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Malignancy Assessment Using Gene Identification in Captured Cells Algorithm for the Prediction of Malignancy in Women With a Pelvic Mass

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OBJECTIVE: To evaluate the detection of malignancy in women with a pelvic mass by using multiplexed gene expression analysis of cells captured from peripheral blood.

METHODS: This was an IRB-approved, prospective clinical study. Eligible patients had a pelvic mass and were scheduled for surgery or biopsy. Rare cells were captured from peripheral blood obtained preoperatively by using a microfluidic cell capture device. Isolated mRNA from the captured cells was analyzed for expression of 72 different gene transcripts. Serum levels for several commonly assayed biomarkers were measured. All patients had a tissue diagnosis. Univariate and multivariate logistic regression analyses for the prediction of malignancy using gene expression and serum biomarker levels were performed, and receiver operating characteristic curves were constructed and compared.

RESULTS: A total of 183 evaluable patients were enrolled (average age 56 years, range 19–91 years). There were 104 benign tumors, 17 low malignant potential tumors, and 62 malignant tumors. Comparison of the area under the receiver operating characteristic curve for individual genes and various combinations of genes with or without serum biomarkers to differentiate between benign conditions (excluding low malignant potential tumors) and malignant tumors showed that a multivariate model combining the expression levels of eight genes and four serum biomarkers achieved the highest area under the curve (AUC) (95.1%, 95% CI 92.0–98.2%). The MAGIC (Malignancy Assessment using Gene Identification in Captured Cells) algorithm significantly outperformed all individual genes (AUC 50.2–65.2%; all $P<.001$) and a multivariate model combining 14 different genes (AUC 88.0%, 95% CI 82.9–93.0%; $P=.005$). Further, the MAGIC algorithm achieved an AUC of 89.5% (95% CI 81.3–97.8%) for stage I–II and 98.9% (95% CI 96.7–100%) for stage III–IV patients with epithelial ovarian cancer.

CONCLUSION: Multiplexed gene expression evaluation of cells captured from blood, with or without serum biomarker levels, accurately detects malignancy in women with a pelvic mass.

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The prevalence of pelvic masses is relatively common, with 5–10% of women being diagnosed with a mass at some point in their lifetime.[1] Annually in the United States, more than 200,000 women undergo surgery for a pelvic mass, with 13–21% being malignant.[2] It is critical to accurately assess the risk for malignancy in women who present with a pelvic mass before surgery, because morbidity and mortality outcomes are significantly improved when women with ovarian cancer have surgery performed by gynecologic oncologists experienced in the management of ovarian cancer.[3–5] Currently, history and physical, imaging, serum CA 125 levels and formal biomarker algorithms are included in the pelvic mass assessment guidelines from the American College of Obstetricians and Gynecologists.[6] Despite these guidelines, only 30–50% of women diagnosed with ovarian cancer are referred to gynecologic oncologists.[7,8] Although improvements have been made in the triage of women presenting with a pelvic mass by the addition of U.S. Food and Drug Administration–cleared predictive algorithms such as ROMA® (Risk of Ovarian Malignancy Algorithm) and OVA1®, further improvements are needed.[6,9–12]

Emerging technologies for the isolation and interrogation of cells captured from blood, comprised of circulating tumor cells (CTCs) and other rare circulating cells (RCCs), present a novel opportunity for the detection and potential characterization of malignancy with a simple peripheral blood test.[13] This study was designed to evaluate multiplexed gene expression of cancer related targets in cells captured from peripheral blood (CTCs and RCCs) using a liquid biopsy system, alone or in combination with serum biomarkers, for detection of malignancy in women with a pelvic mass.

METHODS

This prospective clinical study, registered with clinicaltrials.gov (NCT02781272), was approved by the University of Rochester Wilmot Cancer Institute IRB and conducted through the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Wilmot Cancer Institute at the University of Rochester. Any patient diagnosed with an ovarian cyst or pelvic mass who was scheduled for an imaging guided biopsy, surgical biopsy, or surgical excision for definitive tissue diagnosis of their pelvic mass was eligible for enrollment into the trial. All patients were required to have a pelvic mass documented by imaging (ultrasonography, computed tomography, or magnetic resonance imaging) within 90 days before surgery or biopsy. Patients with a malignancy diagnosed within the prior 5 years were excluded from enrollment into the study, except for patients with skin cancers (squamous cell or basal cell).

A convenience sample of 200 patients with a pelvic mass was selected based on patient availability and the expected incidence of malignancy in this referred population of 20–30%. The goal was to obtain samples from a minimum of 50 women diagnosed with a malignant pelvic mass to evaluate the ability of using multiplexed gene expression evaluation of cells captured by the Parsortix® system from preoperatively collected peripheral blood (CTCs and RCCs) for the identification of malignancy in women with a pelvic mass.

Imaging guided biopsy, surgical biopsy, or surgical excision for evaluation of the pelvic mass was performed by a qualified physician. All tissue samples were evaluated by the gynecologic oncology pathology team at the University of Rochester pathology department. Results of the histopathologic evaluation, including the final diagnosis, along with histologic subtype and stage of cancer (if present), were documented. Only patients with a confirmed histologic diagnosis were considered evaluable. Race, age, and menopausal status information was recorded for all patients to determine the demographics of the population of women included in the study. All data collected were independently source verified.

