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Association Between IHC and MSI Testing to Identify Mismatch Repair–Deficient Patients with Ovarian Cancer

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Objective: In epithelial ovarian cancer, concordance between results of microsatellite instability (MSI) and immunohistochemical (IHC) testing has not been demonstrated. This study evaluated the association of MSI-high (MSI-H) status with loss of expression (LoE) of mismatch repair (MMR) proteins on IHC and assessed for potential factors affecting the strength of the association. *Methods:* Tumor specimens from three population-based studies of epithelial ovarian cancer were stained for MMR proteins through manual or automated methods, and results were interpreted by one of two pathologists. Tumor and germline DNA was extracted and MSI testing performed. Multivariable logistic regression models were fitted to predict loss of IHC expression based on MSI status after adjusting for staining method and reading pathologist. *Results:* Of 834 cases, 564 (67.6%) were concordant; 41 were classified as MSI-H with LoE and 523 as microsatellite stable (MSS) with no LoE. Of the 270 discordant cases, 83 were MSI-H with no LoE and 187 were MSS with LoE. Both IHC staining method and reading pathologist were strongly associated with discordant results. *Conclusions:* Lack of concordance in the current study may be related to inconsistencies in IHC testing methods and interpretation. Results support the need for validation studies before routine screening of ovarian tumors is implemented in clinical practice for the purpose of identifying Lynch syndrome.

Introduction

Ovarian cancer is the fifth most common cancer in terms of incidence and cancer mortality in U.S. women (American Cancer Society 2013). Over 90% of ovarian cancers are of the ''epithelial'' cell type. Currently the classification of epithelial ovarian cancers relies primarily on pathologic and clinical factors that are insufficient to reveal the complex molecular cascade of events that drives the clinical behavior of tumors.

The mismatch repair (MMR) pathway is one of the best-defined molecular pathways involved in both inherited (Leach *et al.*, 1993; Bronner *et al.*, 1994; Papadopoulos *et al.*, 1994; Akiyama *et al.*, 1997; Miyaki *et al.*, 1997) and sporadic (Gras *et al.*, 2001; Strathdee *et al.*, 2001; Geisler *et al.*, 2003) cancer pathogenesis. Defects in the MMR pathway may be an important etiologic factor in an estimated 10%–12% of unselected ovarian cancers (Pal *et al.*, 2008a; Murphy and Wentzensen, 2011). Detection of MMR deficiency in tumors may help to select patients for germline testing for Lynch syndrome (Pal *et al.*, 2008b), a hereditary cancer syndrome that confers increased risks for colorectal, ovarian, endometrial, and several other types of cancer (Engel *et al.*, 2012; Pande *et al.*, 2012; Win *et al.*, 2012).

MMR-deficient tumors are typically identified through (1) immunohistochemistry (IHC) to detect loss of protein expression for one or more MMR proteins (i.e., MLH1, MSH2, MSH6, PMS2) and (2) microsatellite instability (MSI) testing to identify MSI-high (MSI-H) tumors. Although analytical validity, clinical validity, and clinical utility of IHC and MSI testing have been demonstrated for colorectal (Evaluation of Genomic Applications in Practice and Prevention

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Working Group 2009), validity of these tests in ovarian tumors remains uncertain (Aysal *et al.*, 2012; Pal *et al.*, 2012). Concordance rates of greater than 96% between IHC and MSI were demonstrated in two large studies of primarily colorectal tumors (Cicek *et al.*, 2011; Bartley *et al.*, 2012). The objective of the current study was to evaluate concordance between IHC and MSI in a population-based multicenter study of epithelial ovarian cancer.

Materials and Methods

Participant population and analytical samples

Participants for the current study were drawn from three population-based studies of confirmed primary epithelial ovarian cancer: the Familial Ovarian Tumor Study (FOTS) in Toronto, Canada (Risch *et al.*, 2001), the Tampa Bay Ovarian Cancer Study (TBOCS) at the Moffitt Cancer Center in Florida (Pal *et al.*, 2005), and the North Carolina Ovarian Cancer Study (NCOCS) at Duke University in North Carolina (Wenham *et al.*, 2003). Details about study design, populations, and data collection methods have been published previously (Risch *et al.*, 2001; Wenham *et al.*, 2003; Pal *et al.*, 2005). The institutional review board at each study site approved the study protocol, and all participants provided written informed consent.

