Validation of Tissue Microarray Technology in Ovarian Cancer: Results from the Nurses' Health Study

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Abstract

Background: **Tissue microarrays (TMAs) allow high-throughput evaluation of protein expression from archived tissue samples. We identified characteristics specific to ovarian cancer that may influence TMA interpretation.**

Methods: **TMAs were constructed using triplicate core samples from 174 epithelial ovarian cancers. Stains for p53, Ki-67, estrogen receptor-α, progesterone receptor, Her-2, WT-1, cytokeratin 7, and cytokeratin 20 were evaluated by intraclass correlation coefficients, Spearman correlation coefficients, the effect of sample age, and tumor histology on the ability to score the cores, and inter-rater reliability.**

Results: **The interclass correlation coefficient and the mean Spearman correlation coefficients among 3 cores were ≥0.91 and 0.87, respectively. Tissue age and tumor histology were not predictive of an inability to evaluate stains, but borderline tumors had a 2 to 4-fold increase in the risk of having uninterpretable cores over invasive tumors. There was moderate to substantial concordance between the two pathologists for estrogen receptor-α [Cohen's Kappa (κ), 0.79] and Ki-67 (κ, 0.52). The prevalence of positive staining cells by histologic type was comparable with previous studies.**

Conclusion: **TMA is a valid method for evaluating antigen expression in invasive ovarian cancer but should be used with caution for borderline tumors. We suggest several methods of quality control based on intercore comparisons and show that some antigens may be affected by age of the samples.** (Cancer Epidemiol Biomarkers Prev 2008;17(11):3043–50)

Introduction

Tissue microarrays (TMAs), assembled by taking core needle biopsies of pre-existing paraffin-embedded tissues, allow efficient evaluation of protein expression from archived tissue samples. The simultaneous staining of hundreds of cases minimizes the cost, time, and variability in experimental conditions. Acceptance of TMA data presumes that tissue cores are representative of larger sections and a minimal variability in tissue quality among cores. Most validation studies comparing whole sections to core samples have found duplicate and triplicate cores from each case to be representative of the entire tumor (1). Nevertheless, there is no guarantee that this conclusion is true for all immunostains, tumor types, and population samples. Because validation against whole sections is impractical for every new array and stain, an intra-array validation procedure is essential.

We present a novel approach to TMA validation using intercore comparisons to address the question of adequate tumor representation. The optimum number of cores and effect of tissue characteristics on staining including sample age and tumor histology has not been thoroughly explored for ovarian tumors (2). We evaluated staining for several commonly expressed antigens in ovarian tumors using a triplicate-core TMA including 174 women in the Nurses' Health Study (NHS). Our population-based study required the retrieval of tumor blocks from multiple hospitals with variable storage time. Our specific aims were to evaluate the effect of tissue characteristics (i.e., age of sample, histology, borderline, and invasion) on staining, to estimate the number of core biopsies required to ensure adequate representation of the entire tumor, and to calculate inter-rater reliability for stain interpretation.

Materials and Methods

Study Population. The NHS is a prospective cohort study that collects data on cancer incidences and dietary, hormonal, lifestyle, and environmental exposures (3). Cases of epithelial ovarian tumors including borderline (n = 21), invasive (n = 139), and primary peritoneal (n = 14) types were identified by biennial questionnaire from 1976 to 2002 or death certificate followed by diagnostic confirmation and coding of the

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Note: J.L. Hecht and J. Kotsopoulos contributed equally and should be considered co-first authors.

Condensed abstract: TMAs constructed using triplicate core samples can be successfully used to evaluate antigen expression in invasive ovarian cancer but should be used with caution for borderline tumors.

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Table 1. Antibodies and retrieval methods

Antibody (clone)	Dilution	Retrieval
CK7 (M7018)	1:1,000	Proteinase
CK20 (M7019)	1:50	Proteinase
ER (1D5)	1:200	Citrate
Ki-67 (M7240)	1:200	Citrate
p53 (IM1767)	1:1,200	Citrate
PR (PgR 636)	1:50	Citrate
WT-1 (M3561)	1:100	Citrate

NOTE: Citrate, 30-min heat retrieval in 1 × citrate buffer (pH 6.Zymed), pressure cooker. Proteinase, DakoCytomation Proteinase K (code No. S 3020) for 5 min at 25°C.

tumor characteristics (invasive versus borderline, histologic type, and stage) by a gynecologic pathologist (Jonathan L. Hecht; ref. 4). Representative paraffin-embedded tissue blocks from the primary excision were requested for each case. Available blocks were matched to a corresponding H&E-stained slide and the histologic diagnosis was confirmed. The initial fixation, processing, and storage conditions of these tissues is largely unknown as blocks came from multiple hospitals across the United States but are assumed to follow standard practices of clinical labs. Ethical approval for the use of the tissue was approved by the Brigham and Women's Hospital institutional review board.