Blood samples were obtained within 30 days before or on the day of surgery or biopsy of the pelvic mass and before anesthesia. Up to 35 mL of whole blood was collected by venipuncture into the following evacuated blood collection tubes for all patients: one 5-mL serum separating tube followed by three separate 10-mL K_2EDTA tubes. The 5-mL serum separating tube was drawn first and was used for serum biomarker testing, followed by the collection of blood into the three K_2EDTA tubes, which were pooled and used for the capture and harvest of circulating cells. The collection of the serum separating tube first helps to avoid contamination of the K_2EDTA samples with squamous or noncirculating cells that might be collected as a result of the venipuncture. The blood collected into the K_2EDTA tubes was pooled and two equal volume aliquots (7.5–15

mL each, 12.2 mL on average) were processed using Parsortix PR1 Systems for the capture of circulating cells (CTCs and RCCs) based on their size and deformability.[14] The captured cells from each aliquot of blood were harvested into a single tube (each cell harvest consisted of 210 microliters of phosphate-buffered saline, so a combined harvest contained 420 microliters of phosphate-buffered saline and cells), and the captured circulating cells were lysed with RLT buffer containing 1% 2-mercaptoethanol (if a single harvest was collected, 600 microliters of RLT buffer was added to the harvest [total lysate volume of 810 microliters]; if two harvests were combined, 1,200 microliters of RLT buffer was added to the combined harvest [total lysate volume of 1,410 microliters]). The cell lysate from each patient was split into two equal volume aliquots and stored frozen at −180°C in liquid nitrogen. Using the RNeasy Micro Kit, RNA was purified from one of the lysate aliquots and subsequently analyzed using a highly multiplexed gene expression assay to evaluate the expression of 72 different gene transcripts representing 52 different ovarian cancer–associated genes and eight different housekeeping genes (Table 1). The ovarian cancer–associated genes were identified through a literature search as being primarily related to ovarian cancer or to cancer in general. The housekeeping genes were included to help gauge the quality of the RNA obtained, as well as the relative quantity of blood cells (primarily white blood cells) present in the lysates.

Serum obtained from blood collected into the 5-mL serum separating tube was used to measure seven different serum biomarkers related to ovarian cancer and menopausal status that are currently in use in one or more multivariate biomarker algorithms for pelvic mass risk assessment. Serum levels of CA 125, HE4, transthyretin (prealbumin), apolipoprotein A1, transferrin, β-2-microglobulin, and follicle-stimulating hormone were determined for each patient. All serum biomarker testing was performed through the University of Rochester Laboratories and ARUP Laboratories.

The primary objective of the study was to evaluate the ability of cancer-related gene expression in circulating cells captured from peripheral blood using a liquid biopsy system, alone or in combination with serum biomarkers, to detect malignancy in patients with a pelvic mass. Univariate logistic regression and multivariate backward-stepwise logistic regression analyses of the gene expression results and the serum biomarkers, alone and in combination, were performed to develop algorithms for the differentiation of various histopathologic diagnosis groupings of the evaluable patients (eg, benign or low malignant potential vs all cancers, benign vs all cancers). Receiver operating characteristic curves were constructed for each univariate and multivariate model, the area under the curve (ROC-AUC) was determined, and the equality of the ROC-AUC was tested ("roccomp" command in Stata). Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio of a positive test, likelihood ratio of a negative test, and overall accuracy for the multivariate logistic regression models were determined using various thresholds for the predictive probabilities.

RESULTS

A total of 200 patients with an ovarian cyst or pelvic mass were enrolled into the study between June 2016 and April 2017, of which 183 (91.5%) were evaluable. The mean and median age of the evaluable cohort was 56 years (range: 19–91). Of these patients, 68 (37.2%) were premenopausal and 115 (62.8%) were postmenopausal as determined through history and clinical exam. Demographics and final pathology diagnoses for the 183 evaluable patients are summarized in Tables 2 and 3.

Of the 183 evaluable patients, 104 (56.8%) were diagnosed with benign disease, 17 (9.3%) were diagnosed with low malignant potential tumors, 42 (23.0%) were diagnosed with ovarian cancer, 14 (7.6%) were diagnosed with nonovarian gynecologic cancer, and six (3.3%) were diagnosed with nongynecologic metastatic cancers. For the 37 (20.1%) patients diagnosed with invasive epithelial ovarian cancer, 12 (32.4%) had a stage I disease, four (10.8%) had stage II, nine (24.4%) had stage III, and 12 (32.4%) had stage IV.

The areas under the receiver operating characteristic curve (ROC-AUC) of the individual genes and serum biomarkers for their ability to discriminate between various histopathologic diagnosis groupings of the 183 evaluable patients are reported in Table 4. Analysis of the expression of individual genes in the captured circulating cells (CTCs and RCCs) through RNA interrogation for the prediction of benign (excluding low malignant potential tumors) compared with malignancy (all cancers) did not produce any significant results when examining each gene alone. The highest ROC-AUC found for any one individual gene was seen for *FN1* (transcript 1) when differentiating between benign or low malignant potential compared with all cancers, with an ROC-AUC of 65.2% (95% CI 56.6–73.7%), followed by *CCR2* (transcript 2) and *CD274* when differentiating between