A total of 979 IHC testing samples (27%, 22%, and 50% from NCOCS, TBOCS, and FOTS, respectively) and 979 MSI samples were obtained (31%, 22%, and 47%). We excluded 134 participants because MSI results were not obtained for at least three microsatellite markers or IHC results were not obtained for one or more of the three proteins (MLH1, MSH2, and MSH6). Thus, the analysis is based on 834 participants (Table 1).

Table 1. Analytical Samples by Study Design, Staining Method, and Pathologist

Variable	*Samples with both IHC and MSI* (n = *834*)	*IHC samples by slide type*	
		TMA slides (n = *387*)	*Full-section slides* (n = *447*)
Study site			
North Carolina	251 (30.1)	0 (0.0)	251 (56.2)
Tampa Bay, FL	207 (24.8)	203 (52.5)	4 (0.9)
Toronto, Canada	376 (45.1)	184 (47.5)	192 (43.0)
Staining method			
Automated	306 (36.7)	0 (0.0)	306 (68.5)
Manual	528 (63.3)	387 (100)	141 (31.5)
Pathologist			
A	381 (45.7)	203 (52.5)	178 (39.8)
B	453 (54.3)	184 (47.5)	269 (60.2)
Pathologist staining method			
A			
Automated	37 (4.4)	0 (0.0)	37 (8.3)
Manual	344 (41.2)	203 (52.5)	141 (31.5)
B			
Automated	269 (32.3)	0 (0.0)	269 (60.2)
Manual	184 (22.1)	184 (47.5)	0 (0.0)

Values are expressed as number (percentage).
IHC, immunohistochemical testing; MSI, microsatellite instability testing; TMA, tissue microarray.

MSI testing

Tumor-extracted DNA from deparaffinized cells was analyzed by polymerase chain reaction by using the five standardized microsatellite markers (Bat25, Bat26, D2S123, D5S346, and D17S250) developed by the National Cancer Institute for colorectal cancers (Boland *et al.*, 1998) with germline DNA used as the normal control DNA. Tumors were classified as MSI-H if two or more of the five biomarkers were positive for shifts in the allelic bands; in all other instances, tumors were considered microsatellite stable (MSS).

Immunohistochemistry for MMR proteins and staining process

Staining for expression of 3 MMR proteins (i.e., MLH1, MSH2, and MSH6) was performed using archived, formalin-fixed, paraffin-embedded epithelial ovarian cancer tissue blocks that had been stored at room temperature. Each tumor block underwent one of two types of tissue preparation: full-section or tissue microarray (TMA). TMA included three cores per case and were constructed as described previously (Coppola *et al.*, 2012). All TMAs ($n = 387$) were stained at a single laboratory by using a manual method described previously (Coppola *et al.*, 2012). Full-section slides were stained by using one of two methods: (1) the same manual procedure at the same laboratory where TMAs were stained ($n = 141$ full-section slides) or (2) an automated procedure at a second laboratory ($n = 306$ full-section slides).

For the automated staining method, full section slides were dewaxed by heating at 55°C for 30 min and by three washes, 5 min each with xylene. Tissues were rehydrated by a series of 5-min washes in 100%, 95%, and 80% ethanol and distilled water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 20 min. After blocking with universal blocking serum (Ventana Medical Systems Inc., Tucson, AZ) for 30 min, the samples were incubated with the primary antibody at 4°C overnight. The mouse monoclonal antibodies used included anti-hMLH1 (clone G168-15 [BioCare Medical, Concord CA], dilution 1:40), anti-hMSH2 (clone FE11 [Zymed/Invitrogen, Carlsbad, CA] dilution 1:200), anti-hMSH6 (clone BC/44 [BioCare Medical]; dilution 1:70). The samples were then incubated with biotin-labeled secondary antibody and streptavidin-horseradish peroxidase for 30 min each (Ventana Medical Systems Inc., Tucson, AZ). The slides were developed with 3,3̀- diaminobenzidine tetrahydrochloride substrate (Ventana Medical Systems Inc.) and counterstained with hematoxylin (Ventana Medical Systems Inc.). The tissue samples were dehydrated and coverslipped, and standard cell conditioning following the Ventana proprietary recommendations was used for antigen retrieval.

IHC slides were scored by one of two senior board-certified pathologists (referred to here as pathologist A and pathologist B), both of whom have over 20 years of experience in IHC methods. The expression reactions were scored into four grades, according to the intensity of the staining: 0, 1, 2 and 3. The percentages of positive cells were also scored

into four categories: 0 (0%), 1 (1–33%), 2 (34–66%), and 3 (67–100%). The product of the intensity and percentage scores was then used as the final score and classified as follows: 0, absent; 1–3, weak; 4–6, moderate; and 7–9, strong. For full-section slides, any value of ≤3 was categorized as having loss of expression (LoE) of MMR protein. For TMA slides, an average score of ≤3 from the three cores was categorized as having LoE. The IHC status, as the final outcome variable, was then defined as LoE of any one of the three (MLH1, MSH2, and MSH6) proteins.