TMA Construction. The TMA was assembled using a manual tissue arrayer (Beecher Instruments). Three 0.6-mm tissue cores were taken from each targeted lesion and placed into a recipient block with a spacing of 0.8 mm from core center to core center. After construction, 4-μm sections were cut and stained with H&E on the initial slides to verify the histologic diagnosis. Slides were cut from the TMA block for immunohistochemical staining.

Immunohistochemistry. Microarray slides were processed and stained within 2 weeks of cutting. Five-micrometer sections were soaked in Xylene overnight to remove any adhesive from the tape transfer system. Slides were deparaffinized and antigens were retrieved and stained with the primary antibodies at the dilutions presented in Table 1. The antibodies used were monoclonal (mouse) with p53 purchased from Immunotech and all others from DakoCytomation. The primary antibodies were detected either using a biotin-free, horseradish peroxidase enzyme–labeled polymer conjugated to either goat anti-mouse or anti-rabbit secondary antibodies (EnVision+ Systems; Dako).

Scoring. Levels of staining were subjectively graded by a gynecologic pathologist (JLH). For each stain, this was based on the number of reactive versus total cells and were categorized as 0%, 1% to 10%, 11% to 25%, 26% to 50%, and >50%. Three spots from the same case were independently assessed and analyzed. Spots where tissue was missing from the slide or where only a few cell clusters (an arbitrary cutoff was set at <20 cells) were present, were subsequently designated as not interpretable and are called ''missing'' (hereafter called the term ''Missingness'') in the remainder of the article (see Fig. 1). For the estrogen receptor (ER)-α and Ki-67 immunostaining, one of the two TMA slides was graded by a second pathologist (not listed as an author) to assess inter-rater reliability. Staining intensity was not used in our analysis due to the variability in tissue processing at the originating hospitals.

Statistical Analysis. The within- and between-person variances were estimated for each stain using scores from the three core specimens per tumor using a random effects model. Using these variances, the intraclass correlation coefficient (ICC) was estimated for each stain and, in the context of this paper, provides an estimate of the reproducibility across the three core specimens per tumor sample. The ICC was calculated by dividing the between-person variance by the sum of the between-person and the within-person variance and is a measure of reproducibility of replicate measures from the same subject (5). An ICC of <0.4 indicates poor reproducibility, 0.4 to 0.75 indicates fair to good reproducibility, and ≥0.75 indicates excellent reproducibility. The natural logarithm of the stain values was used for the ICC calculations because the transformed values were more normally distributed. The Spearman correlation coefficient (ρ) was used to evaluate whether staining between the three cores for each protein were correlated. The mean ρ of the three cores subsequently was used in the Spearman-Brown formula to estimate the number of cores required for staining to obtain a representative measure reflecting the entire tumor sample (6). For comparability with the ICC calculations, we used ≥0.75 as a cut-point for high reliability. We also stratified by invasive versus borderline tumor morphology and by the age of the tumor block (ages ≤10 y old versus >10 y).

The Fisher's exact test was used to examine positive staining for the seven stains by histologic type, as well as by the age of the tumor block, using the categories defined above. Positive staining was expressed as the percentage of positively stained cells using the maximum value of the three cores. This value was highly correlated to the mean and median of the three cores. The cores were dichotomized into positive if >10% of cells stained positive and negative if 10% or less stained.

Unconditional logistic regression was used to evaluate whether the age of the tumor block (ages ≤10, >10 y), presence of invasion (invasive, borderline) or histology (serous including poorly differentiated and primary peritoneal, endometrioid, mucinous, clear cell, other) were associated with missingness, which was defined as having one or more of the TMA cores from a particular tumor sample be assigned as missing. Borderline tumors, blocks age ≤10 y, and tumors of serous histology were used as the reference categories. The odds ratios and 95% confidence intervals are reported.