Table 1. Gene Transcripts Analyzed

Gene Name	Description
*AFP**	Alpha fetoprotein
*AGR2**	Anterior gradient 2
*CCNE2**	Cyclin E2
CCR2 (transcript 1)*	C-C chemokine receptor type 2
CCR2 (transcript 2)*	C-C chemokine receptor type 2
*CD274**	Programmed death-ligand 1 (PDL1)
CD45 (transcript 1)†	Protein tyrosine phosphatase receptor type C (PTPRC)
CD45 (transcript 2)†	Protein tyrosine phosphatase receptor type C (PTPRC)
*CDH1**	Epithelial cadherin (E-cadherin)
*CDH2**	Neuronal cadherin (N-cadherin)
*CDH3**	Placental cadherin (P-cadherin)
CDH5 (transcript 1)*	Vascular endothelium cadherin (VE-cadherin)
CDH5 (transcript 2)*	Vascular endothelium cadherin (VE-cadherin)
*CEACAM5**	Carcinoembryonic antigen-related cell adhesion molecule 5
*CHI3L1**	Chitinase-3-like protein 1 (YKL-40)
*CLDN3**	Claudin 3
*CXCR4**	C-X-C chemokine receptor type 4 (CD184)
*EMP2**	Epithelial membrane protein 2
EN2 (transcript 1)*	Homeobox protein Engrailed-2
EN2 (transcript 2)*	Homeobox protein Engrailed-2
*EPCAM**	Epithelial cell adhesion molecule
*ERBB2**	Receptor tyrosine-protein kinase erbB-2 (HER2)
ERBB3 (transcript 1)*	Receptor tyrosine-protein kinase erbB-3 (HER3)
ERBB3 (transcript 2)*	Receptor tyrosine-protein kinase erbB-3 (HER3)
*ERCC1**	DNA excision repair protein ERCC-1
*ESR1**	Estrogen receptor 1 (ER)
FN1 (transcript 1)*	Fibronectin 1
FN1 (transcript 2)*	Fibronectin 1
*FOXJ1**	Forkhead box protein J1
*FXYD3**	FXYD domain containing ion transport regulator 3
*GPX8**	Glutathione peroxidase 8 (putative)
*HE4**	Human epididymis protein 4
*HJURP**	Holliday junction recognition protein
HSP90AB1†	Heat shock protein 90 alpha family class B member 1
*HUWE1**	HECT, UBA and WWE domain containing 1, E3 ubiquitin protein ligase
*INHA**	Inhibin alpha
*INHBA**	Inhibin subunit beta A
*KRT20**	Keratin 20 (CK20)
*KRT7**	Keratin, type II cytoskeletal 7 (CK7)
*LAMB1**	Laminin subunit Beta-1
*MAL2**	T-cell differentiation protein 2
*MSLN**	Mesothelin
*MUC1**	Cell surface–associated mucin 1 (EMA)
*MUC16**	Cell surface–associated mucin 1 (CA 125)
*NOTCH1**	Notch homolog 1, translocation-associated (drosophila)
PAX8 (transcript 1)*	Paired box gene 8
PAX8 (transcript 2)*	Paired box gene 8
PAX8 (transcript 3)*	Paired box gene 8
*PGR**	Progesterone receptor (PR)
*PLAT**	Plasminogen activator, tissue type
PPIA†	Peptidylprolyl isomerase A
*PPIC**	Peptidyl-prolyl cis-trans isomerase C
*PRAME**	Preferentially expressed antigen in melanoma
RPL13A†	60S ribosomal Protein L13a
RPL4 (transcript 1)†	60S ribosomal protein L4
RPL4 (transcript 2)†	60S ribosomal protein L4
RPLP0 (transcript 1)†	60S acidic ribosomal protein P0

(*continued*)

Table 1. Gene Transcripts Analyzed (*continued*)

Gene Name	Description
RPLP0 (transcript 2)†	60S acidic ribosomal protein P0
*S100A16**	S100 calcium-binding protein A16
*SCGB2A2**	Secretoglobin family 2A member 2 (Mammaglobin-A)
*SEPT2**	Septin 2
*SERPINE2**	Serpin family E member 2
*SLC6A8**	Sodium and chloride-dependent creatine transporter 1
TBP†	TATA box binding protein
*TFF1**	Trefoil factor 1
TPT1 (transcript 1)†	Translationally controlled tumor protein
TPT1 (transcript 2)†	Translationally controlled tumor protein
TPT1 (transcript 3)†	Translationally controlled tumor protein
*TUSC3**	Tumor suppressor candidate 3
*VCAM1**	Vascular cell adhesion molecule 1
*VEGFA**	Vascular endothelial growth factor A
*VIM**	Vimentin

* Ovarian cancer–associated genes.
† Housekeeping genes.

benign compared with all cancers, with ROC-AUCs of 64.4% (95% CI 55.9–72.9%) and 64.2% (95% CI 55.6–72.9%), respectively. The individual serum biomarkers had higher ROC-AUCs than any of the individual genes, with the highest being HE4 in all cases, but particularly when differentiating between benign or low malignant potential compared with epithelial ovarian cancer only with an ROC-AUC of 89.4% (95% CI 83.4–95.3%).

Backward-stepwise multivariate logistic regression analysis of the gene expression results alone, the serum biomarkers alone, and the gene expression results and serum biomarkers in combination for their ability to discriminate between various histopathologic diagnosis groupings of the 183 evaluable patients are reported in Table 5. Comparison of the ROC-AUCs between the gene-only and serum biomarkers–only multivariate algorithms for the discrimination between the various histopathologic diagnosis groupings showed nonsignificant differences (all $P>.38$). However, combining gene expression analysis with serum biomarker analysis produced the highest ROC-AUCs compared with either serum markers alone or gene expression results alone (Table 5). In most instances, a comparison of the ROC-AUCs for the gene-only and serum biomarkers–only algorithms to the combination algorithms showed significant differences ($P<.05$). The combination of genes and serum biomarkers to differentiate benign compared with all cancers showed that a multivariate model combining the expression of eight genes and four serum protein biomarkers achieved the highest ROC-AUC. This algorithm, referred to hereafter as the MAGIC (Malignancy Assessment using Gene Identification in

Table 2. Patient Demographics

Demographic	All Evaluable Patients (N=183)	Normal or Benign Tumors (n=104, 56.8%)	LMP Tumors (n=17, 9.3%)	All Cancers (n=62, 33.9%)
Age (y)	56±13 56 (19–91)	53±12 53 (20–82)	51±12 52 (24–67)	62±14 62 (19–91)
Menopausal status				
Premenopausal	68 (37.2)	49 (47.1)	8 (47.1)	11 (17.7)
Postmenopausal	115 (62.8)	55 (52.9)	9 (52.9)	51 (82.3)
Race				
Black	13 (7.1)	8 (7.7)	1 (5.9)	4 (6.5)
White	166 (90.7)	94 (90.4)	15 (88.2)	57 (91.9)
None of the above	4 (2.2)	2 (1.9)	1 (5.9)	1 (1.6)

LMP, low malignant potential.
Data are average±SD, median (range), or n (%).

Table 3. Summary of Histologic Subtypes for Benign and Malignant Tumors

Classification	Histology	Premenopausal	Postmenopausal	All
Benign		49 (47.1)	55 (52.9)	104 (56.8)
Benign condition	Serous cystadenoma, cystadenofibroma	7	22	29
	Mucinous cystadenoma, cystadenofibroma	4	8	12
	Endometriosis	13	2	15
	Dermoid, teratoma	1	3	4
	Fibrothecoma	0	3	3
	Fibroids (leiomyoma)	7	8	15
	Cysts (eg, corpus luteum, paratubal, follicular, hemorrhagic, simple)	17	9	26
Borderline or LMP		8 (47.1)	9 (52.9)	17 (9.3)
LMP tumor	Serous	4	5	9
	Mucinous	4	4	8
Malignant		11 (17.7)	51 (82.3)	62 (33.9)
Ovarian cancer	EOC serous	3	16	19
	EOC mucinous	0	4	4
	EOC endometrioid	2	4	6
	EOC clear cell	0	3	3
	EOC mixed	2	3	5
	Sex cord, stromal (granulosa cell)	2	3	5
Nonovarian gynecologic cancer	Endometrial	0	9	9
	Uterine sarcoma	0	5	5
Nongynecologic metastatic cancer	Colon	0	1	1
	Appendix	1	0	1
	Gastric	0	2	2
	Other (lymphoma, inflammatory myofibroblastic tumor)	1	1	2

LMP, low malignant potential; EOC, epithelial ovarian cancer.
Data are n (%) or n unless otherwise specified.