Table 1 summarizes the number of IHC slides read by each of the two pathologists per slide type (TMA versus full section) and staining method (automated versus manual). Approximately 37% of samples were stained by an automated method, and pathologist A evaluated 46% of all samples. Notably, all TMA slides were manually stained, with slightly more than half being read by pathologist A. For the full-section slides, pathologist A read all of the 141 manually stained slides but only 12.1% of the 306 automatically stained slides.

Data analysis

Data were summarized by slide type (full-section versus TMA) using descriptive statistics. The 95% confidence interval (CI) for binomial proportion was calculated on the basis of the exact binomial distribution. The frequency and percentage of tumor samples with concordant and discordant MMR status based on IHC and MSI results were calculated overall, by specimen type, by staining method, and by pathologist. The extent of concordance was tested using Cohen κ correlation coefficient (κ) with its 95% CI, the maximum value κ (κ_{max}) given the observed distribution, and exact *p* value. Multivariable logistic regression models for IHC loss of expression were fit with MSI status as the predictor of interest stratifying by slide type (full-section versus TMA) and adjusting for staining method (manual versus automated) and/or pathologist (A versus B). All statistical tests were two-sided, and statistical significance was set at a *p* value of 0.05. Analysis was conducted by using SAS software, version 9.3 (SAS Institute, Cary, NC), and R 2.15.1 (http://cran.r-project.org/src/base/R-2/).

Results

Demographic and clinical characteristics of participants are presented in Table 2. Women ranged in age from 20 to 80 years, with a mean ± standard deviation of 56 ± 11.8 years; 92% of patients were white. Stage 1 or 2 cancer was diagnosed in approximately one third, and almost 40% of tumors had nonserous histologic features. Patient demographic and clinical characteristics did not significantly differ according to slide type used for IHC, except for race (white versus other; exact $p = 0.02$).

Tumor classifications based on IHC and MSI results are presented in Table 3. Overall, there were 124 (14.9%) MSI-H tumors, including 42 of 447 (9.4%) full-section slides and 82 of 387 (21.2%) TMA slides. The percentage of tumors demonstrating LoE of at least one of the three MMR proteins was 27.3%, with LoE for each protein as follows: 22.1%, 8.2%, and 6.0% for MLH1, MSH2, and MSH6, respectively. LoE varied by slide type, with 19.1% of the TMA slides and 34.5% of the full-section slides demonstrating IHC LoE for one or more of the MMR proteins.

Table 2. Demographic and Clinical Characteristics of Participants

Characteristic	*All samples with both IHC and MSI (n = 834)*	*TMA slides (n = 387)*	*Full-section slides (n = 447)*
Mean age ± SD (y)	56.0 ± 11.8	56.3 ± 11.8	55.8 ± 11.8
Stage, *n* (%)			
1–2	292 (35)	130 (33.6)	162 (36.2)
3–4	534 (64)	256 (66.1)	278 (62.2)
Unknown	8 (1)	1 (0.3)	7 (1.6)
Race			
White	766 (91.8)	365 (94.3)	401 (89.7)
African American	28 (3.4)	2 (0.5)	26 (5.8)
Asian	35 (4.2)	18 (4.7)	17 (3.8)
Other	5 (0.6)	2 (0.5)	3 (0.7)
Histologic features, *n* (%)			
Borderline	100 (12)	39 (10.1)	61 (13.6)
Invasive	734 (88)	348 (89.9)	386 (86.4)
Serous	440 (59.9)	197 (56.6)	243 (63)
Nonserous	294 (40.1)	151 (43.4)	143 (37)
Nonserous histology, *n* (%)			
Clear cell	64 (21.8)	24 (15.9)	40 (28)
Endometrioid	119 (40.5)	67 (44.4)	52 (36.4)
Mucinous	35 (11.9)	19 (12.6)	16 (11.2)
Other[a]	76 (25.9)	41 (27.2)	35 (24.5)

[a]Other histologies include: carcinoma unspecified; mixed cell; peritoneal; transitional cell carcinoma.
SD, standard deviation.