For the validation study, we calculated the percent concordance between the scores of the two pathologists for ER-α and Ki-67 staining and the (κ) statistic, which takes into account the agreement occurring by chance. Overall concordance was evaluated for each of the three cores in a case and also by using the core with the maximum value for each tumor. The weighted Cohen's κ (κ) coefficient and associated 95% confidence interval are reported. Agreement was considered poor if κ is <0.0, slight if κ is 0.00 to 0.20, fair if κ is 0.21 to 0.40, moderate if κ is 0.41 to 0.60, substantial if κ is 0.61 to 0.80, and almost perfect if κ is >0.80 (7). The maximum value of the 3 cores for ER-α and Ki-67 was then dichotomized (positive if >10% of cells stained). Percent agreement was calculated

using these binary scores. All analyses were done using SAS version 9.1 (SAS Institute, Inc.).

Results

Study Population. A total of 672 ovarian cancer cases were eligible for tumor block collection (Table 2). Of these, we received tissue from 346 (51%) women, including 254 (38%) tumor blocks and 279 (42%) tumor slides. We did not receive a block or slide for 326 (49%) of the eligible cases for various reasons, including that the tissue had been destroyed (n = 177; 54%), the patient was deceased (n = 36; 11%), various hospital-specific reasons (i.e., they could not find the requested sample, required a fee, did not respond, refused to send a sample; n = 109; 34%), and other miscellaneous reasons (n = 4; 1%). For the 346 cases for which we received tissue, 172 were not included in the TMA because no tumor block was available (i.e., only a slide was sent from hospital; n = 76; 44%), we did not receive the primary tumor sample

