Published in final edited form as:
Anticancer Res. 2012 November ; 32(11): 4963–4969.

Uncertainty in the Utility of Immunohistochemistry in Mismatch Repair Protein Expression in Epithelial Ovarian Cancer

Domenico Copppola, MD[1,4,*], **Santo V. Nicosia, MD**[5], **Andrea Doty, MPH**[2], **Thomas A Sellers, PhD, MPH**[2], **Ji-Hyun Lee, PhD**[3], **Jimmy Fulp, MS**[3], **Zachary Thompson, PhD**[2], **Sanja Galeb, BS**[2], **John McLaughlin, PhD**[6], **Steven A Narod, MD**[7], **Joellen Schildkraut, PhD**[8], and **Tuya Pal, MD**[2]

[1]Department of Anatomic Pathology, H. Lee Moffitt Cancer Center, 12902 Magnolia Drive Tampa, FL 33612

[2]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center, 12902 Magnolia Drive Tampa, FL 33612

[3]Department of Biostatistics, H. Lee Moffitt Cancer Center, 12902 Magnolia Drive Tampa, FL 33612

[4]Department of Experimental Therapeutics, H. Lee Moffitt Cancer Center, 12902 Magnolia Drive Tampa, FL 33612

[5]Department of Pathology and Cell Biology, University of South Florida, College of Medicine 12901 Bruce B. Downs Blvd. MDC 3 Tampa, FL 33612

[6]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue Toronto, ON, Canada M5G 1X5

[7]Centre for Research in Women's Health, Women's College Research Institute, 76 Grenville Street Toronto, ON, Canada M5S 1B2

[8]Duke University, Duke School of Medicine, 10 Bryan Searle Drive DUMC Box 3917 Durham, NC 27710

Abstract

Background—Utility of immunohistochemistry (IHC) for mismatch repair (MMR) protein expression has been demonstrated in colorectal cancer but remains incompletely defined in ovarian cancer. We evaluated MMR protein expression in three population-based samples of epithelial ovarian cancers.

Methods—IHC staining was performed on full section (FS) or tissue microarray (TMA) slides for MLH1, MSH2, and MSH6 expression.

Results—Of 487 cases, 147 and 340 were performed through FS and TMA, respectively. Overall, Loss of Expression (LoE) of at least one MMR protein was observed in 12.7% based on an expression score of ≤3 (on a scale of 9). Notably, LoE was significantly higher in TMAs (17.9%) compared to FS cases (0.7%) ($p < 0.001$).

Corresponding Author (to whom reprints should be requested): Domenico Coppola, MD, Department of Anatomic Pathology, Moffitt Cancer Center, Departments of Oncologic Sciences, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612, Domenico.Coppola@moffitt.org.

Financial Disclosures: none

Conclusions—A substantial proportion of epithelial ovarian cancers have a loss of MMR protein expression. Protein expression results vary significantly by the tissue sampling methodology utilized, raising concerns about the clinical utility of this test for ovarian tumors.

Keywords

immunohistochemistry; IHC; ovarian cancer; mismatch repair; MLH1; MSH2; MSH6

INTRODUCTION

Ovarian cancer ranks fifth in both cancer incidence and cancer mortality in U.S. women, having one of the highest mortality rates among gynecologic cancers[1]. Defects in the mismatch repair (MMR) pathway, one of the best defined molecular pathways involved in both inherited and sporadic cancer pathogenesis,[2] may be etiologically important in a substantial proportion of ovarian cancers [3]. Established methods to classify tumors as MMR-deficient cancers include: 1) immunohistochemistry (IHC) to measure loss of MMR protein expression; and 2) microsatellite instability analysis to identify those with a microsatellite instability-high (MSI-H) phenotype.

