endometriosis [10] and obesity [11]. Other factors are protective and include oral contraceptive use [12], tubal ligation [13], breast feeding [14], and parity [15]. The objective of this study was to evaluate whether ovarian cancer patients with MSI-high cancers have the same risk and protective factors as patients with MSI-low and MSI-stable ovarian cancers. A secondary objective was to evaluate the distribution of the specific histologic subtypes of ovarian cancer among MSI-high, MSI-low and MSI stable ovarian cancer patients.

METHODS

Participant Population

Participants for the current study were drawn from three population-based studies of cases of primary invasive epithelial ovarian cancer: the Familial Ovarian Tumor Study (FOTS) in Ontario at the University of Toronto, the Tampa Bay Ovarian Cancer Study (TBOCS) at the Moffitt Cancer Center, and the North Carolina Ovarian Cancer Study (NCOCS) at Duke University. Details about study design, populations, and data collection methods have been published previously.[16-18]

The study protocol was approved by the institutional review board at each study site, and written informed consent was obtained from all subjects prior to participation. Inclusion criteria for study enrolment involved diagnosis of newly incident, pathologically confirmed primary epithelial ovarian cancer, either borderline or invasive, age 20 years or above, and residence in the defined study geographic area. All women completed risk factor questionnaires at study entry. We evaluated the prevalence of specific known risk and protective factors among the study population, including adult BMI, smoking history, parity, *BRCA1* and *BRCA2* status, past oral contraceptive use and tubal ligation.

Microsatellite instability (MSI) Testing

Tumor-extracted DNA from deparaffinized cells was analyzed by polymerase chain reaction (PCR), using the five standardized microsatellite markers developed by the National Cancer Institute (NCI) for colorectal cancers[9], with germline DNA used as normal control DNA. The standardized markers consisted of two mononucleotide repeats (Bat25 and Bat26) and three dinucleotide repeats (D2S123, D5S346 and D17S250). As stated earlier, tumors were classified as demonstrating high microsatellite instability (MSI-high) if two or more of the five markers were positive for shifts in the allelic bands; if one of the markers was positive tumors were classified as MSI-low; if no marker was positive, tumors were considered MSI-stable.

BRCA1 and BRCA2 testing

Mutation detection and classification was performed using a range of techniques, but all suspected nucleotide alterations were confirmed by direct sequencing of DNA [17].

Data analyses

Prevalence of each risk factor was calculated for each MSI group. Comparisons between groups according to MSI status were analyzed using descriptive statistics, with chi-square values for comparing proportions between independent groups of categorical variables. All reported P-values are two-sided. All analyses were carried out with SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Case characteristics

In total, 917 ovarian cancer patients were included, among whom the mean age at ovarian cancer diagnosis was 56.3 years. Of cancers in these individuals, 127 (13.8%) were MSI-high, 221 (24.1%) were MSI low and 569 (62.0%) were MSI-stable.

Histologic subtypes

Among 127 MSI-high ovarian cancers, 54.0% were classified as serous, compared to 59.7% serous among MSI-low and 53.3% among MSI-stable cancers. Of the 127 MSI-high, 15.7% were endometrioid type, compared to 16.3% endometrioid among MSI-low and 16.1% endometrioid among MSI-stable. The proportions of mucinous type were 13.3% among MSI-high, 6.3% among MSI-low and 11.5% among MSI-stable tumors. Lastly, among MSI-high cancers, 4.7% were clear cell type compared to 5.9% clear cell among MSI-low and 7% clear cell among MSI-stable. No statistically significant difference was observed between the three groups in terms of histology (Table 1).

Breast-ovarian cancer syndrome (BRCA)

We evaluated *BRCA1* and *BRCA2* mutation carrier status. A total of 438 patients underwent genetic testing. Twenty nine patients were identified as *BRCA1* mutation carriers and 17 patients were *BRCA2* mutation carriers. Among the 29 *BRCA1* carriers, 20.7% had MSI-high cancers compared with 5.9% MSI-high cancers among 17 *BRCA2* carriers and 11.7% MSI-high cancers among 392 patients who were negative for these mutations (Table 1). The proportions of *BRCA1* patients who were either MSI-high or MSI-low was 55.1%, while the proportion of patients who were negative for either mutation who were MSI-high or MSI-low was 38.5% ($p=0.08$).