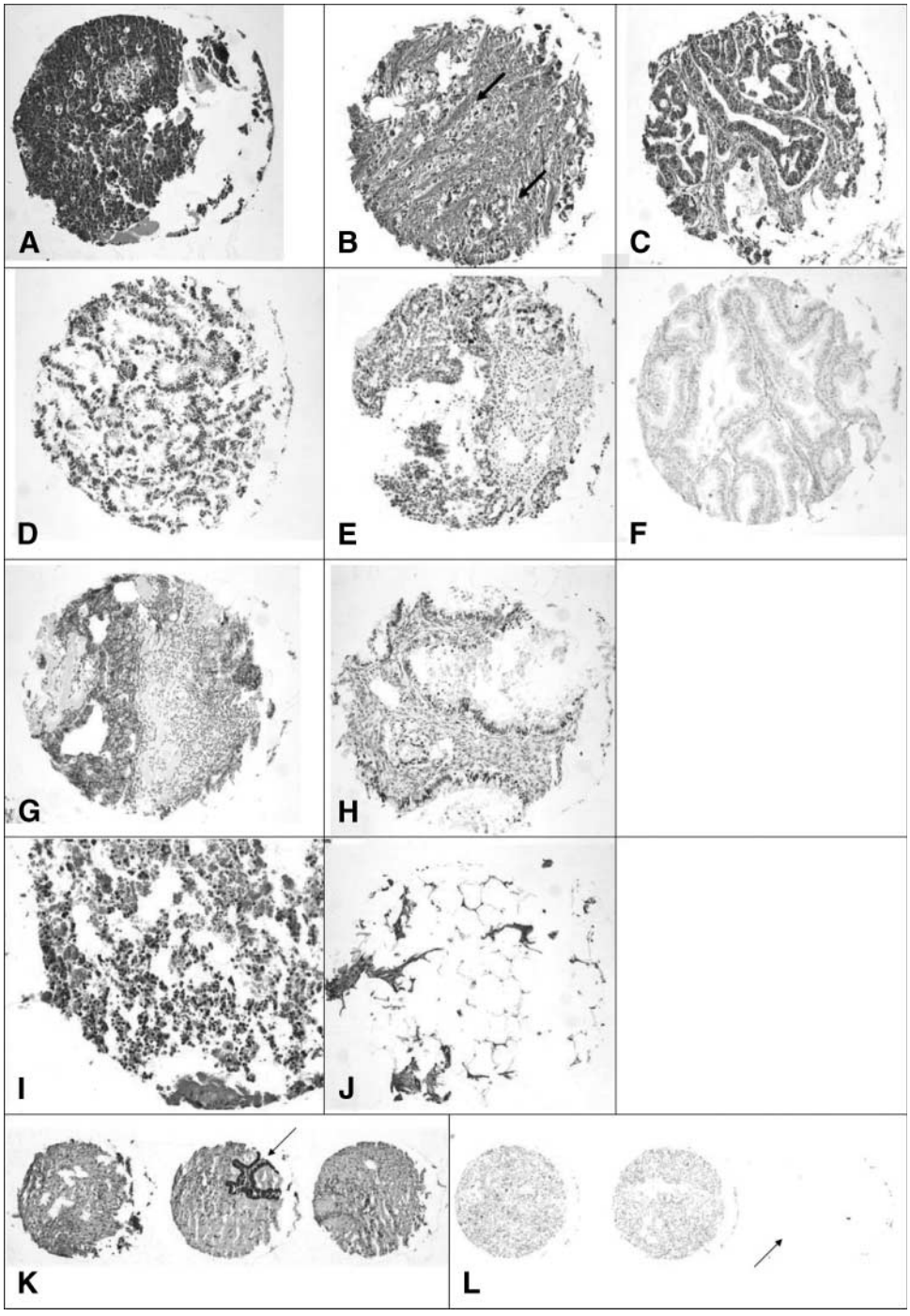

Figure 1. Missingness in the tissue array. Tissue preservation varied among samples. Minimal criteria for evaluation of a "spot" on the tissue array required the presence of viable and representative tumor (**A,** serous carcinoma, sheet-like growth pattern; **B,** serous carcinoma, infiltrative pattern. **C,** mucinous carcinoma. **D** to **F,** examples of nuclear staining for ER with 100%, 15%, and 100% positive cells, respectively. Notice that although **F** shows less intense staining, scoring is based on the proportion of cells. **G** to **H,** examples of membranous staining for cytokeratin CK7 with 100% and <10%, respectively). Multiple cores are taken from each sample to increase the chance of including tumor, but tumor volume varies among spots [**I,** the tumor present is entirely necrotic. **J,** tumor in this core has been exhausted, only fat from deeper in the tissue block is present. **K,** only one of three cores from the same tissue block of this serous borderline tumor shows epithelial cells, (*arrow*). **L,** the third core of this tumor was lost during processing of an immunostain]. Original magnification, ×100. H&E or immunoperoxidase stain with hematoxalyn counterstain.

Table 2. Characteristics of Nurses' Health Study sample eligible for inclusion in the TMA

Characteristic	Eligible cases in the NHS	Cases in the NHS with a tumor block or slide	Cases included in the final TMA analysis
N (%)	672 (100%)	346 (51%)	174 (26%)
Age at diagnosis, mean (SD)	59.4 (9.3)	61.7 (9.2)	61.2 (8.9)
Lapse between date of diagnosis and date of tissue collection, years, mean (SD)	12.6 (6.8)	10.2 (5.9)	10.0 (6.0)
Lapse between date of diagnosis and date of tissue collection, *n* (%)*			
0 to 5 y	112 (17%)	80 (23%)	43 (25%)
>5 to 10 y	160 (24%)	124 (36%)	61 (35%)
≥10 to 27 y	385 (59%)	142 (41%)	70 (40%)
Parity, mean (SD) †	3.8 (1.7)	3.8 (1.7)	3.8 (1.7)
Oral contraceptive use, ever, *n* (%)	248 (37%)	137 (40%)	71 (41%)
Tubal ligation, ever, *n* (%)	70 (10%)	45 (13%)	22 (13%)
Body mass index in 1990, mean (SD)	25.6 (5.3)	25.4 (5.0)	25.1 (5.0)
Histology, *N* (%) ‡			
Serous	430 (64%)	215 (62%)	102 (59%)
Endometrioid	97 (14%)	55 (16%)	30 (17%)
Mucinous	64 (10%)	29 (8%)	15 (9%)
Clear cell	32 (5%)	16 (5%)	12 (7%)
Other§	49 (7%)	31 (9%)	15 (9%)
Morphology, *n* (%)			
Borderline	72(11%)	35 (10%)	21 (12%)
Invasive	598 (89%)	309 (89%)	153 (88%)
Unknown	2 (<0.3%)	2 (1%)	0
Disease stage, *n* (%)			
I, II	231 (34%)	120 (35%)	74 (43%)
III, IV	426 (64%)	220 (63%)	100 (57%)
Other/unknown	15 (2%)	6 (2%)	0

Abbreviation: NHS, Nurses' Health Study.
*The total is not 672 due to missing information (tissue collection date).
† Among parous women.
‡ Histologic classification based on pathology report. Samples included in the TMA were verified by microscope review.
§ Other includes transitional, carcinosarcoma, mixed histology, or unknown.