Concordant and discordant cases are shown in Table 3. Overall, 67.7% (95% CI, 64.4–70.8%) were concordant (i.e., MMR deficiency status of the tumor was the same on the basis of results of both IHC and MSI testing): 41 (5%) were classified as MMR-deficient according to results of both testing methods (MSI-H with IHC LoE), and 523 (63%) showed no evidence of MMR deficiency (MSS with no LoE on IHC) ($\kappa = 0.05$; $p = 0.127$; $\kappa_{max} = 0.63$). Of the manually stained slides, 75.6% were concordant ($\kappa = 0.08$; $p = 0.069$; $\kappa_{max} = 0.88$) versus 53.9% of the automatically stained slides ($\kappa = 0.08$; $p = 0.039$; $\kappa_{max} = 0.21$). Concordance rates also differed according to which pathologist interpreted the IHC result, with 82.6% ($\kappa = 0.20$; $p < 0.001$; $\kappa_{max} = 0.63$) and 56.8% ($\kappa = 0.02$; $p = 0.56$; $\kappa_{max} = 0.31$) concordant cases for pathologists A and B, respectively.

Results of multivariable logistic regression for IHC LoE of at least one MMR protein as the outcome and MSI status as the predictor variable (adjusting for staining method and pathologist if applicable) are summarized in Table 4. In the analysis of full-section slides, the unadjusted odds ratio (OR) of MSI-H status was statistically significant (OR, 2.27; 95% CI, 1.20–4.32), but after adjustment for pathologist and staining method, the OR became only marginally significant (OR, 2.19; 95% CI, 0.98–4.93). After adjustment for MSI-H status, the automatically stained slides read by pathologist B were 14 times more likely to be interpreted as showing loss of MMR protein expression as were the automatically stained slides read by pathologist A (adjusted OR, 14.0; 95% CI, 4.2–46.7). Also, the manually stained slides read by pathologist A

Table 3. Overview of Concordant and Discordant Samples Based on Immunohistochemical Testing and Microsatellite Instability Testing Analyses

Variable	Overall[a]	Specimen type		Staining method		Reading pathologist	
		TMA	Full-section	Automated	Manual	A	B
All samples (*n*)	834	387	447	306	528	381	453
MSI-H, *n* (%)	124 (14.9)	82 (21.2)	42 (9.4)	32 (10.5)	92 (17.4)	70 (18.4)	54 (11.8)
IHC LoE, *n* (%)	228 (27.3)	74 (19.1)	154 (34.5)	153 (50.0)	75 (14.2)	36 (9.4)	192 (42.0)
Concordant cases, *n* (%)							
MSI-H/IHC LoE	41 (4.9)	19 (4.9)	22 (4.9)	22 (7.2)	19 (3.6)	16 (4.2)	25 (5.5)
MSS/IHC no LoE	523 (62.7)	250 (64.6)	273 (61.1)	143 (46.7)	380 (72.0)	291 (76.4)	232 (51.3)
Discordant cases, *n* (%)							
MSI-H/IHC no LoE	83 (10.0)	63 (16.3)	20 (4.5)	10 (3.3)	73 (13.8)	54 (14.2)	29 (6.4)
MSS/IHC LoE	187 (22.4)	55 (14.2)	132 (29.5)	131 (42.8)	56 (10.6)	20 (5.2)	167 (36.7)
κ-value	0.05	0.05	**0.09**	**0.08**	0.08	**0.20**	0.02
(95% CI)	(−0.02 to 0.12)	(−0.05 to 0.16)	**(0.01–0.17)**	**(0.01–0.15)**	(−0.01 to 0.18)	**(0.08 to 0.32)**	(−0.05 to 0.09)
Exact *p*-value	0.127	0.342	**0.011**	**0.039**	0.069	**<0.001**	0.560
Maximum κ	0.634	0.936	0.329	0.209	0.879	0.634	0.311

Boldface indicates statistical significance.
[a]All percentages were calculated on the basis of the entire sample size of 834.
TMA, tissue microarray; MSI-H, high microsatellite instability; IHC LoE, loss of expression of at least one mismatch repair protein on immunohistochemistry; MSS, microsatellite stable; CI, confidence interval.

were 92% less likely to be interpreted as demonstrating loss of MMR protein expression as were the automatically stained slides read by the same pathologist (OR, 0.08; 95% CI, 0.01–0.79). Although the race distribution differed between TMA and full-section slides in the univariate analysis, race was not significant in the multivariable models; thus, it was dropped from all models.

