

# Ovarian Mucinous Neoplasms: Can Gene Expression Microarray-Based Technology Distinguish Primary Versus Metastatic Tumors?

MAYO CLINIC Fabiola Medeiros, Kevin C. Halling, Christopher P. Kolbert, Fariborz Rakhshan Rohakhtar, Tina M. Kane Lindgren, Marcia J. Wilson, Cynthia M. Rendler, Matthew P. Goetz, Debra A. Bell  
Mayo Clinic, Rochester, MN, USA

## Abstract

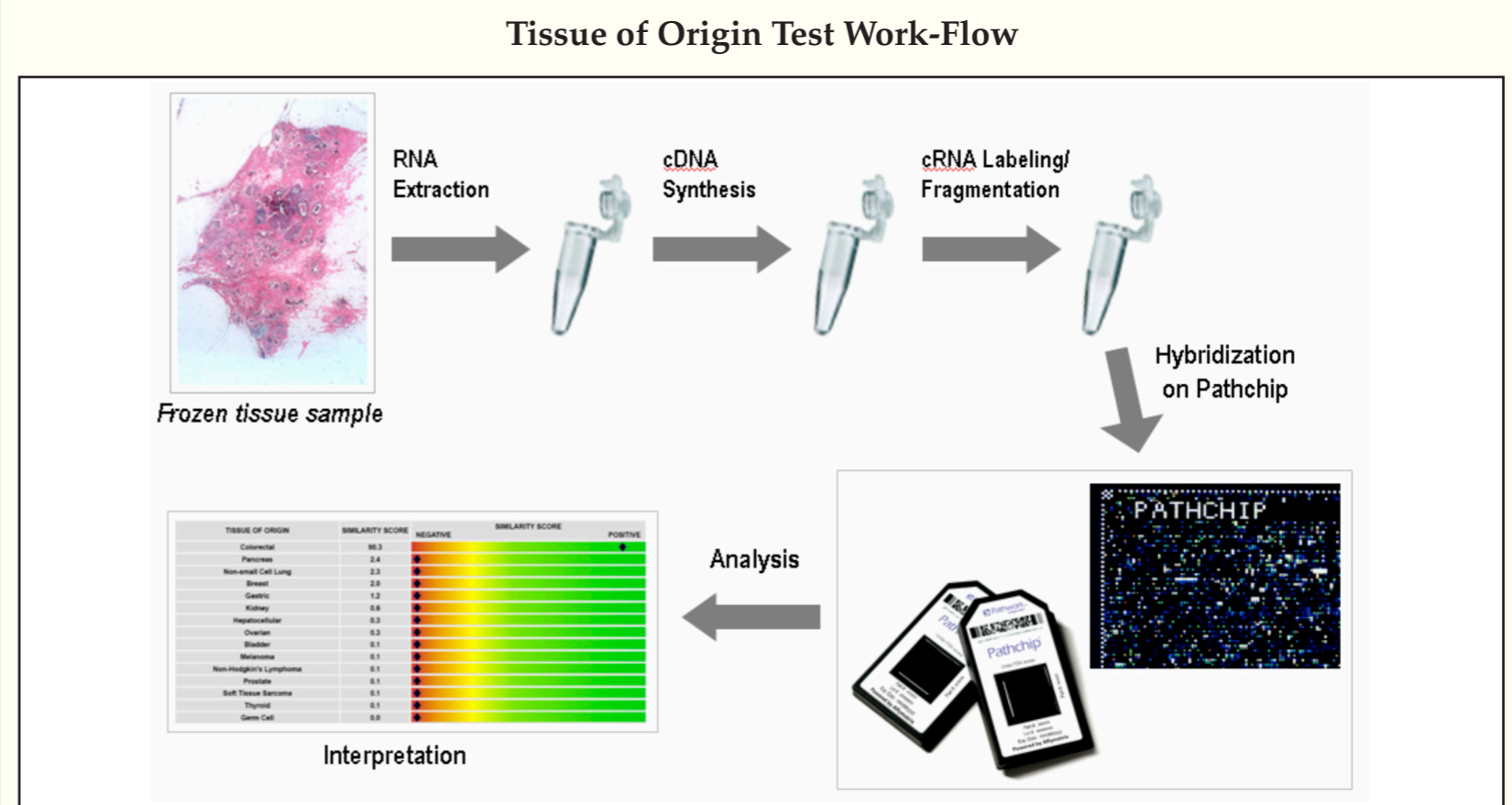
**Background:** It has been estimated that metastatic tumors to the ovary account for 7% of all ovarian tumors and the majority of these represent spread from primary lesions in the gastrointestinal tract. The histopathologic distinction between primary ovarian malignancy and metastasis is usually challenging, particularly for mucinous lesions. Immunohistochemical studies have shown considerable overlap for all currently available markers, including CK7, CK20 and CDX2. A correct diagnosis is critical for staging, therapeutic, and prognostic reasons. This study aims to determine the utility of a gene expression microarray-based test to differentiate primary versus metastatic mucinous tumors of the ovary.