(*n* = 30; 17%), the tumor was not an ovarian or peritoneal primary (*n* = 17; 10%), or the material was insufficient for analysis or was noncancerous tissue (*n* = 49; 28%). Thus, for the current study, 174 (26%) of eligible tumor blocks were included in the TMA.

The average time lapse between the date of cancer diagnosis and the date of tissue collection was 12.6 years for all the eligible cases, 10.2 years for the cases from whom we obtained a tissue sample, and 10.0 years among the cases that were included in the final analysis (Table 2). The eligible cases, those with any tissue sample, and those in the TMA analysis, were similar with respect to their age at diagnosis and various other known risk factors for ovarian cancer including parity, oral contraceptive use, tubal ligation, and body mass index. The number of invasive carcinomas from each histologic subtype included in the array were 102 (59%) for serous (ovarian, peritoneal, and poorly differentiated), 30 (17%) for endometrioid, 15 (9%) for mucinous, 12 (7%) for clear cell, and 15 (9%) for other subtypes and was similar to the distribution observed in the total cohort of eligible cases (64% serous, 14% endometrioid, 10% mucinous, 5% clear cell, and 7% for the other histologic subtypes).

Homogeneity Among Cores. We evaluated the ICC and Spearman correlation coefficients, in all the tumors combined, and stratified by morphology (invasive or borderline) and age of the tissue sample, for each stain (Table 3). The ICCs were ≥0.91 for all tumors combined, ≥0.92 when limited to invasive tumors, and ≥0.79 for borderline tumors, for all of the 7 stains. When stratified by the age of the sample, the ICCs were ≥0.89 for samples ages ≤10 years and ≥0.91 for samples ages >10 years. Furthermore, the mean Spearman correlation coefficients between the three cores from each tumor sample were high for all the tumors combined ($\rho \geq 0.87$),

Table 3. ICCs for the reliability of the three tissue core samples

Antigen	All tumors*		Invasive		Borderline		≤10 y †		>10 y	
	ICC ‡	ρ§	ICC	ρ	ICC	ρ	ICC	ρ	ICC	ρ
Ki-67	0.94	0.93	0.93	0.92	0.96	1.00	0.89	0.88	0.95	0.94
PR	0.95	0.93	0.95	0.93	0.92	0.93	0.95	0.93	0.95	0.93
p53	0.97	0.96	0.97	0.96	0.80	0.75	0.98	0.97	0.96	0.95
ER-α	0.91	0.91	0.92	0.92	0.79	0.76	0.89	0.92	0.91	0.90
WT-1	0.98	0.98	0.99	0.99	0.85	0.93	>0.99	0.99	0.96	0.96
CK7	0.94	0.96	0.94	0.95	0.92	0.96	0.95	0.96	0.93	0.95
CK20	0.97	0.87	0.97	0.87	n/a‖	n/a	n/a	n/a	0.97	0.87

Abbreviation: n/a, not applicable.
*Range for the number of observations used (with nonmissing data): all tumors 413 to 486; invasive 376 to 400; borderline 45 to 52; ages ≤10 y, 154 to 181; ages >10 y, 178 to 303.
† Age was dichotomized into ages ≤10 and >10 y at of the time of staining.
‡ ICC were calculated using the using the log transformed values for each stain.
§ ρ is the mean Spearman correlation of three cores from each tumor sample.
‖ Too many missing observations (*n* = 47) to perform the calculation.

Table 4. Percentage of tissue samples staining positive, by histologic type

Antigen	Positive staining, *n* (%)*					
	Serous invasive	Serous borderline	Mucinous	Endometrioid	Clear cell	*P* †
	n = 68	*n* = 12	*n* = 17	*n* = 35	*n* = 17	
Ki-67	39 (57%)	1 (8%)	2 (12%)	14 (40%)	6 (35%)	0.001
PR	12 (18%)	5 (42%)	5 (29%)	18 (51%)	0 (0%)	<0.001
p53	39 (57%)	1 (8%)	4 (24%)	12 (34%)	1 (6%)	<0.001
ER-α	36 (53%)	6 (50%)	6 (35%)	19 (54%)	4 (24%)	0.26
WT-1	61 (90%)	8 (67%)	3 (18%)	13 (37%)	1 (6%)	<0.001
CK7	57 (84%)	12 (100%)	14 (82%)	16 (46%)	13 (76%)	<0.001
CK20	6 (9%)	0 (0%)	7 (41%)	1 (3%)	0 (0%)	0.001