The assessment of MMR protein expression is well-accepted for identification of Hereditary Non-Polyposis Colorectal Cancer (HNPCC)-associated cancers (i.e., cancers of the colorectum, endometrium, stomach, ovaries, urinary tract, other gastrointestinal sites and brain) [4]. In fact, loss of MMR protein expression may help to identify those with germline MMR gene mutations, which in turn may provide individuals with an opportunity for cancer prevention through colorectal, endometrial and ovarian cancer risk management options as outlined in the NCCN guidelines [5]. In addition, it provides at-risk relatives the opportunity to prevent cancers through increased cancer screening and/or prophylactic surgeries [6, 7]. Furthermore, MMR deficiency has the potential to identify a specific pathway to tumorigenesis, which could have implications to diagnosis and treatment.

Most studies have evaluated MMR deficiency in colorectal and endometrial cancers. The few studies of MMR deficiency in ovarian cancer have either only focused on IHC in a small series of cases, [8–10] or only among cases identified as microsatellite instability high (MSI-H) [11, 12]. Moreover, the methods to define MMR deficiency have varied, with some performed on paraffin-embedded full section slides [8] and others based on tissue microarray (TMA) slides [9, 10]. Additionally, the scoring system by which loss of expression (LoE) of proteins on IHC was defined has varied across studies, and has been only partly been described in many instances. Furthermore, prior reports include limited characterization of these tumors on demographic and clinical variables. There are data to suggest that MMR deficient tumors tend to occur prior to age 50, particularly those associated with germline mutations in the MMR genes [13]. Furthermore, Sood et al reported that MMR deficient ovarian tumors (based on MSI-H status) had a mean age at diagnosis that was five years earlier than ovarian tumors without MSI-H [14]. Another finding observed in most studies is overrepresentation of non-serous histologies, such as endometrioid, mucinous and clear cell subtypes, in those with MMR-deficient ovarian tumors [3, 15]. Although a recent meta-analysis estimated a frequency of MMR deficiency of approximately 10% based on IHC and/or MSI results, [3] the data remain insufficient to inform the clinical utility of this test for ovarian cancer.

The purpose of the current study was to quantify the frequency of ovarian tumors with MMR deficiency through IHC for MMR protein expression through use of a mix of full section and tissue microarray (TMA) slides, as well as demographic and clinical predictors

of the loss of expression phenotype. A secondary objective was to compare full section and TMA-based results, to explore the clinical utility of IHC for MMR protein expression.

METHODS

Participants

Participants for this study were drawn from three population-based studies of epithelial ovarian cancer: the Familial Ovarian Tumor Study (FOTS) in Toronto, [16] the Tampa Bay Ovarian Cancer Study (TBOCS) at the Moffitt Cancer Center, [17] and the North Carolina Ovarian Cancer Study (NCOCS) at Duke University, [18] with details about study design, populations, and data collection methods published previously. Briefly, FOTS cases were identified through monitoring of pathology reports submitted to the population-based Ontario Cancer Registry for province-wide recruitment. TBOCS cases were recruited through a rapid case ascertainment mechanism in the two most populous counties in the Tampa Bay region. NCOCS cases were identified through a rapid case ascertainment mechanism in a 48-county region located in the central portion of North Carolina. The study protocol was approved by the institutional review board at each center, and written informed consent was obtained from all participants.

Eligibility criteria for study enrollment were defined as incident, pathologically confirmed primary epithelial ovarian cancer, either borderline or invasive, aged 20 years and above. Through each of the studies, data collection included: administration of a questionnaire, collection of demographic, clinical, and family history information, and review of medical records for histopathology of the ovarian tumor. Formalin-fixed paraffin-embedded epithelial ovarian tumor blocks were obtained from the hospital at which the participant had their surgery for 487 participants: 142 from Duke, 219 from Moffitt, and 126 from Toronto.

Construction of TMA

One representative H&E stained full section slide of each tumor was reviewed by expert pathologists (DC, SVN) to confirm the diagnosis. For all Duke cases and 5 Moffitt cases, full sections representative of the tumor were reviewed and used for the immuno-histochemical analysis. For the remaining 214 Moffitt cases and all Toronto cases, the pathologists selected 3 representative areas of tumor to be included on the TMA blocks.