Among the TMA slides, the MSI-H group showed 1.6 times higher odds of MMR protein expression loss on IHC than the MSS group, while adjusting for the pathologist, but the result was not statistically significant (OR, 1.56; 95% CI, 0.85–2.87). The adjusted OR for pathologist B compared with pathologist A was 1.71 (95% CI, 1.01–2.89), which implies that TMA slides read by pathologist B were 71% more likely to be read as showing loss of expression than TMA slides read by pathologist A after adjustment for MSI-H status.

Discussion

To our knowledge, this is the first study to evaluate concordance between IHC and MSI testing for identifying MMR deficiency in epithelial ovarian tumors. Most prior studies evaluating MMR defects in ovarian tumors performed testing sequentially via one of two methods: (1) IHC was done on all tumors, followed by MSI testing on those samples with loss of MMR protein expression (Malander *et al.*, 2004; Rosen *et al.*, 2006; Domanska *et al.*, 2007), or (2) MSI was performed on all tumors, followed by IHC testing on those that demonstrated MSI-H (Ichikawa *et al.*, 1999; Chiaravalli *et al.*, 2001; Cai *et al.*, 2004; Liu *et al.*, 2004; Singer *et al.*, 2004; Ueda *et al.*, 2005). Geisler *et al*. (2003) evaluated a series of 125 ovarian tumors to determine concordance between MMR status by using MSI testing and mRNA expression of MMR genes. Their study revealed a 91% overall concordance between the two methods (Geisler *et al.*, 2003); however, concordance among their subset of 21 MSI-H tumors was poor, with only 10 of the 21 (48%) MSI-H tumors demonstrating loss of mRNA expression (Geisler *et al.*, 2003). Given that loss of mRNA expression is expected to result in loss of protein expression, we questioned whether results of IHC and MSI would be concordant. Our data revealed poor overall concordance (about 68%) between MSI and IHC, with even poorer concordance among the subset of 124 MSI-H tumors (41 of 124 [33%]).

In contrast to our study of ovarian tumors, concordance rates of IHC and MSI for colorectal and endometrial tumors have generally been very high (>95%) (Cicek *et al.*, 2011; Bartley *et al.*, 2012). Nevertheless, lower concordance rates have been found on a subset of MSI-H colorectal tumors (Bartley *et al.*, 2012) and among colorectal tumors from a patient population with moderate to high risk for Lynch syndrome (Baudhuin *et al.*, 2005). Differences in tissue type could translate into differences in the validity of MSI and IHC results for identifying patients who may have Lynch syndrome. For instance, similar rates of MSI have been found in normal ovarian tissue, cystadenomas, and cystadenocarcinomas, possibly because of the ovulatory process (Caliman *et al*., 2012). Additionally, ovarian tumors have been shown to demonstrate high rates of genetic heterogeneity (Khalique *et al*., 2007). Consequently, tumor heterogeneity could be contributing to the high rates of discordance between IHC and MSI results in our current study.

Table 4. Logistic Regression Results for Immunohistochemistry Loss of Mismatch Repair Protein Expression Stratified by Slide Type

	Full-section (n = *447*)			*TMA* (n = *387*)		
Variable	n	*Univariate OR (95% CI)*	*Adjusted OR (95% CI)*	n	*Univariate OR (95% CI)*	*Adjusted OR (95% CI)*
MSI-H[a]		**2.27 (1.20–4.32)**	2.19 (0.98–4.93)		1.37 (0.76–2.47)	1.56 (0.85–2.87)
Pathologist (A or B) staining method[b]						
A: Automated	37	Reference	Reference	0	–	–
A: Manual	142	**0.08 (0.01–0.80)**	**0.08 (0.01–0.79)**	203	–	–
B: Automated	269	**14.29 (4.28–47.65)**	**13.95 (4.17–46.66)**	0	–	–
B: Manual	0	NA[c]	NA[b]	184	–	–
Pathologist B vs. A (reference)[d]		–		1.58 (0.95–2.63)	**1.71 (1.01–2.89)**	

Conclusion: After controlling for MSI-H status, manual staining was significantly less likely to result in IHC loss of MMR expression for full-section slides read by pathologist A. Additionally, pathologist B was more likely than pathologist A to interpret results as IHC loss of expression for both slide types (i.e., full-section and TMA).

Boldface indicates statistical significance.

[a]MSI-H was included as the independent variable in the logistic regression models.

[b]Pathologist and staining method were included as control variables in the full-section logistic regression models.

[c]One of the two pathologists (B) did not read any full-section slides that were manually stained.