Captured Cells) algorithm, was composed of the gene expression of *PPIA*, *TBP*, *TPT1* (transcripts 2 and 3), *WFDC-2* (HE4), *INHA*, *VEGFA*, *CCR2*, and *SEPT2*, plus serum levels of β-2-microglobulin (B2M), transferrin, CA 125 and HE4, and achieved an ROC-AUC of 95.1% (95% CI 92.0–98.2%) for the discrimination of benign tumors from all cancers. The MAGIC algorithm significantly outperformed all individual genes (ROC-AUC 50.2–65.2%; all $P<.001$), the serum biomarkers–only algorithm (ROC-AUC 89.6%, 95% CI 84.3–95.0%), and the gene-only algorithm (ROC-AUC 88.0, 95% CI 82.9–93.0%, $P=.005$) for the discrimination of benign tumors from all cancers (Table 5 and Fig. 1).

Table 6 provides the estimates of sensitivity, specificity, negative predictive value, positive predictive value, accuracy, likelihood ratio of a positive test, and likelihood ratio of a negative test for the MAGIC algorithm, the serum biomarkers–only algorithm, and the gene expression–only algorithm at set specificities of approximately 65%, approximately 75%, approximately 85%, 95%, and 100% for the discrimination of benign tumors (excluding low malignant potential tumors) compared with any malignancy. Further, the MAGIC algorithm achieved an ROC-AUC of 89.5% (95% CI 81.3–97.8%) for patients with stage I–II epithelial ovarian cancer and 98.9% (95% CI 96.7–100%) for patients with stage III–IV disease (data not shown).

DISCUSSION

Accurate risk stratification for patients presenting with a pelvic mass is essential for optimum management. Surgical intervention for patients with ovarian cancer performed by a gynecologic oncologist is associated with improved morbidity and mortality and better overall survival.[5,7,15–20] Despite this, it is estimated that only 30–40% of patients with an ovarian malignancy undergo their initial surgery by a gynecologic oncologist.[7,20–23] In recent years, there has been significant research in the development multiple biomarker algorithms to predict malignancy in patients with a pelvic mass. The serum biomarkers HE4 and CA 125 havebeen shown to be the markers with the most value for detecting malignancies when used in multiple marker algorithms.[12,24–26] Analysis of serum biomarkers measured in the current trial found similar

Table 4. Area Under the Receiver Operating Characteristic Curve Analysis of the Individual Genes and Serum Biomarkers for Their Ability to Differentiate Between Various Histopathologic Diagnosis Groupings of the Evaluable Patients