The TMA blocks were constructed using the same protocol for the FOTS patients (based in Toronto) and the TBOCS patients (based at Moffitt) with a precision instrument (Chemicon model ATA-100, Chemicon Int'l, Temecula, CA, USA) as previously described [19]. For each case, three replicate cores (1 mm in size for Moffitt TMA cases and 0.6 mm in size for Toronto cases) were sampled from the donor tissue block and placed side-by-side on a separate recipient block. Normal control tissue (fallopian tissue) was included in the block. A heated glass slide was used to even the surface of the recipient block. Sample tracking was based on coordinate positions for each tissue core in the TMA recipient block; 4 μm sections were transferred onto separate TMA slides for IHC staining of each of the three MMR proteins under investigation (hMLH1, hMSH2, and hMSH6).

Immunohistochemistry for MMR Proteins

Deparaffinized, formalin-fixed paraffin-embedded tissues were microwaved in 1X EDTA (Chemicon Int'l, Temecula, CA) (*hMSH2*) or Borg Decloaker (BioCare Medical, Concord, CA) (*hMLH1* and *hMSH6*), cooled at room temperature for 20 minutes, rinsed with deionized water and placed in TBS/Tween for 5 minutes. Immunostaining was carried out on the Dako Autostainer using the Vector Elite Mouse IgG – HRP detection kit (Vector Laboratories, Burlingame, CA) following avidin/biotin blocking (Vector Laboratories).

Slides were incubated in mouse monoclonal *hMLH1* (clone G168-15, BioCare Medical, Concord, CA) at 1:40 or *hMSH6* (clone BC/44, BioCare, Concord, CA) at 1:70 overnight at 4°C or *hMSH2* (Clone FE11, Zymed/Invitrogen, Carlsbad, CA) at 1:200 for 30 minutes at room temperature. For overnight incubations, slides were removed from the autostainer, placed in a humid chamber in the refrigerator, and returned to the autostainer the following day. 3,3'-Diaminobenzidine (Dako, Carpinteria, CA) was the chromogen. Slides were counterstained with modified Mayer's hematoxylin, dehydrated through ascending grades of ethanol, cleared with xylene and mounted with resinous mounting medium.

Immunohistochemistry analysis

Loss of MMR expression was defined as absence of detectable nuclear staining of tumor cells in the presence of retained nuclear staining in lymphocytes and/or in non-neoplastic epithelial or stromal cells, which served as internal positive controls. The stained tissue full sections slides and TMA cores were examined by senior board-certified pathologists (DC, SVN). Stainings were classified based on nuclear staining intensity and distribution using a semi-quantitative ordinal scoring system to generate a combined expression score. The intensity and staining were scored on a scale of 0–3. Specifically, the intensity score was graded based on least intense (score of 0) to most intense (score of 3) and the staining was graded based on the percentage of positive cells as follows: 0(0%), 1(1–33%), 2(34–66%) and 3(67–100%). The product of the intensity and staining was used as the final score. The score was calculated for each of the 3 cores of each sample and the mean of the 3 scores was used as the final score. The final scores were classified as: 0 negative, 1–3 weak, 4–6 moderate and 7–9 strong.

Data collection

All participants completed a study questionnaire and interview, by which demographic and family history variables were collected. Family history information included collection of all ages and types of cancer diagnosed in first, second and third degree relatives. Medical records were retrieved on all participants to abstract information on tumor histology. In addition, information on date of diagnosis (based on pathology report) and date of study enrollment (based on date consent form signed) were collected, in order to determine time between diagnosis and enrollment (calculated in days).

Statistical analyses

Differences in demographic, clinical, and pathological variables across the specimen types (full section, 1.0 mm TMA core, 0.6 mm TMA core) were evaluated using Kruskal-Wallis tests for continuous variables and Pearson Chi-square exact tests based on Monte Carlo estimation for categorical variables. Frequencies/proportions of LoE were calculated overall and also according to specimen type, including LoE as defined by cut-off scores of 3 and below and 4 and below. The 95% confidence intervals for the proportions of LoE were calculated using the Wilson confidence (score) limits method. Further, demographic and clinical variables were compared between LoE and no LoE patient subgroups using Wilcoxon sum rank tests and Pearson Chi-Square exact tests. For this analysis, full section cases were excluded due to the limited sample size (as only 1/147 of these samples had LoE). All reported p-values are two-sided. All analyses were carried out with R version 2.13.1.