[d]Given that all tissue microarrays were manually stained, only pathologist was controlled for in the tissue microarray models.

OR, odds ratio; NA, not available.

Despite concerns about the reliability and validity of IHC results in general (O'Leary, 2001; Seidal *et al.*, 2001; Wick and Mills, 2001; Goldstein and Bosler, 2007), neither MSI nor IHC has been established as the gold standard for identifying MMR deficiency in colorectal or endometrial tumors. Our study does not allow us to conclude which method is better at detecting MMR status in ovarian tumors, but the prevalence of MMR deficiency based on our IHC results (27%) was substantially higher than the 10% prevalence found in a prior meta-analysis (Murphy and Wentzensen, 2011). This suggests our study suffered from a large false-positive rate for IHC tests. In contrast, the prevalence of MSI-H in our study was 15%, which is more consistent with prior estimates (Pal *et al.*, 2008a; Murphy and Wentzensen, 2011).

Our statistical models helped us identify several factors that may have contributed to variability in IHC results. These factors include subjectivity of IHC scoring, differences in MLH1 probes used for manual versus automatic staining, and potential problems from using the same cut-off score to determine loss of MMR protein expression. Given the concern about IHC scoring, we performed post hoc analyses to assess agreement between MSI and IHC using a set of different cutpoints for IHC. For automatic staining, the κ value was highest when an IHC cutpoint of 1 was used. For manual staining, a cutpoint of 3 produced the highest κ value. Regardless of the cutpoint, κ values remained below 0.11. These results suggest that IHC cutpoints were not responsible for the poor concordance between IHC and MSI results.

With regard to staining methods, published studies validating and/or comparing automated versus manual IHC staining appear to be limited. One study suggests that automation improves the accuracy of HER2 detection in breast cancer tissue (Bankfalvi *et al.*, 2004). Analogous studies were not identified for MMR detection in colon or ovarian cancer, and our results do not provide evidence to determine whether either method was superior because concordance between IHC and MSI was poor for both methods. From a clinical standpoint, a high false-positive rate indicates that a large proportion of ovarian tumors would be misclassified as MMR deficient. Thus, if the automated IHC procedures and scoring system in the current study were used to select patients with ovarian cancer who should be offered confirmatory germline testing for Lynch syndrome, it could lead to unnecessary healthcare expenditure. Alternatively, manual staining may lead to a higher false-negative rate, resulting in the failure to identify cases of Lynch syndrome. These rates would further be influenced by the reading pathologist.

Another issue raised after initial data analysis involved the potential impact of age on our findings. Data from two of the three population-based studies in which the approximate age of tumor specimens was available revealed an average tumor age of 5 and 10 years, respectively. However, post hoc analyses looking at concordance stratified by tumor age revealed no evidence that tumor age contributed to variability in concordance (data not shown).

The current study had several strengths, including the large sample size, population-based design of the parent studies, and comprehensive collection of clinical and demographic data on study participants. Despite these strengths, the parent studies were not designed to validate IHC and MSI testing or determine the reliability of these two methods in identifying MMR-deficient tumors or Lynch syndrome. Nevertheless, the heterogeneity in our IHC testing is important clinically because it mimics ''real world'' testing practices, where testing is performed in different laboratories using various methods, with results interpreted by a single pathologist. Adding to our heterogeneity in IHC testing was the use of two different sample slide types (full-section and TMAs); thus, results were reported separately for the two-slide types.

Another study limitation was the inclusion of only three of the four MMR proteins for IHC, as PMS2 protein expression was not standard at the time testing was performed. In addition, the National Cancer Institute panel used for MSI testing does not always identify patients with germline

mutations in *MSH6,* and therefore these patients may have a discordant result (Wu *et al.*, 1999). Nevertheless, most MMR-deficient tumors have mutations in *MLH1* and *MSH2* (Chiaravalli *et al.*, 2001). Therefore, we would presume these explanations would contribute little to the high rates of discordance.