Risk factors analyses

Among patients with MSI-high tumors, 10% identified themselves as current smokers; among those with MSI-low, 14.3% were current smokers, and of those with MSI-stable, 15.6% were current smokers. The rate of past smoking for MSI-high cancer patients was 39.2% compared to 32.4% among MSI-low cancer patients and 34.9% for MSI-stable cancer patients. The percentages of never smoking among MSI-high, low and stable cancer patients were 50.8%, 53.3%, and 49.5% respectively. No statistically significant difference was observed among the groups with regard to smoking (Table 2).

Subgroup analyses according to adult BMI did not show any statistically significant differences. Four hundred seventeen (46.8%) patients had a BMI below 25, 262 (29.4%) had a BMI of 25-30 and 215 (24.0%) had a BMI above 30. The mean BMI among MSI-high patients was 26.4, compared to 25.5 among MSI-low patients and 26.8 was among MSI-stable patients. If obesity is defined as a BMI of 30 or greater, the proportions of obese patients were 25.2% among patients with MSI-high, 17.8% among MSI-low and 26.3% among MSI-stable cancers (Table 2).

Ninety one (9.9%) patients were nulliparous and 826 (90.1%) parous. Mean parity was 2.5. Among 116 individuals with MSI-high tumors, 12.1% were nulliparous, compared to 9.7% among MSI-low and 11.1% among MSI-stable. The mean parity was 2.7 among patients with MSI-high cancers, 2.7 among MSI-low, and 2.4 among MSI-stable (Table 2).

Five hundred thirty four women (58.2%) reported having ever used oral contraceptives, 58.8% among patients with MSI-high cancers, 56.7% among MSI-low and 64.1% among MSI-stable. Two hundred six women reported having undergone past tubal ligation; the

rates among patients with MSI-high cancers were 22.6% compared to 22.0% among MSI-low and 23.7% among MSI-stable (Table 2).

Discussion

In the current study, we sought to evaluate risk factor distributions among ovarian cancer patients with MSI-high, MSI-low and MSI-stable tumors. Our findings indicate that there are no statistically significant differences in the prevalence of any of the considered risk factors (obesity, low parity and smoking) or any of the protective factors (history of oral contraceptive use, tubal ligation) for ovarian malignancy.

Pathogenic mechanisms that explain the link between many of the risk factors and development of epithelial ovarian carcinoma have not been determined. However, several reproduction and non-reproduction risk factors have been linked to the development of this disease. Traditionally, two main hypotheses have been proposed: incessant ovulation-repeated ovulations that result in minor trauma to the ovarian epithelium, in turn the repeated repair leading to malignant transformation, and excess gonadotropins-persistent ovarian exposure to the resulting elevated estradiol concentrations which may be carcinogenic.

With regard to reproduction, risk of ovarian cancer is increased in women with primary infertility, and reduced in women that have taken oral contraceptives or are multiparous. The European Prospective Investigation into Cancer found a significantly decreased risk in parous versus nulliparous women (Relative Risk [RR] 0.71, 95% CI 0.59-0.87), and among women with at least one full term pregnancy, the risk of ovarian cancer decreased by 8 percent for each additional pregnancy (95% CI 0.85-0.99) [19]. The number of full-term births seems most influential, but several studies have found lesser but significant decreased risks associated with an increasing number of incomplete pregnancies [20, 21]. In our study the distributions of reproductive risk factors parity and oral contraceptive use among MSI-high, low and stable were similar.

Non-reproduction risk factors for ovarian cancer have also been examined. Cigarette smoking has been observed to be associated with mucinous but not serous, endometrioid or clear cell cancer [22]. In our study no statistically differences in the prevalence of smoking among MSI-high, low and stable were noticed.