RESULTS

The demographic, clinical, and pathological features of the participants are summarized in Table 1. Tumor tissue was examined on a total of 487 participants with a mean age of 56.1 years. Proportions of invasive and borderline cases were 85% and 15%, respectively. Of the

invasive cases, 61.8% were of serous histology. Family history of an HNPCC-associated cancer was reported by 34.5% of participants. The time to study enrollment was highest in the TMA cases with 0.6 mm cores (i.e., which represents cases from Toronto), compared to the full section and 1 mm core cases which had comparable median time from diagnosis to enrollment. Similarly, for the full section cases, there were a higher proportion of borderline tumors, likely attributable to survival bias resulting from the higher median time from diagnosis to enrollment.

The frequency of LoE defined as a score of 3 and below on samples based on full section slides, 1 mm TMA cores and 0.6 mm TMA cores was 0.7%, 15.9% and 21.4%, respectively (Table 2). When using a cut-off score of 4, all LoE frequencies were higher for all specimen types, as expected (i.e., 13.6%, 27.5%, and 26.2%, respectively). The estimated frequencies were statistically significantly different between full sections, 1 mm TMA and 0.6 mm TMA regardless of cut point used.

Evaluation of demographic and clinical variables was limited to TMA-based cases (n=340) since analysis based on full section specimens yielded only 1 case with LoE. Overall, cases with LoE were similar to cases without LoE for all variables except one: histologic subtype. In particular, subgroup analyses of histologic subtypes in those with invasive tumors revealed an association of tumors with LoE and non-serous subtypes ($P = 0.003$), with the highest frequency seen in those with mucinous cancers (38.9%), followed by clear cell (35.3%) and endometrioid (23.1%) subtypes.

DISCUSSION

In this study we evaluated MLH1, MSH2, and MSH6 protein expression through IHC in 472 samples of ovarian carcinomas of diverse histologic subtypes. Our findings indicate an overall frequency of loss of MMR protein expression of 12.7%. Furthermore, the frequency of LoE observed varied by specimen type, with a lower overall frequency of LoE in full section slides (0.7%) compared to TMA slides (15.9% and 21.4% for 1 mm and 0.6 mm cores, respectively), which suggest that specimen type is important to consider when assessing IHC in ovarian samples. Finally, our data suggests LoE may be more common in ovarian epithelial tumors with non-serous histologies.

Our overall TMA-based estimates (17.9%) are higher than that reported in the few studies to date that have evaluated loss of MMR protein expression based on IHC in unselected invasive epithelial ovarian carcinomas, whereas our full section estimates (0.7%) are lower. [8–10] The study by Domanska et al [8] represents the only report of IHC on full section ovarian cancer slides; loss of MLH1, MSH2, MSH6 and PMS2 expression was 6.1% in patients age 40 or below at diagnosis. Two studies have performed IHC on TMA sections. Malander et al [9] evaluated MLH1, MSH2, MSH6 and PMS2 and noted loss of protein expression in 2.3% (3/128) of cases. Rosen et al [10] reported a loss of expression of MLH1 and MSH2 based on TMA sections in 2.2% (7/322) of unselected ovarian cancers. More recently, a meta-analysis estimated a frequency of MMR deficiency of approximately 10% based on IHC and/or MSI results in ovarian cancers [3].

Although our study is the largest to date of MMR protein deficiency in ovarian cancer based on IHC, it is also the first multi-center study to include both TMAs and full sections, thus the lack of consistency in MMR frequency found in our study could not have been detected in prior efforts. Furthermore, the limited details on IHC methodology (such as detailed protocol, equipment and antibodies, and the scoring system) in prior reports preclude our ability to fully compare our results with prior studies. Of note, the LoE frequency varied

based on whether a cut-off score of 3 versus 4 was used, suggesting it is imperative to report scoring methodologies in published reports to allow cross comparisons between studies.