	Benign or LMP vs All Cancers			Benign vs All Cancers			Benign or LMP vs EOC Only			Benign vs Ovarian Cancers Only		
Gene or Biomarker Name	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)
AFPc	183	53.2	49.6–56.8	166	53.0	49.4–56.7	158	53.2	48.6–57.8	146	52.6	48.4–56.7
AGR2	183	52.7	46.7–58.8	166	52.1	45.8–58.3	158	52.6	45.2–60.0	146	51.7	44.6–58.9
CCNE2	183	50.4	41.7–59.1	166	52.1	43.2–61.0	158	53.1	43.2–63.0	146	55.4	45.6–65.2
CCR2 (transcript 1)	183	63.9	55.6–72.1	166	64.3	55.8–72.8	158	61.5	51.9–71.1	146	61.3	51.5–71.1
CCR2 (transcript 2)	183	64.3	56.0–72.5	166	64.4	55.9–72.9	158	61.9	52.1–71.6	146	61.5	51.6–71.4
CD274	183	64.1	55.7–72.6	166	64.2	55.6–72.9	158	61.7	51.5–72.0	146	60.3	50.2–70.5
CD45 (transcript 1)	183	52.9	44.1–61.7	166	54.1	45.1–63.2	158	51.3	40.8–61.8	146	51.1	40.7–61.4
CD45 (transcript 2)	183	58.6	49.9–67.3	166	59.1	50.2–68.0	158	55.4	44.8–66.0	146	57.0	46.7–67.2
CDH1	183	53.1	45.4–60.8	166	53.8	45.8–61.8	158	55.3	46.3–64.3	146	56.7	48.0–65.4
CDH2	183	50.5	41.9–59.1	166	50.3	41.3–59.3	158	50.6	40.0–61.2	146	50.4	39.9–60.8
CDH3	183	57.4	50.0–64.8	166	57.2	49.5–64.9	158	57.4	48.6–66.2	146	57.5	48.8–66.2
CDH5 (transcript 1)	183	57.3	50.1–64.5	166	57.4	50.1–64.7	158	54.5	45.9–63.1	146	55.2	47.0–63.5
CDH5 (transcript 2)	183	58.3	50.8–65.8	166	57.7	50.1–65.4	158	54.0	45.2–62.8	146	55.3	46.6–64.0
CEACAM5	183	56.0	47.3–64.7	166	56.2	47.1–65.3	158	57.8	47.1–68.5	146	56.6	45.9–67.3
CHI3L1	183	56.3	47.6–64.9	166	57.6	48.8–66.5	158	58.3	48.0–68.5	146	56.9	46.6–67.2
CLDN3	183	53.6	44.8–62.4	166	52.8	43.8–61.9	158	50.5	39.6–61.4	146	52.1	41.8–62.4
CXCR4	183	51.5	42.8–60.3	166	51.8	42.7–60.8	158	50.6	40.0–61.3	146	50.4	40.1–60.7
EMP2	183	50.3	41.4–59.2	166	50.6	41.6–59.7	158	54.1	43.4–64.8	146	54.4	44.2–64.6
EN2 (transcript 1)	183	56.0	48.9–63.1	166	57.1	50.0–64.3	158	54.7	46.1–63.3	146	56.4	48.2–64.7
EN2 (transcript 2)	183	51.6	42.6–60.6	166	50.9	41.7–60.1	158	52.2	41.3–63.1	146	52.5	41.8–63.2
EPCAM	183	53.0	45.0–60.9	166	52.8	44.5–61.0	158	50.9	41.3–60.4	146	50.6	41.5–59.7
ERBB2	183	50.4	41.3–59.5	166	50.6	41.3–59.9	158	50.9	39.8–62.1	146	50.8	40.1–61.5
ERBB3 (transcript 1)	183	51.0	44.5–57.5	166	51.1	44.5–57.7	158	50.1	42.1–58.1	146	51.6	44.1–59.1
ERBB3 (transcript 2)	183	50.3	41.8–58.7	166	51.0	42.3–59.7	158	55.8	45.9–65.7	146	57.6	48.0–67.2
ERCC1	183	58.1	49.5–66.7	166	58.7	49.8–67.6	158	53.3	43.3–63.3	146	55.1	45.1–65.1
ESR1	183	52.3	43.8–60.8	166	51.5	42.7–60.3	158	54.3	44.5–64.1	146	52.7	43.0–62.4
FN1 (transcript 1)	183	65.5	57.1–73.8	166	65.2	56.6–73.7	158	58.6	48.5–68.7	146	60.2	50.2–70.2
FN1 (transcript 2)	183	62.2	53.3–71.0	166	62.7	53.6–71.7	158	55.0	44.6–65.5	146	58.0	47.6–68.4
FOXJ1	183	59.0	50.4–67.5	166	60.8	52.1–69.6	158	53.5	43.1–64.0	146	56.0	45.9–66.2
FXYD3	183	59.1	50.8–67.3	166	59.9	51.4–68.3	158	50.4	40.7–60.2	146	54.9	45.2–64.6
GPX8	183	55.5	46.2–64.9	166	55.9	46.5–65.4	158	50.0	38.5–61.6	146	50.7	39.7–61.6
HE4	183	60.2	51.5–68.8	166	60.9	52.0–69.8	158	56.1	45.4–66.8	146	56.6	46.3–67.0
HJURP	183	55.7	47.0–64.4	166	57.0	48.1–65.9	158	52.5	42.5–62.5	146	50.7	40.7–60.7
HSP90AB1	183	56.1	47.5–64.8	166	55.8	46.9–64.7	158	53.9	43.7–64.1	146	54.8	44.7–64.9
HUWE1	183	56.0	47.4–64.5	166	55.7	46.9–64.6	158	52.5	42.2–62.7	146	52.6	42.5–62.7
INHA	183	59.7	50.8–68.6	166	60.1	50.9–69.3	158	57.7	46.4–69.0	146	58.6	47.8–69.4
INHBA	183	50.5	43.2–57.8	166	50.2	42.7–57.7	158	54.1	45.7–62.5	146	54.3	46.2–62.4
KRT20	183	51.0	47.1–54.9	166	51.2	47.1–55.3	158	51.5	47.1–55.9	146	52.0	47.8–56.2
KRT7	183	56.8	48.3–65.4	166	58.5	49.7–67.3	158	53.3	43.4–63.3	146	53.8	44.0–63.7
LAMB1	183	61.0	52.4–69.7	166	61.8	52.9–70.6	158	55.6	44.7–66.5	146	57.2	46.7–67.7
MAL2	183	51.2	47.6–54.8	166	51.4	47.7–55.0	158	50.7	46.5–54.9	146	50.5	46.7–54.3
MSLN	183	55.3	47.1–63.5	166	55.7	47.2–64.1	158	54.4	44.6–64.2	146	56.8	47.2–66.5
MUC1	183	61.5	52.8–70.1	166	61.9	53.0–70.8	158	57.5	47.1–68.0	146	58.6	48.5–68.8
MUC16	183	52.1	46.8–57.4	166	52.2	46.8–57.6	158	50.4	44.4–56.4	146	50.9	45.0–56.8
NOTCH1	183	55.7	46.9–64.5	166	56.6	47.5–65.6	158	52.1	41.1–63.0	146	52.3	41.7–62.9
PAX8 (transcript 1)	183	54.7	45.4–63.9	166	54.8	45.2–64.4	158	53.8	42.6–65.0	146	51.1	40.1–62.0
PAX8 (transcript 2)	183	55.3	49.0–61.6	166	55.8	49.3–62.1	158	51.2	44.3–58.1	146	51.7	45.0–58.3
PAX8 (transcript 3)	183	50.2	42.4–58.0	166	50.3	42.2–58.3	158	52.7	43.6–61.8	146	51.8	42.8–60.8
PGR	183	52.1	48.9–55.3	166	52.2	48.8–55.6	158	52.3	48.7–55.9	146	52.6	49.1–56.1
PLAT	183	62.1	54.6–69.6	166	62.3	54.6–69.9	158	59.6	50.6–68.7	146	58.6	50.0–67.2
PPIA	183	55.2	46.5–63.9	166	56.6	47.7–65.5	158	51.5	41.4–61.6	146	55.4	45.2–65.5
PPIC	183	63.3	54.8–71.7	166	62.7	54.0–71.4	158	58.9	48.5–69.4	146	58.3	48.2–68.5
PRAME	183	52.3	45.2–59.3	166	53.6	46.5–60.8	158	50.7	42.4–59.1	146	50.5	42.7–58.3
RPL13A	183	53.2	44.4–62.1	166	53.8	44.7–62.9	158	50.6	40.0–61.2	146	52.8	42.3–63.3
RPL4 (transcript 1)	183	54.8	46.1–63.5	166	56.0	47.0–64.9	158	51.0	40.8–61.3	146	52.9	42.7–63.2
RPL4 (transcript 2)	183	57.3	48.7–65.8	166	59.5	50.7–68.2	158	52.1	41.9–62.2	146	55.9	45.8–66.0
RPLP0 (transcript 1)	183	57.4	48.9–66.0	166	57.7	48.9–66.5	158	55.0	44.9–65.1	146	56.2	46.3–66.2
RPLP0 (transcript 2)	183	55.1	46.4–63.8	166	54.6	45.6–63.7	158	55.2	45.0–65.4	146	56.0	45.9–66.2
S100A16	183	55.6	47.0–64.2	166	54.6	45.7–63.4	158	54.0	43.6–64.5	146	52.9	42.8–63.1
SCGB2A2	183	52.0	45.3–58.7	166	52.0	45.1–58.9	158	52.7	44.6–60.9	146	52.6	44.7–60.5