*Calculated using the maximum value of the three cores that was then dichotomized (positive if staining in >10% of cells).
† *P* value calculated using Fisher's exact test using the maximum value of the three cores from each tumor block.

as well as by the presence of invasion ($\rho \geq 0.87$ and $\rho \geq 0.75$ for invasive and borderline tumors, respectively) and age of the tumor block ($\rho \geq 0.87$ for both age categories) for each of the stains. Using the Spearman-Brown formula, we estimated that one core is sufficient to obtain a reliability of ≥0.75 for each antigen in all of the strata evaluated (data not shown).

Factors Affecting Staining Positivity. The proportion of cells staining positive varied by histology for Ki-67, progesterone receptor (PR), p53, WT-1, CK7, and CK20 ($P \leq 0.001$) but were relatively consistent across histology for ER-α (Table 4). Staining positivity for CK7 was relatively high across all the subtypes (46-100%). More than half of the serous invasive, 40% of the endometrioid, and 35% of the clear cell tumors expressed Ki-67. Expression of Ki-67 was low among serous borderline and mucinous tumors. Ninety percent of serous invasive, 67% of serous borderline, and 37% of endometrioid tumors expressed WT-1; however, expression in the other histologic subtypes was <20%. For CK20, 41% of mucinous samples stained positive, with <10% expression among the other subtypes. Fifty-seven percent of the serous invasive tumors expressed p53 and >40% of the endometrioid and serous borderline tumors expressed PR; however expression of these two antigens was fairly low among the other subtypes.

The age of the sample had no effect on the proportion of cells staining positive for PR, p53, WT-1, CK7, and CK20; however, for Ki-67 and ER-α, there was a significantly lower proportion of samples that stained positive among the stains ages >10 years (data not shown). For Ki-67, 62% of the samples ages <10 years stained positive versus 31% of those ages >10 years ($P = 0.04$). Similarly for ER-α, 62% of the samples obtained in the last 10 years stained positive compared with 40% of the older samples ($P = 0.05$). In our multivariate analyses, simultaneous adjustment for age, invasion, and histology did not substantially alter the results. Each staining protocol was optimized based on the staining intensity of the most reactive spots on a ''tester'' array containing cores from a subset of the study cases. No attempt was made to modify the protocol to compensate for our additional analysis by age of the stain and histologic subtype.

Factors Affecting Stain Missingness. We also examined whether the presence of invasion, specific tumor histology, or age of the tissue sample were associated with stain missingness (data not shown). There was a significant association between tumor invasion and missingness (e.g., the inability to interpret a stain). Borderline tumor samples were more likely to have missing stains on one or more TMA cores for p53, Ki-67, PR, WT-1, and CK20 stains than invasive samples, with a 2- to 4-fold increase in the risk of having missing cores compared with invasive tumors. However, this association only reached statistical significance for Ki-67 staining (RR, 4.0; 95% confidence interval, 1.5-11.0). The presence of invasion did not influence missingness for the ER-α and CK7 stains. Neither histologic classification nor the age of the tissue block was associated with missingness for any of the antigens ($P > 0.10$ for all antigens). For invasive ovarian cancer, we found that 76% to 90% of cases showed loss of no cores, 4% to 11% of cases showed loss of one core, 1 to 3% of cases showed

Table 5. Concordance and percent agreement between two pathologists

	ER-α	Ki-67
Between Individual Cores	*n* = 288	*n* = 267
Cohen's κ (95% CI)	0.79 (0.74-0.83)	0.52 (0.45-0.59)
Percent agreement*	192 (73%)	141 (53%)
Between Maximum Stain of Three Cores	*n* = 101	*n* = 96
Cohen's κ (95% CI)	0.73 (0.65-0.82)	0.55 (0.44-0.66)
Percent agreement	63 (66%)	50 (52%)
Between dichotomized value †	*n* = 96	*n* = 96
κ (95% CI)	0.65 (0.50-0.81)	0.67 (0.53-0.82)
Percent agreement	81 (84%)	81 (84%)

Abbreviation: 95% CI, 95%, confidence interval.
* Number of cores that were scored the same by the two pathologists.
† The maximum stain of the three cores was dichotomized (positive if staining in >10% of cells).

loss of 2 cores, and 1% to 18% of cases showed loss of all 3 cores. For borderline tumors, we found that 60% to 75% of cases showed loss of no cores, 5% to 15% of cases showed loss of one core, 5% to 15% of cases showed loss of two cores, and 0% to 20% of cases showed loss of all 3 cores.