Validation of the use of TMA for MMR protein expression has been evaluated in a single colorectal cancer study in which IHC for MLH1 and MSH2 was conducted in 263 colorectal cases through TMA (3 cores per case) and full section. Results indicated comparable frequencies of 98.8% and 99.2%, respectively [20]. In ovarian cancers, validation of TMA technology has been performed on several markers, including p53, Ki-67, estrogen and progesterone receptors, among others, [21, 22] however to our knowledge there are no published studies that have validated IHC for MMR proteins expression in ovarian cancer. Specifically, the study by Rosen et al represents the only published report in which full sections and TMAs were compared in high-grade serous ovarian cancers through IHC studies of Ki-67, estrogen receptors and p53 and correlation coefficients were reported as 0.86, 0.93, and 0.82, respectively [23]. In general, correlation coefficients of greater than 0.8 are considered evidence of excellent correlation. In a subsequent study, Hecht et al performed IHC for Ki-67, estrogen receptors, and p53 among others using triplicate core samples from 174 epithelial ovarian cancers and demonstrated high intraclass correlation between the cores [21]. Ultimately, there have been studies to validate use of TMAs for MMR protein expression in colorectal cancer as well as studies to validate use of TMAs to evaluate protein expression in ovarian cancers (which have not included MMR proteins). Consequently, studies to evaluate MMR protein expression in ovarian cancers using either TMA or full section slides have been conducted under the assumption that either a valid approach. Our study differs from prior efforts as we used a combination of TMAs and full section slides to evaluate MMR protein expression in ovarian cancers. Based on published reports, we presumed TMA and full section cases were equivalent, however our results pertaining to MMR expression suggest they may not be directly comparable due to differences observed by specimen type. These findings suggest that validity of TMA may vary by antigen, and clearly demonstrate the need for large scale studies to validate the use of IHC for MMR protein expression specifically in ovarian cancers, prior to their use in the clinical setting. Moreover, in an effort to further clarify these differences, tumor samples on 19 participants in the study population were evaluated through both full section and TMA slides for expression of three MMR proteins based on IHC. Results demonstrated poor correlation of full section and TMA-based samples, with a LIN correlation coefficient of 0.4, 0.3 and 0.7 for MLH1, MSH2 and MSH6, respectively (data not shown).

Possible reasons for the discrepancy observed by specimen type include the large size at diagnosis of ovarian tumors compared to colorectal tumors, which may contribute to their heterogeneity as they may be composed of different clonal populations exhibiting different MMR reactivity. Another possible explanation is that the fixative does not adequately penetrate the large blocks of cancer typical of ovarian cancer which may impact IHC for some proteins more than others. Interestingly, the frequency of LoE inversely correlated with the size of surface area of the tissue examined (0.7% in full sections (usually several mm in diameter) vs.15.9% in TMA cores of 1mm in diameter vs. 21.4% in TMA cores measuring 0.6mm in diameter). It is possible that when evaluating larger size tissues, there is a higher likelihood of identifying even a small area strongly staining for the marker evaluated, thus potentially leading to a lower frequency of LoE. Conversely, when a small size sample of a large tumor is evaluated, a negative stain will not completely exclude the possibility that a non-sampled area of the same tumor be positive. Finally, another possibility considered was whether discrepancy in results may have occurred due to considerable differences between the three study sites. However, comparison of demographic variables between study participants did not suggest any underlying differences in the study populations at the three sites to account for our findings. In fact, all three sites encompassed primarily a Caucasian population and each study was population-

based thus collected specimens from multiple hospitals within their study catchment area. Ultimately, future efforts focused on differences between full section and TMA slides are needed on a larger range of antibodies, to determine their validity in each antibody prior to use in the clinical setting.