(*continued*)

Table 4. Area Under the Receiver Operating Characteristic Curve Analysis of the Individual Genes and Serum Biomarkers for Their Ability to Differentiate Between Various Histopathologic Diagnosis Groupings of the Evaluable Patients (*continued*)

	Benign or LMP vs All Cancers			Benign vs All Cancers			Benign or LMP vs EOC Only			Benign vs Ovarian Cancers Only		
Gene or Biomarker Name	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)	n	ROC-AUC (%)	95% CI (%)
SEPTIN2	183	58.9	50.4–67.4	166	59.1	50.3–67.8	158	53.0	43.0–63.1	146	53.0	43.0–63.1
SERPINE2	183	54.9	45.9–63.9	166	55.0	45.8–64.2	158	52.1	41.5–62.7	146	51.3	40.9–61.7
SLC6A8	183	57.6	48.8–66.5	166	57.5	48.4–66.7	158	55.7	45.1–66.2	146	57.5	47.3–67.8
TBP	183	57.0	48.5–65.4	166	56.8	48.0–65.6	158	54.9	45.0–64.7	146	54.9	45.0–64.8
TFF1	183	54.4	45.5–63.3	166	54.0	44.8–63.2	158	50.3	39.7–60.9	146	50.6	40.2–61.1
TPT1 (transcript 1)	183	59.7	51.1–68.4	166	61.7	53.0–70.5	158	55.7	45.0–66.4	146	57.1	46.8–67.4
TPT1 (transcript 2)	183	58.2	49.6–66.8	166	59.5	50.7–68.3	158	55.3	44.8–65.7	146	55.9	45.8–66.0
TPT1 (transcript 3)	183	58.2	49.8–66.7	166	58.4	49.7–67.2	158	57.5	47.8–67.2	146	57.9	48.3–67.6
TUSC3	183	59.5	51.6–67.5	166	59.7	51.4–67.9	158	54.6	45.1–64.0	146	56.5	47.1–65.8
VCAM1	183	57.5	49.6–65.4	166	57.2	49.0–65.3	158	52.4	43.2–61.6	146	51.0	42.1–59.9
VEGFA	183	62.1	53.8–70.3	166	63.1	54.6–71.7	158	56.9	46.9–66.8	146	59.7	49.9–69.4
VIM	183	56.9	48.1–65.6	166	57.7	48.7–66.6	158	56.7	46.3–67.1	146	58.1	47.8–68.4
Prealbumin	183	75.6	68.0–83.2	166	76.0	68.3–83.7	158	73.8	65.2–82.4	146	73.3	64.3–82.3
Apolipoprotein A1	181	64.3	56.0–72.6	164	66.6	58.1–75.1	156	56.0	45.6–66.4	144	59.9	49.8–70.0
Transferrin	183	75.9	68.5–83.3	166	77.5	70.0–85.0	158	71.9	61.8–82.0	146	74.5	65.2–83.8
B2M	183	70.3	62.2–78.3	166	69.9	61.6–78.2	158	68.9	58.7–79.0	146	66.4	56.4–76.4
FSH	183	51.6	42.6–60.6	166	50.4	41.2–59.6	158	63.2	53.6–72.8	146	54.5	44.0–65.1
CA 125	183	80.0	73.2–86.8	166	82.6	76.0–89.2	158	81.0	72.3–89.7	146	83.8	76.1–91.5
HE4	183	83.6	76.9–90.4	166	85.1	78.4–91.7	158	89.4	83.4–95.3	146	85.1	77.3–93.0

LMP, low malignant potential; EOC, epithelial ovarian cancer; FSH, follicle-stimulating hormone.

results to previous studies, which showed that the addition of other biomarkers to HE4 and CA 125 do not significantly improve the performance over the dual marker combination of HE4 and CA 125 for the differentiation of benign masses from malignant pelvic masses.[25–30] The additive properties of serum biomarkers to detect malignancy may open the door to markers that are associated with, but perhaps not specific to, epithelial ovarian cancer. Although this could contribute to overall improvement in sensitivity of an algorithm, it could also lead to decreased specificity and little incremental value to the statistical performance of a dual marker algorithm.[25] For these reason,s novel methods for detecting malignancies outside of serum biomarker analysis is needed.

Circulating tumor cells are cells that originate and detach from solid tumors and can be detected in the peripheral circulation of patients with malignancies. Circulating tumor cells are rare and have been estimated to have a ratio of 1 in 100 million to 1 in 1 billion cells in circulating blood.[31,32] Multiple techniques and newer technologies have been developed to capture CTCs, which have now been demonstrated to be present in the blood of nearly all late-stage solid tumor patients and have also been shown to be present in early-stage disease.[33–35] The presence of CTCs in blood has been shown to be a useful prognostic indicator in patients with primary and metastatic cancers.[36–38] Gene expression profiling has been used in prognostic calculators in malignancies, such as multiple myeloma, breast cancer, and gliomas.[39–42] The Parsortix system used in the current study employs a microfluidic based particle separation technology, with a gradient stepwise cassette that captures cells based on their size and deformability. Most blood cells pass across the steps and through the terminal gap and cells with increased size and rigidity are captured on the cassette and preserved. This system has been shown to efficiently capture RCCs and CTCs that can then be harvested and used for gene expression analysis.[14,43] The liquid-biopsy and multiplex gene expression technologies used in the current trial are being investigated for detection of multiple cancer types, including ovary, endometrial, breast, prostate, and lung cancer.