**Design:** The study comprised 24 ovarian mucinous tumors, including 10 primary ovarian mucinous adenocarcinomas, 7 metastatic adenocarcinomas from gastrointestinal primaries and 7 ovarian borderline mucinous tumors. RNA was extracted from fresh-frozen tumor specimens and processed with the Pathwork® Tissue of Origin (TOO) Test (Pathwork Diagnostics, Sunnyvale, CA). The TOO Test has been validated to detect tissue from 15 different primary sites including ovarian, pancreas, gastric and colorectal. Each patient underwent a full clinical and imaging assessment. Detailed histopathologic evaluation and immunohistochemistry for CK20, CK7 and CDX2 were performed in all cases using formalin-fixed, paraffin-embedded sections. TOO Test results were blindly compared to clinical, histopathologic and immunohistochemical findings in order to evaluate the potential of the test to correctly indicate the site of tumor origin, whether ovarian or gastrointestinal.

**Results:** The combination of clinical, histopathologic and immunohistochemical features confidently indicated ovarian origin in 10 cases and gastrointestinal origin in 7 cases. There were 7 cases in which the tumor origin was uncertain. Clinically these 7 cases were considered likely to be primary ovarian mucinous adenocarcinomas. However, they had unusual histopathologic features or extensive extra-ovarian disease at diagnosis also suggesting a non-ovarian primary. The TOO test made a tissue of origin determination for 87% of the specimens (21/24) that was either ovarian, pancreas, colorectal, or gastric. The TOO test assigned an ovarian origin to 5 of 10 cases that were ovarian primaries and assigned gastrointestinal origin to 5 of the 7 cases that were gastrointestinal primaries. The discordant cases were most commonly primary ovarian mucinous adenocarcinoma or borderline tumors that were interpreted by the TOO Test as pancreatic tumors metastatic to the ovary. The TOO test indicated a pancreatic or gastric origin for 6 of 7 cases with uncertain primary site based on clinicopathologic features.

**Conclusions:** The Pathwork TOO Test tissue calls were concordant with the clinicopathologic findings for the majority of the cases that were considered to be mucinous tumors metastatic to the ovary or primary ovarian neoplasms. All uncertain primary cases were determined by the TOO Test to be of gastric or pancreatic origin. Considering the lack of reliable criteria to differentiate primary versus metastatic mucinous ovarian tumors and the great impact of this distinction, the TOO test may be of significant clinical utility. However, additional studies that correlate treatment outcomes with current clinicopathologic classification vs. TOO classifications are needed to clarify the clinical utility of the TOO assay.