The expected proportions of histopathologic subtypes for epithelial ovarian cancers in the general population are: approximately 55–70% with serous histology, followed by the mucinous (3–9%), endometrioid (8–15%), clear cell (7–13%), mixed (4–6%) and undifferentiated subtypes.[24] Our finding of overrepresentation of non-serous histologies, particularly mucinous, clear cell, and endometrioid subtypes, in MMR-deficient ovarian tumors is consistent with prior studies [3, 8–10, 15].

There were a number of strengths in the current study, including the large sample size, population-based design of the parent studies, as well as the comprehensive collection of clinical and demographic data on study participants. Despite these strengths, it is important to acknowledge that our study was designed to evaluate MMR protein deficiency in ovarian tumors, not validate the use of TMA specific to MMR proteins. Nevertheless, our findings that MMR LoE frequency varies by specimen type (i.e., full section slides versus TMA) is of broad clinical relevance as it serves to demonstrate the limitation for the use of IHC as a reliable methodology for the assessment of MMR deficiency in ovarian cancer. Given our findings, it is critical to evaluate the validity of results of MMR deficiency in ovarian cancers, prior to its use as a predictive or prognostic marker. Other factors that may affect IHC results performed on paraffin-embedded tissues include type and time of fixation, thickness of tissue sections, age of the parental tissue block, and tumor heterogeneity. In fact, multiple studies to evaluate HER2 in breast cancer have demonstrated the multitude of factors that may significantly affect the results of immunostains [25]. As a result of how this may significantly impact patient care, both American Society of Clinical Oncology (ASCO) and College of American Pathology (CAP) have provided guidelines for the processing of breast specimens to be stained for Her2 [26]. Ultimately, the implementation of similar guidelines for ovarian cancer specimens will improve the immunohistochemical evaluation of these tumors, and will improve our ability to better determine the basis of discrepant results, as they arise. Finally, IHC was limited to the three more commonly stained proteins, MLH1, MSH2, and MSH6, at the time at which testing was done. PMS2 was not done, as it has more recently been included in routine IHC analyses.

In conclusion, our study suggests that a significant portion of human ovarian cancers exhibit defects in the mismatch repair pathway, proposing this pathway as a potential contributor to the development of epithelial ovarian cancers. The results also indicate that immunohistochemical techniques for the assessment of MMR proteins deficiency may have limited diagnostic accuracy in ovarian cancer and require further validation studies to assess utility. Future efforts focused on inter and intra-institutional standardization of IHC methodologies is crucial for the successful utilization of this technique for clinical usage in ovarian tumors.

Acknowledgments

This work was supported by grants #R01CA111914 (TP) and #K07CA108987 (TP) from the National Cancer Institute

REFERENCES

1. American Cancer Society, I. Cancer Facts & Figures 2007. 2007 [Internet] [cited 2007 2007]; Available from: http://www.cancer.org/docroots/STT/stt_0.asp.