In ovarian cancer, gene expression in CTCs is an emerging field not yet incorporated into existing clinical risk-stratification models. Recently, research has identified that in addition to transcoelomic spread, there is significant hematogenous spread, which may play a significant role in the metastatic spread to the omentum.[44,45] Circulating tumor cell isolation, enrichment, and molecular characterization has been reported in patients with ovarian cancer. Kolostova et al reported on 40 patients using a cytomorphologic approach to identify captured cells for gene expression analysis. Comparison of the relative gene

Table 5. Area Under the Receiver Operating Characteristic Curve Analysis and Comparisons of the Multivariate Algorithms for the Gene Expression Results Alone, the Serum Biomarkers Alone, and the Combination of Gene Expression and Serum Biomarkers Results for Their Ability to Differentiate Between Various Histopathologic Diagnosis Groupings of the Evaluable Patients

Multivariate Logistic Regression Algorithms		Benign or LMP vs All Cancers			Benign vs All Cancers			Benign or LMP vs EOC Only			Benign vs Ovarian Cancers Only		
		n	ROC-AUC (%)	ROC-AUC (95% CI) (%)	n	ROC-AUC (%)	ROC-AUC (95% CI) (%)	n	ROC-AUC (%)	ROC-AUC (95% CI) (%)	n	ROC-AUC (%)	ROC-AUC (95% CI) (%)
Serum biomarkers only	Included in algorithm	Prealbumin, transferrin and HE4			Prealbumin, transferrin and HE4			Prealbumin and HE4			B2M, transferrin, CA 125 and HE4		
Genes only	Performance	183	87.8	82.2–93.3	166	89.6	84.3–95.0	158	88.4	81.2–95.5	146	89.6	83.7–95.4
	Included in algorithm	*EMP2, PPIA, CDH1, CDH5, PLAT, TPT1, MUC16, HUWE1, CHI3L1, CCR2, CD274* and *CXCR4*			*ERBB2, RPL4, ERBB3, CDH1, FN1, GPX8, PLAT, PAX8, TPT1, HUWE1, INHBA, CHI3L1, CCR2* and *CXCR4*			*EMP2, PPIA, PPIC, ERBB3, CDH1, CDH5, FN1, TPT1, MUC16, HUWE1, CHI3L1, CCR2* and *SEPT2*			*PPIA, PPIC, ERBB3, CDH1, CDH3, CDH5, FN1, GPX8, TFF1, PAX8, RPL13A, HSP90AB1, TPT1, MSLN, HUWE1, CHI3L1, CCR2* and *SEPT2*		
Genes and serum biomarkers	Performance	183	84.5	78.8–90.2	166	88.0	82.9–93.0	158	84.9	78.5–91.3	146	87.9	82.0–93.7
	Included in algorithm	*EPCAM, PPIA, CDH1, TPT1, HE4, HUWE1, INHBA, VEGFA* and *CCR2* plus B2M, transferrin and HE4			*PPIA, TBP, TPT1, HE4, INHA, VEGFA, CCR2* and *SEPT2* plus B2M, transferrin, CA 125 and HE4			*EMP2, CDH1, CDH5, FN1, TPT1, HUWE1* and *CCR2* plus prealbumin and HE4			*PPIA, RPLP0, TBP, TPT1* and *VEGFA* plus B2M, transferrin, CA 125 and HE4		
P for comparison of ROC-AUCs	Performance	183	92.6	88.2–97.0	166	95.1	92.0–98.2	158	94.7	91.5–97.8	146	93.9	89.8–98.1
	Serum vs genes	0.3896			0.6219			0.4805			0.6638		
	Serum vs combo	0.0722			0.0113			0.0317			0.0113		
	Genes vs combo	0.0089			0.0054			0.0018			0.0765		

LMP, low malignant potential; EOC, epithelial ovarian cancer; ROC-AUC, area under the receiver operating characteristic curve.

expression level in peripheral blood samples confirmed a statistically significant difference for gene expression of *KRT7*, *WT1*, *EPCAM*, *MUC16*, *MUC1*, *KRT18* and *KRT19*. The combination of these genes could suggest the presence of ovarian cancer CTCs in the peripheral blood of patients.[46]

The role of CTCs, and multiple CTC gene expression identification and quantification analyses in epithelial ovarian cancer have been explored. However, no studies have incorporated the CTC gene expression analysis and serum biomarker levels into a risk assessment tool such as the one constructed in this study. We evaluated the potential of combining the gene expression of circulating cells captured from blood by liquid biopsy (CTCs and RCCs) with serum biomarker levels for the detection of cancer in women with a pelvic mass before surgical intervention. Single genes and their predictive performances were analyzed. The highest performing individual gene was *FN1* with an AUC of 65.2%, followed by *CCR2* with an AUC of 64.4%. *CCR2* is the receptor for haptoglobin, which has been used as a nonspecific marker for inflammation, infection, and malignancy, including ovarian cancer. *CCR2* may have relation to the tumor microenvironment and cell migration, which could be why it performed so well compared with other single genes. However, its significance and clinical application in ovarian cancer are still unclear.[47] Similarly, multivariate analysis of the gene expression results alone compared with the serum biomarkers alone revealed that no combination outperformed the dual marker combination of HE4 and CA 125 for the prediction of benign (excluding low malignant potential tumors) compared with malignancy (all cancers and ovarian cancers only).

However, the unique grouping of eight genes with a total of nine different transcripts (*PPIA*, *TBP*, *TPT1* transcripts 2 and 3, *WFDC-2* [HE4], *INHA*, *VEGFA*, *CCR2*, and *SEPT2*) and four serum proteins (β-2-microglobulin, transferrin, CA 125, and HE4) comprising the MAGIC algorithm produced the highest ROC-AUC in this study (95.1%), which significantly outperformed any other gene or biomarker combination, including HE4 and CA 125. The MAGIC algorithm not only detected epithelial ovarian cancer, but also detected nonovary primary cancers, including metastatic cancers. At the same time, the MAGIC algorithm did not lose its performance regarding detection of early stage epithelial ovarian cancer, with an ROC-AUC for stage I–II disease of 89.5%. The inclusion of the *WFDC-2* gene, which produces the protein HE4, is of interest as HE4 has recently been