In conclusion, our results showed poor concordance between MSI and IHC results in epithelial ovarian cancers, in contrast to the high concordance rates in colorectal tumors previously reported by other groups (Cicek *et al.*, 2011; Bartley *et al.*, 2012). Furthermore, unlike colorectal tumors (for which sufficient evidence exists to support the clinical validity and clinical utility of routine MSI or IHC testing to identify patients who should be offered germline testing for Lynch syndrome [Evaluation of Genomic Applications in Practice and Prevention Working Group, 2009; Palomaki, *et al*., 2009]), the clinical validity and utility of these tests for identification of Lynch syndrome among patients with ovarian cancer remain uncertain. Despite the paucity of validity and quality assurance studies, laboratories are already performing IHC on ovarian tumors in a clinical setting (Cicek *et al.*, 2011). Thus, our findings clearly demonstrate the need to (1) further assess IHC testing methods (e.g., automated versus manual), (2) demonstrate validity and clinical utility of IHC testing in ovarian tumors, and (3) ensure interrater reliability in scoring slides to standardize IHC processes before offering widespread screening of ovarian tumors as a clinical test to identify patients who should be offered germline testing for Lynch syndrome.

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Author Disclosure Statement

No competing financial interests exist.

References

Akiyama Y, Sato H, Yamada T, *et al.* (1997) Germ-line mutation of the hMSH6/GTBP gene in an atypical hereditary nonpolyposis colorectal cancer kindred. Cancer Res 57:3920–3923.

American Cancer Society (2013) Cancer facts and figures 2013. Available at: http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf . Accessed May 20, 2013.

Aysal A, Karnezis A, Medhi I, *et al.* (2012) Ovarian endometrioid adenocarcinoma: incidence and clinical significance of the morphologic and immunohistochemical markers of mismatch repair protein defects and tumor microsatellite instability. Am J Surg Pathol 36:163–72.

Bankfalvi A, Boecker W, Reiner A (2004). Comparison of automated and manual determination of HER2 status in breast cancer for diagnostic use: a comparative methodological study using the Ventana BenchMark automated staining system and manual tests. Int J Oncol 25:929–935.

Bartley AN, Luthra R, Saraiya DS, *et al.* (2012) Identification of cancer patients with Lynch syndrome: clinically significant discordances and problems in tissue-based mismatch repair testing. Cancer Prev Res (Phila) 5:320–327.

Baudhuin LM, Burgart LJ, Leontovich O, *et al.* (2005) Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. Fam Cancer 4:255–265.

Boland CR, Thibodeau SN, Hamilton SR, *et al.* (1998) A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248–5257.

Bronner CE, Baker SM, Morrison PT, *et al.* (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368:258–261.

Cai KQ, Albarracin C, Rosen D, *et al.* (2004) Microsatellite instability and alteration of the expression of hMLH1 and hMSH2 in ovarian clear cell carcinoma. Hum Pathol 35:552–559.

Caliman LP, Tavares RL, Piedade JB, *et al.* (2012) Evaluation of microsatellite instability in women with epithelial ovarian cancer. Oncol Lett 4:556–560.

Chiaravalli AM, Furlan D, Facco C, *et al.* (2001) Immunohistochemical pattern of hMSH2/hMLH1 in familial and sporadic colorectal, gastric, endometrial and ovarian carcinomas with instability in microsatellite sequences. Virchows Arch 438:39–48.

Cicek MS, Lindor NM, Gallinger S, *et al.* (2011) Quality assessment and correlation of microsatellite instability and immunohistochemical markers among population- and clinic-based colorectal tumors results from the Colon Cancer Family Registry. J Mol Diagn 13:271–281.

Coppola D, Nicosia SV, Doty A, *et al.* (2012) Uncertainty in the utility of immunohistochemistry in mismatch repair protein expression in epithelial ovarian cancer. Anticancer Res 32: 4963–4969.

Domanska K, Malander S, Masback A, *et al.* (2007) Ovarian cancer at young age: the contribution of mismatch-repair defects in a population-based series of epithelial ovarian cancer before age 40. Int J Gynecol Cancer 17:789–793.

Engel C, Loeffler M, Steinke V, *et al.* (2012) Risks of less common cancers in proven mutation carriers with Lynch syndrome. J Clin Oncol. 30:4409–4415.

Evaluation of Genomic Applications in Practice and Prevention Working Group (2009) Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med 11:35–41.

Geisler JP, Goodheart MJ, Sood AK, *et al.* (2003) Mismatch repair gene expression defects contribute to microsatellite instability in ovarian carcinoma. Cancer 98:2199–2206.

Goldstein NS, Bosler D (2007) An approach to interpreting immunohistochemical stains of adenocarcinoma in small needle core biopsy specimens: the impact of limited specimen size. Am J Clin Pathol 127:273–281.

Gras E, Catasus L, Arguelles R, *et al.* (2001) Microsatellite instability, MLH-1 promoter hypermethylation, and frameshift mutations at coding mononucleotide repeat microsatellites in ovarian tumors. Cancer 92:2829–2836.