Concordance and Percent Agreement between the Two Pathologists. There was moderate agreement between scoring by the two pathologists for Ki-67 overall, as well as when the maximum value from the three cores was used ($\kappa = 0.52$ and $\kappa = 0.55$, respectively; Table 5). For Ki-67, percent agreement was 53% between the individual cores, 52% when using the maximum stain value of the 3 cores, and 84% using the binary scores. There was substantial agreement between the individual cores for the scoring of ER-α ($\kappa = 0.79$ and $\kappa = 0.73$, respectively). Percent agreement for ER-α was 73% between the individual cores, 66% using the maximum stain value of the 3 cores, and 84% using the binary scores. Discordance between the individual cores was 27% for ER-α and 47% for Ki-67. When we independently considered those observations for which the scoring of the two pathologists disagreed, the scoring differed by only one category for 85% of discordant observations for individual cores, and 100% when using the binary scores, for both Ki-67 and ER-α.

Discussion

We present a novel approach to TMA validation using intercore comparisons to address the question of adequate tumor representation. TMA technology is a valid method of evaluating protein expression in invasive ovarian tumors. Our results suggest that the use of one core specimen per tumor sample provides an accurate representation of the entire tumor as assessed by three cores. We also observed moderate to substantial concordance for ER-α and Ki-67 staining across different raters, demonstrating acceptable inter-rater reliability of this technique. Tumor invasion was a significant predictor of having one or more cores with missing stains and thus use of TMA should be considered carefully when evaluating borderline tumors.

Immunohistochemistry on TMAs has been validated for several tumor types with the general conclusion that three 0.6-mm cores produce adequate representation of whole sections (8). In the largest of the studies that include ovarian cancer, Rosen et al. (9) evaluated the number of cores required to adequately represent the expression patterns of Ki-67, ER-α, and p53 in whole sections of ovarian tumors. In that study, the probability that the analysis of a single core corresponded to the staining pattern of a whole section was 91%, and the analysis of 2 and 3 cores increased the reliability to 95% and 96%, respectively. Analysis of additional cores did not improve the reliability. We showed concordant staining among three cores representing each tumor, suggesting that staining is uniform and thus likely to be representative of whole sections. Overall, the ICCs were high for all of the 7 stains (>0.90), suggesting minimal within-tumor variation. Future studies that plan to evaluate the expression of other, less characterized, proteins should similarly evaluate reliability and ICC over multiple cores from the same tumor.

Loss of tissue spots for selected cores may be due to processing of individual TMA sections or to heterogeneity of the tumor tissue in individual cores (10, 11). The degree of variation is specific to tumor type and architectural complexity. In the current study, morphology was strongly predictive of stain missingness; whereas, histologic subtype and the age of the tumor sample were not. Because borderline tumors are more often cystic or papillary in architecture (12) and more prone to tissue loss, TMAs should be used cautiously when evaluating ovarian tumors of borderline origins.

The age of the sample, tissue preparation, and storage conditions are all factors that may affect antigen preservation in tissue arrays constructed from archival blocks (13). Antigen preservation may vary substantially throughout the tissue samples. We did not find such heterogeneity in staining as suggested by the high ICCs between cores, and most antigens were not affected by the storage time of the original tumor block. Two stains, ER-α and Ki-67, however, showed less staining in the older cases suggesting an age effect; curiously but reassuringly, the proportion of cases in each histologic subtype was not affected by age. This could not clearly be explained by time trends, tumor histology, or tumor invasion. It is possible that temporal trends in factors such as postmenopausal hormones may affect the hormone receptor status of ovarian tumors, as is seen with breast cancer where postmenopausal hormone use is predictive of ER-positive cancers (14). Although postmenopausal hormone use has declined in the years since the publication of the results from the Women's Health Initiative (15), the annual number of prescriptions for hormone replacement therapy in 1975 was 36 million in 1992, 58 million in 1995, and 90 million in 1999 through 2002, indicating wide-spread use during the period of diagnosis of the cases in the current study (16). Further studies evaluating a role of various exposures and ovarian tumor hormone status are warranted.