High body mass index (BMI) appears to increase ovarian cancer risk. A systematic review of 28 studies reported a small, but statistically significant, association between obesity (BMI 30 kg/m² or more) and risk of ovarian cancer (Odds Ratio [OR] 1.3, 95% CI 1.1-1.5) [23]. This association may be stronger for endometrioid cancer than serous cancer.

Protective factors have been implicated as well in the pathogenesis of ovarian cancer. We have discussed above the reduced risk according to parity. For oral contraceptives, studies have consistently shown that prolonged use reduces the risk of ovarian cancer. An analysis of 45 epidemiological studies from 21 countries found that, compared with women who had never used OCs, any use of OCs was associated with a statistically significant reduction in risk (RR 0.73, 95% CI 0.70-0.76) [12]. Women with a history of tubal ligation had a reduction in ovarian cancer risk (RR 0.69, 95% CI 0.64-0.75) in a meta-analysis of 13 case-control studies [24]. Various risk factors have also been examined in the context of specific high risk populations. Our group previously evaluated the effect of parity, breastfeeding and use of oral contraceptives on the risk of ovarian cancer in women who carry mutations in the *BRCA1* and *BRCA2* genes. Although parity has a known protective effect on sporadic ovarian cancer, it has also been observed to be associated with reduced risk among carriers

of *BRCA1* mutations (RR 0.67, 95% CI 0.46–0.96), but with increased risk among those with *BRCA2* mutations (RR 2.74, 95% CI 1.18–6.41) [25].

In specific high risk groups, risks may be modified by other risk factors, as shown by Moorman and colleagues who found that risk of breast cancer among *BRCA1* or *BRCA2* carriers was modified by reproduction characteristics and alcohol use [26]. Therefore, we hypothesized that MSI-high tumor status might represent a specific case population that harbors distinct risks of ovarian cancer associated with the various risk and protective factors. However, among ovarian cancers, the distributions with and without microsatellite instability were similar.

We also did subgroup analyses of the specific histologic types among MSI-high, MSI-low and MSI-stable tumors, and did not find differences in the distributions. In our current study the fraction of serous histologies was 54.0% among MSI-high cancers compared to 59.7% and 53.3% among MSI-low and MSI-stable, respectively, which is comparable to the known distributions of histologic types among sporadic ovarian cancer: serous histology accounts for as much as 70% of ovarian cancer, mucinous and endometrioid tumors are less common (about 10-15% each), followed by clear cell tumors, Brenner and transitional cell tumors, and undifferentiated carcinomas [27].

A previous study examined histological types of ovarian carcinoma and microsatellite status, but was much too small and underpowered to draw adequate conclusions [28]. King and colleagues performed a polymerase chain reaction-based microsatellite analysis of DNA extracted from neoplastic and non-neoplastic tissues of 41 ovarian cancer patients, seven of which had microsatellite alterations. Twenty-four of the 41 epithelial ovarian neoplasms were classified as serous adenocarcinomas. Only two of these (2/24 or 8%) were found to have microsatellite alterations. The remaining five tumors in which microsatellite instability was observed were classified as endometrioid carcinomas (2), mixed serous and mucinous carcinomas (1), malignant mixed Mullerian tumor (1) and immature teratoma (1) [29]. Murphy et al. in their review on frequency of mismatch repair deficiency in ovarian cancer also evaluated MSI frequency in ovarian cancer subtypes. They concluded that among the different subtypes the frequency of MSI was the same [30].

In a meta-analysis done to estimate the frequency of MMR phenotype in unselected ovarian cancers and in various histologic subtypes, MSI-high was seen in approximately 12%. MMR-deficient ovarian cancers also seem to be characterized by an overrepresentation of non-serous histologic subtypes [8]. While non-serous histologies appear to be associated with MMR deficiency, when using MSI-high as a surrogate for MMR deficiency no overrepresentation of non-serous histologies is seen.