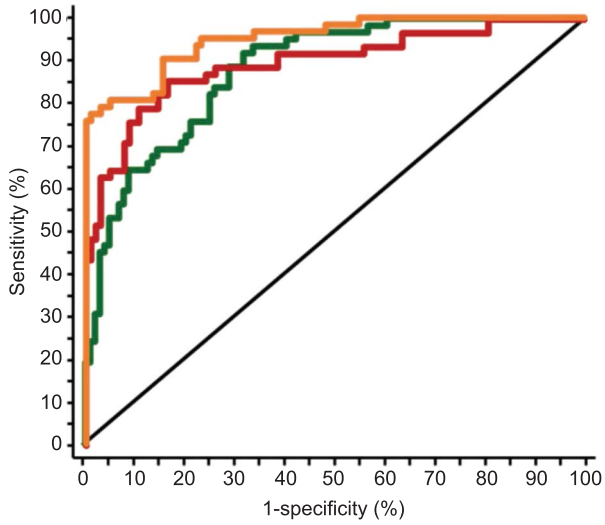

Fig. 1. Comparison of areas under receiver operating characteristic curves for the genes-only algorithm (*green*), the serum biomarkers–only algorithm (*red*), and the MAGIC (Malignancy Assessment Using Gene Identification in Captured Cells) algorithm (genes and serum biomarkers) (*orange*). Benign (n=104) vs all cancers (n=62).

Moore. MAGIC Algorithm for Prediction of Malignancy. Obstet Gynecol 2022.

shown to play an inhibitory role of $CD8^+$ infiltrating lymphocytes and inducing macrophages to the M2 nonactive form in the ovarian cancer tumor microenvironment.[48] Approximately 80% of epithelial ovarian cancers overexpress the HE4 protein, which may speak to the importance of the *WFCD-2* gene in pathogenesis.

A major strength of this study is that it is a large prospective clinical trial employing novel technology with the ability to capture circulating cells from blood (RCCs and CTCs) and analyze their gene expression. Often, biomarker and gene expression research are executed retrospectively, by using tissue biopsy samples through microarray analysis. All the patients in this study had gene expression testing from a prospective liquid biopsy of peripheral blood samples, in addition to serum testing using blood samples collected just before surgery. Moreover, the nature of biomarker and gene expression testing is inherently objective. The key measures for this model are not reliant on subjective factors such as physician assessment or imaging interpretation, which have been used by other risk-assessment models and, thus, subject to interpretive variation.[49–51] Additionally, the MAGIC algorithm has the advantage that it can also detect cancers of nonovarian origin, including metastatic cancers. A primary limitation of this study is that it was performed at a single tertiary care institution with patients who had been referred to the division of gynecologic oncology, and, therefore, the cohort may not represent the

Table 6. Performance Parameters of Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy, Likelihood Ratio of a Positive Test, and Likelihood Ratio of a Negative Test for the Following Multivariate Algorithms for the Differentiation of Benign Compared With All Cancers: the MAGIC (Malignancy Assessment Using Gene Identification in Captured Cells) Algorithm, the Serum Biomarkers–Only Algorithm, and the Gene Expression–Only Algorithm

Benign (n=104) vs All Cancers (n=62)	Predictive Probability Threshold (%)	Estimates (95% CI) (%)				
		Sensitivity	Specificity	Accuracy	LR+	LR−
MAGIC algorithm	10 or greater	95 (87–99)	66 (56–75)	77 (70–83)	2.8 (2.1–3.7)	13.8 (4.5–42.2)
	16 or greater	95 (87–99)	76 (67–84)	83 (77–88)	4.0 (2.8–5.6)	15.8 (5.5–66.6)
	30 or greater	90 (80–96)	85 (76–91)	87 (81–92)	5.9 (3.7–9.3)	8.7 (4.1–18.7)
	48 or greater	81 (69–90)	95 (89–98)	90 (84–94)	16.8 (7.1–39.9)	4.9 (3.0–8.2)
	67 or greater	76 (63–86)	100 (97–100)	91 (86–95)	—	4.1 (2.7–6.4)
Serum biomarkers–only algorithm	18 or greater	89 (78–95)	65 (55–74)	74 (67–81)	2.6 (1.9–3.4)	5.8 (2.8–11.8)
	20 or greater	87 (76–94)	74 (65–82)	79 (72–85)	3.4 (2.4–4.7)	5.7 (3.0–11.1)
	31 or greater	82 (70–91)	85 (76–91)	84 (77–89)	5.3 (3.4–8.5)	4.8 (2.8–8.2)
	60 or greater	65 (51–76)	95 (89–98)	84 (77–89)	13.4 (5.6–32.3)	2.7 (1.9–3.8)
	88 or greater	44 (31–57)	100 (97–100)	79 (72–85)	—	1.8 (1.4–2.2)
Gene expression–only algorithm	18 or greater	94 (84–98)	64 (62–80)	75 (69–83)	2.6 (2.0–3.4)	9.9 (3.8–25.8)
	20 or greater	79 (67–88)	75 (66–83)	77 (69–83)	3.2 (2.2–4.5)	3.6 (2.2–5.9)
	31 or greater	69 (56–80)	84 (75–90)	78 (71–84)	4.3 (2.7–6.8)	2.7 (1.9–4.0)
	60 or greater	52 (39–65)	95 (89–98)	79 (72–85)	10.8 (4.4–26.2)	2.0 (1.5–2.6)
	88 or greater	19 (10–31)	100 (97–100)	70 (62–77)	—	1.2 (1.1–1.4)

LR+, likelihood ratio of positive test; LR−, likelihood ratio of negative test; MAGIC, Malignancy Assessment using Gene Identification in Captured Cells.

general population. The MAGIC algorithm needs to be verified and validated in a separate real-world cohort comprised of patients from the general community with a pelvic mass of unclear risk of malignancy. Proof of efficacy in a prospective validation data set would be necessary before clinical application.

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Authors' Data Sharing Statement

Will individual participant data be available (including data dictionaries)? *No.*

What data in particular will be shared? *Not available.*

What other documents will be available? *Not available.*

When will data be available (start and end dates)? *Not applicable.*

By what access criteria will data be shared (including with whom, for what types of analyses, and by what mechanism)? *Not applicable.*

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