Rimm et al. (1) have shown that archival tissue samples retain their antigenicity over decades for ER-α, PR, Her2, Ki-67, and CK in breast carcinoma. It is not clear why our findings differ but may be related to tissue storage practices. A warm basement location for long-term block storage is not unusual. The 10-year interval may also be significant because many states have laws requiring storage of blocks for only 10 years; it may be that blocks are transferred to different storage after that time.

To evaluate interrater reliability, concordance between the scoring of the two pathologists for the ER-α and Ki-67 stains was compared. We observed substantial concordance for ER-α and moderate concordance for Ki-67. The results were similar when agreement was evaluated between the triplicate cores and when using the maximum value of the three cores, and most scores differed by only one unit. It is reassuring, however, that the percent agreement improved substantially for both ER-α and Ki-67 staining when the binary values were used, suggesting that it may be difficult for the pathologist to distinguish between those stains that decrease within the intermediate categories. One would expect a decrease in the κ statistic when using a dichotomous value versus a continuous or categorical variable because of an increase in agreement attributed to chance. As expected, we observed increased agreement

for ER-α and Ki-67 but a decrease in κ only for ER-α and increase for Ki-67. The increased variability between raters suggests that future epidemiologic studies using TMAs for ovarian cancer should either use one pathologist to maintain consistency, or alternatively, ensure that the pathologists follow well-characterized guidelines and use standardized protocols.

We evaluated the expression of commonly described antigens expressed by ovarian cancer and the pattern of staining was comparable with that described in the literature. As expected, invasive serous expression of p53 and Ki-67 was high, whereas expression in borderline tumors was low (17, 18). Mucinous tumors express CK7 and CK20 with higher frequency than other histologies (19), but they have infrequent expression of WT-1, p53, and Ki-67 (20). Endometrioid carcinomas (all grades) normally show moderate expression of p53 and Ki-67 and a similar CK7/CK20 pattern to serous tumors (21), yet CK7 expression in our population was slightly lower compared with the serous cancers. Clear cells rarely express p53, WT-1 or CK20 but do express CK7 (22, 23). This pattern of expression was confirmed in our study. Similar to other studies, ER-α and PR were variably expressed across all the tumor histologies (2, 22, 24, 25).

There were 672 eligible ovarian cancer cases available for this study; however, we were only able to obtain a tissue sample of any kind for 52% of these cases and a tumor block for TMA analysis in 26%. This relatively low retrieval rate was primarily attributed to the fact that almost 60% of the diagnoses were made >10 years in the past, and hence, the tissue had already been destroyed when the request was made. The decrease in the time lapse between the diagnosis date and the date of tissue collection for samples included in the TMA suggests that we were more likely to receive blocks or slides from the hospital when the time lapse between diagnosis and collection was smaller. Moreover, the patient characteristics of all the eligible cases were similar to those used in the present article, suggesting that the cases in the TMA are representative of the overall eligible case set.

The limitations associated with our study include the small sample size despite the large number of women initially eligible for analysis. Although we were not able to retrieve a tissue block for more than half of the eligible cases in this study, the introduction of bias in our results is minimal because the proportion of tumors of each histology are similar to the whole cohort. The small sample size after stratification by histologic type may have hindered our ability to detect significant differences between the subgroups.

Conclusion. The current study adds experience to the use of TMAs for ovarian tumors. Unlike the majority of array validation studies that compare core staining to whole sections, we evaluated the expression of common antigens in ovarian cancer by comparing the staining among three cores from each tumor. Overall, we observed that the analysis of protein expression in at least one 0.6-mm core in TMA blocks was sufficient to represent the whole tumor section. Inter-rater reliability of stains was reasonable and the staining of p53, ER-α, PR, Ki-67, WT-1, CK7, and CK20 by histologic subtype was similar to that observed in previous studies. In addition, we observed that borderline morphology, but not age of the tumor block or tumor histology, was associated with the inability to interpret several stains, suggesting caution when analyzing borderline tumors. Overall, TMAs seem to be a valid method for analyzing protein expression via immunohistochemistry for ovarian and peritoneal carcinomas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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