Finally, our previous study exploring the association between Lynch syndrome germline mutations and ovarian cancer histology did find overrepresentation of cancers with non-serous histologies in syndrome-positive patients, yet this group of patients may differ from those of the current study, in whom not all of Lynch syndrome germline mutations were confirmed [31]. *BRCA1* and *BRCA2* are tumor suppressor genes and play a number of roles in the maintenance of genome integrity; they are involved in repair of double-strand DNA breaks, control of cell cycle checkpoint responses, and chromosomal segregation. The association between MSI and the *BRCA* genes has not been reported previously. Evaluating the association between *BRCA1* and *BRCA2* status and MSI, we found the rate of MSI-high as 20.7% among carriers of *BRCA1* mutations and 11.7% among non-carriers. Our numbers of carrier subjects were small, however.

A limitation of this study includes the possibility of misclassification of MSI status. This would likely be non-differential according to the epidemiologic risk factors, which would be

expected to lead to attenuation in the associations that may exist, and to reduce power to detect associations.

To summarize, in the current study we found similar distributions of risk and protective factors among MSI-high, MSI-low and MSI-stable tumor groups. To our knowledge, this is the first study to evaluate risk factors for ovarian cancer according to MSI test result. We also found that distributions of specific histologic groups of invasive ovarian cancer are the same among the three MSI groups. The increased fraction of MSI-high tumors among 29 *BRCA1* mutation carriers (20.7%) needs to be reevaluated in larger *BRCA1*-carrier populations.

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Table 1

Histology and BRCA mutations distributions according to MSI status, among ovarian cancer patients.

		MSI status						p-value
		High		Low		Stable		
Age at diagnosis		55.1		56.8		53.7		
Histology	Serous	69	13.7%	132	26.2%	303	60.1%	0.48
	Mucinous	17	17.5%	14	14.4%	66	68.0%	
	Endometrioid	20	13.5%	36	24.3%	92	62.2%	
	Clear cell	6	10.1%	13	22.0%	40	67.8%	
	Other	15	13.8%	26	23.8%	68	62.4%	
		127	13.8%	221	24.1%	569	62.1%	
BRCA	<i>BRCA1</i>	6	20.6%	10	34.5%	13	44.8%	0.34
	<i>BRCA2</i>	1	5.9%	4	23.5%	12	70.6%	
	Either mutation	7	15.2%	14	30.4%	25	54.3%	
	Negative for mutation	46	11.7%	105	26.8%	241	61.4%	
	Total	53	12.1%	119	27.2%	266	60.7%	

Table 2

Distribution of Risk factors by MSI status among ovarian cancer patients

Risk factor- non reproductive		MSI status						p- value
		High		Low		Stable		
Smoking	Current	12	10.0%	30	14.3%	86	15.6%	0.46
	Past	47	39.2%	68	32.4%	192	34.9%	
	Never	61	50.8%	112	53.3%	272	49.5%	
	Total	120		210		550		
Adult BMI	<25	61	48.0%	112	52.0%	244	44.2%	0.16
	25-30	34	26.7%	65	30.2%	163	29.5%	
	>30	32	25.2%	38	17.8%	145	26.3%	
	Mean BMI	26.4		25.5		26.86		0.11
	Total	123		215		552		
Risk factorrepr- oductive								
Parity	0	14	12.1%	19	9.7%	58	11.1%	0.15
	Mean Parity	2.6		2.7		2.4		
	Total	116		195		524		
Past oral contraceptive use	Yes	70	58.8%	119	56.7%	345	64.1%	0.10
	No	49	41.2%	91	43.3%	193	35.9%	
	Average duration of use in months	49 (SD=64)		43 (SD=69)		42 (SD=65)		0.79
	Total	119		210		538		
Past tubal ligation	Yes	28	22.6%	47	22.0%	131	23.7%	0.81
	No	96	77.4%	167	78.0%	422	76.3%	
	Total	124		214		553		