2. Pal T, Permuth-Wey J, Sellers TA. A review of the clinical relevance of mismatch-repair deficiency in ovarian cancer. Cancer. 2008; 113(4):733–742. [PubMed: 18543306]
3. Murphy MA, Wentzensen N. Frequency of mismatch repair deficiency in ovarian cancer: A systematic review. Int J Cancer.
4. Lynch HT, et al. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2009; 76(1):1–18. [PubMed: 19659756]
5. Lynch PM. Current approaches in familial colorectal cancer: a clinical perspective. J Natl Compr Canc Netw. 2006; 4(4):421–430. [PubMed: 16569392]
6. Jarvinen HJ, et al. Ten years after mutation testing for Lynch syndrome: cancer incidence and outcome in mutation-positive and mutation-negative family members. J Clin Oncol. 2009; 27(28): 4793–4797. [PubMed: 19720893]
7. 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. 2009; 11(1):35–41. [PubMed: 19125126]
8. Domanska K, et al. 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. 2007; 17(4):789–793. [PubMed: 17343610]
9. Malander S, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. Gynecol Oncol. 2006; 101(2):238–243. [PubMed: 16360201]
10. Rosen DG, et al. Immunohistochemical staining of hMLH1 and hMSH2 reflects microsatellite instability status in ovarian carcinoma. Mod Pathol. 2006; 19(11):1414–1420. [PubMed: 16941012]
11. Ichikawa Y, et al. 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. 1999; 112(1):2–8. [PubMed: 10432927]
12. Chiaravalli AM, et al. Immunohistochemical pattern of hMSH2/hMLH1 in familial and sporadic colorectal, gastric, endometrial and ovarian carcinomas with instability in microsatellite sequences. Virchows Arch. 2001; 438(1):39–48. [PubMed: 11213834]
13. Ketabi Z, et al. Ovarian cancer linked to Lynch syndrome typically presents as early-onset, non-serous epithelial tumors. Gynecol Oncol. 121(3):462–465. [PubMed: 21388660]
14. Sood AK, et al. Application of the National Cancer Institute international criteria for determination of microsatellite instability in ovarian cancer. Cancer Res. 2001; 61(11):4371–4374. [PubMed: 11389062]
15. Pal T, et al. Systematic review and meta-analysis of ovarian cancers: estimation of microsatellite-high frequency and characterization of mismatch repair deficient tumor histology. Clin Cancer Res. 2008; 14(21):6847–6854. [PubMed: 18980979]
16. Risch HA, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet. 2001; 68(3):700–710. [PubMed: 11179017]
17. Pal T, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005; 104(12):2807–2816. [PubMed: 16284991]
18. Wenham RM, et al. Polymorphisms in BRCA1 and BRCA2 and risk of epithelial ovarian cancer. Clin Cancer Res. 2003; 9(12):4396–4403. [PubMed: 14555511]
19. Fedor HL, De Marzo AM. Practical methods for tissue microarray construction. Methods Mol Med. 2005; 103:89–101. [PubMed: 15542899]
20. Jourdan F, et al. Tissue microarray technology: validation in colorectal carcinoma and analysis of p53, hMLH1, and hMSH2 immunohistochemical expression. Virchows Arch. 2003; 443(2):115–121. [PubMed: 12802583]
21. Hecht JL, et al. Validation of tissue microarray technology in ovarian cancer: results from the Nurses' Health Study. Cancer Epidemiol Biomarkers Prev. 2008; 17(11):3043–3050. [PubMed: 18990746]
22. Rosen DG, et al. Validation of tissue microarray technology in ovarian carcinoma. Mod Pathol. 2004; 17(7):790–797. [PubMed: 15073602]

23. Rosen B, et al. Systematic review of management options for women with a hereditary predisposition to ovarian cancer. Gynecol Oncol. 2004; 93(2):280–286. [PubMed: 15099934]

24. Seidman JD, et al. The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. Int J Gynecol Pathol. 2004; 23(1):41–44. [PubMed: 14668549]

25. Jacobs TW, et al. HER-2/neu protein expression in breast cancer evaluated by immunohistochemistry. A study of interlaboratory agreement. Am J Clin Pathol. 2000; 113(2): 251–258. [PubMed: 10664627]

26. Wolff AC, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007; 25(1):118–145. [PubMed: 17159189]

Anticancer Res. Author manuscript; available in PMC 2014 February 10.

Table I

Demographic and Clinical Variables (IHC testing) of Ovarian Cancer Cases by Tumor Specimen Type