Ichikawa Y, Lemon SJ, Wang S, *et al.* (1999) Microsatellite instability and expression of MLH1 and MSH2 in normal and

malignant endometrial and ovarian epithelium in hereditary nonpolyposis colorectal cancer family members. Cancer Genet Cytogenet 112:2–8.

Khalique L, Ayhan A, Weale ME, *et al.* (2007). Genetic intra-tumour heterogeneity in epithelial ovarian cancer and its implications for molecular diagnosis of tumours. J Pathol 211:286–295.

Leach FS, Nicolaides NC, Papadopoulos N, *et al.* (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215–1225.

Liu J, Albarracin CT, Chang KH, *et al.* (2004) Microsatellite instability and expression of hMLH1 and hMSH2 proteins in ovarian endometrioid cancer. Mod Pathol 17:75–80.

Malander S, Ridderheim M, Masback A, *et al.* (2004) One in 10 ovarian cancer patients carry germ line BRCA1 or BRCA2 mutations: results of a prospective study in Southern Sweden. Eur J Cancer 40:422–428.

Miyaki M, Konishi M, Tanaka K, *et al.* (1997) Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 17:271–272.

Murphy MA, Wentzensen N (2011) Frequency of mismatch repair deficiency in ovarian cancer: a systematic review. Int J Cancer 129:1914–1922.

O'Leary TJ (2001) Standardization in immunohistochemistry. Appl Immunohistochem Mol Morphol 9:3–8.

Pal T, Akbari MR, Sun P, *et al.* (2012) Frequency of mutations in mismatch repair genes in a population-based study of women with ovarian cancer. Br J Cancer 107:1783–1790.

Pal T, Permuth-Wey J, Betts JA *et al.* (2005) BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer 104:2807–2816.

Pal T, Permuth-Wey J, Kumar A, *et al.* (2008a) Systematic review and meta-analysis of ovarian cancers: estimation of microsatellite-high frequency and characterization of mismatch repair deficient tumor histology. Clin Cancer Res 14:6847–6854.

Pal T, Permuth-Wey J, Sellers TA (2008b) A review of the clinical relevance of mismatch-repair deficiency in ovarian cancer. Cancer 113:733–742.

Palomaki GE, McClain MR, Melillo S, *et al.* (2009) EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 11:42–65.

Pande M, Wei C, Chen J, *et al.* (2012) Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based Lynch syndrome registry. Fam Cancer 11:441–447.

Papadopoulos N, Nicolaides NC, Wei YF, *et al.* (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263:1625–1629.

Resnick KE, Hampel H, Fishel R, *et al.* (2009) Current and emerging trends in Lynch syndrome identification in women with endometrial cancer. Gynecol Oncol 114: 128–134.

Risch HA, McLaughlin JR, Cole DE, *et al.* (2001) Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 68:700–710.

Rosen DG, Cai KQ, Luthra R, *et al.* (2006) Immunohistochemical staining of hMLH1 and hMSH2 reflects microsatellite instability status in ovarian carcinoma. Mod Pathol 19:1414–1420.

Seidal T, Balaton AJ, Battifora H (2001) Interpretation and quantification of immunostains. Am J Surg Pathol 25:1204–1207.

Singer G, Kallinowski T, Hartmann A, *et al.* (2004) Different types of microsatellite instability in ovarian carcinoma. Int J Cancer 112:643–646.

Strathdee G, Appleton K, Illand M, *et al.* (2001) Primary ovarian carcinomas display multiple methylator phenotypes involving known tumor suppressor genes. Am J Pathol 158: 1121–1127.

Ueda H, Watanabe Y, Nakai H, *et al.* (2005) Microsatellite status and immunohistochemical features of ovarian clear-cell carcinoma. Anticancer Res 25:2785–2788.

Wenham RM, Schildkraut JM, McLean K, *et al.* (2003) Polymorphisms in BRCA1 and BRCA2 and risk of epithelial ovarian cancer. Clin Cancer Res 9:4396–4403.

Wick MR, Mills SE (2001) Consensual interpretive guidelines for diagnostic immunohistochemistry. Am J Surg Pathol 25: 1208–1210.

Win AK, Lindor NM, Young JP, *et al.* (2012) Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. J Natl Cancer Inst 104:1363–1372.

Wu Y, Berends MJ, Mensink RG, *et al.* (1999) Association of hereditary nonpolyposis colorectal cancer-related tumors displaying low microsatellite instability with MSH6 germline mutations. Am J Hum Genet 65:1291–1298.

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