	Overall (N=487)	Full Sections (n=147)	0.6 mm cores (n=126)	1 mm cores (n=214)	P-value
Age (Mean (SD))	56.1 (11.8)	55.0 (11.3)	55.7 (13.0)	57.0 (11.5)	0.286
Time to diagnosis in days - Median (range)	153 (0, 1729)	118 (43, 884)	663.5 (0, 1729)	97 (3, 830)	<0.001
Histology					
Borderline, n (%)	73 (15.0)	34 (23.1)	18 (14.3)	21 (9.8)	
Invasive, n (%)	414 (85.0)	113 (76.9)	108 (85.7)	193 (90.2)	
Serous, n (%)	256 (61.8)	78 (69)	59 (54.6)	119 (61.7)	0.031
Non-Serous, n (%)	110 (26.6)	23 (20.4)	39 (36.1)	48 (24.9)	
Clear cell	23 (5.5)	6 (5.3)	5 (4.6)	12 (6.2)	0.264
Endometriod	61 (14.7)	9 (7.9)	26 (24.1)	26 (13.5)	
Mucinous	26 (6.3)	8 (7.1)	8 (7.4)	10 (5.2)	
Other*	48 (11.6)	12 (10.6)	10 (9.3)	26 (13.4)	
Family History (based on 1st and 2nd degree relatives)					
# (%) of subjects with relatives with CRC	86 (17.6)	22 (15)	25 (19.8)	39 (18.2)	0.565
# (%) of subjects with relatives with EC	28 (5.7)	9 (6.1)	4 (3.2)	15 (7)	0.335
# (%) of subjects with relatives with any HNPCC cancers**	168 (34.5)	52 (35.4)	39 (31)	77 (36)	0.632

* Other includes the following histologies: Carcinoma, unspecified (26), Mixed cell (13), Peritoneal (3), and Transitional cell carcinoma (6);

** HNPCC cancers included: colorectum, endometrium, other gastrointestinal tract, urinary tract, ovary and brain;

Abbreviations: CRC: colorectal cancer; EC: endometrial cancer.

Table II

IHC Results of MMR Expression in Epithelial Ovarian Cancer by Specimen Type

LoE Score Cut-Off Value	Overall (n=487)		Full Sections (n=147)	1 mm cores (n=214)	0.6 mm cores (n=126)	P-value	P-value (1 mm vs. 0.6 mm)
	LoE (n (%))	95% CI	LoE (n (%))	LoE (n (%))	LoE (n (%))		
LoE defined as ≤3	62 (12.7)	10.0–16.1	1 (0.7)	34 (15.9)	27 (21.4)	<0.001	0.234
LoE defined as ≤4	112 (23.0)	9.4–27.0	20 (13.6)	59 (27.5)	33 (26.2)	0.003	0.795

Table III

Loss of MMR Protein Expression (LoE) in Epithelial Ovarian Cancer Cases with TMA Slides

	Row Total, n	LoE	No LoE	P-value
Proportions in TMA slides - n (%)	340	61/340 (17.9)	279/340 (82.1)	**<0.001**
Age - Mean (SD)	340	56.4 (13.1)	56.5 (11.8)	0.927
Histology				
Borderline, n (%)	39	6 (1.8)	33 (9.7)	0.821
Invasive, n (%)	301	55 (16.2)	246 (72.4)	
Serous, n (%)	178	24 (9.1)	154 (58.1)	**0.003****
Non-Serous, n (%)	87	25 (9.4)	62 (23.4)	
Clear cell	17	6 (6.9)	11 (12.6)	0.364
Endometriod	52	12 (13.8)	40 (46.0)	
Mucinous	18	7 (8.0)	11 (12.6)	
Other	36	6 (16.7)	30 (83.3)	-
Family History				
Relatives with Colorectal Cancer (CRC)				
# (%) of subjects with relatives with CRC	64	14 (4.1)	50 (14.7)	0.466
# (%) of subjects with no relatives with CRC	276	47 (13.8)	229 (67.4)	
Relatives with Endometrial Cancer (EC)				
# (%) of subjects with relatives with EC	19	3 (0.9)	16 (4.7)	0.955*
# (%) of subjects with no relatives with EC	321	58 (17.1)	263 (77.4)	
Relatives with any HNPCC cancers				
# (%) of subjects with relatives with any HNPCC cancers	116	23 (6.8)	93 (27.4)	0.544
# (%) of subjects with no relatives with any HNPCC cancers	224	38 (11.2)	186 (54.7)	

** Test between Serous and Non Serous, excluding other.