## Figure 2



## Background

- Metastasis to the ovary account for 7% of all ovarian tumors.
- The majority of these are metastatic mucinous adenocarcinomas from the gastrointestinal tract, including colon, appendix, pancreas and stomach.
- Although some clinicopathologic findings can be suggestive of a primary or metastatic mucinous tumor, there are no entirely reliable features for the diagnosis.
- Both primary ovarian mucinous adenocarcinomas and mucinous borderline tumors can be confused with metastatic mucinous adenocarcinomas as they can have a deceptively bland morphologic appearance.
- Immunohistochemical studies have shown considerable overlap for all currently available markers aimed to indicate a possible site of origin, including CK7, CK20 and CDX2.
- The Pathwork Tissue of Origin Test (TOO) (Pathwork Diagnostics, Sunnyvale, CA) evaluates the expression of 1,550 genes using a custom-designed microarray and the Affymetrix® platform. TOO Test is a microarray and analytics test that uses gene expression to quantify the similarity of gene expression of poorly differentiated, undifferentiated and metastatic tumor specimens to the gene expression profiles of 15 cancers of known tissue of origin, including ovary, colorectum, stomach and pancreas.
- This study aims to determine the utility of this gene expression microarray-based test to differentiate primary versus metastatic mucinous tumors of the ovary.

## Results

- The combination of clinical, histopathologic and immunohistochemical features confidently indicated ovarian origin in 10 cases and gastrointestinal origin in 7 cases.
- The clinicopathologic and immunohistochemical features are summarized in Tables 1 and 2.
- There were 7 cases in which the tumor origin was uncertain upon microscopic review. The original pathologic diagnosis for all these cases was a primary ovarian mucinous tumor (6 adenocarcinomas and 1 MBT). However, they all had unusual histopathologic features that could be encountered in metastatic tumors. In addition, one of these cases had extensive extra-ovarian disease at diagnosis (Case 1) and 2 had intra-abdominal progression after surgery (Cases 1 and 12) also suggesting a non-ovarian primary.
- The TOO test made a tissue of origin determination for 87% of the specimens (21/24) as either ovarian (n = 7), pancreas (n = 10), colorectal (n = 3), or gastric (n = 1).
- The TOO test assigned gastrointestinal origin to 4 of the 7 cases (Cases 1, 3 and 4) that were considered to be gastrointestinal primaries. In 2 cases, tumors with known GI primaries yielded an ovarian signature, most likely due to contamination of the tumor with normal ovarian tissue (Cases 6 and 7).
- The TOO test assigned an ovarian origin to 5 of 10 cases that were considered to be ovarian primaries (Cases 14-18). All these cases had müllerian differentiation.
- All ovarian mucinous adenocarcinomas or borderline tumors of the intestinal type were interpreted by the TOO Test as pancreatic or gastric origin (Cases 9-13 and 20).
- The TOO results in conjunction with the original diagnosis and histopathologic review are displayed in Table 3.

## Design

- The study included 24 ovarian mucinous tumors.
- The original diagnosis for these tumors were:
  - 10 primary ovarian mucinous adenocarcinomas
  - 7 metastatic mucinous adenocarcinomas from gastrointestinal (GI) primary
  - 7 ovarian mucinous borderline tumors (MBT)
- One hematoxylin-eosin stained section from the frozen tissue block was reviewed by a pathologist (FM) and circled for areas with at least 60% viable tumor.
- Each patient underwent a full clinical and imaging assessment. Follow-up was recorded when available.
- Detailed histopathologic evaluation was performed by an experienced gynecologic pathologist (DAB). The cases were reclassified based solely on histopathologic features as (FIGURE 1):
  - Primary ovarian mucinous tumor
  - Metastatic mucinous tumor from GI primary
  - Mucinous tumor of indeterminate origin
- Immunohistochemistry for CK20, CK7 and CDX2 was performed on all cases using formalin-fixed, paraffin-embedded sections.
- RNA was extracted from fresh-frozen tumor, processed, hybridized to the Pathchip® microarray and scanned according to previously published methods (Dumur et al, 2008) by the Mayo Clinic Molecular Core Laboratories. The resulting data files were processed by the Pathwork® System Software and the final TOO test results were sent to Mayo Clinic for interpretation (FIGURE 2).
- The TOO reports were blindly compared with the available clinicopathologic diagnosis and to newly assigned histopathologic category.

## Table 1

Clinical Features				Extravarian disease at diagnosis	
Case no.	Age	Laterality	Tumor size (cm)		
1	59	Bilateral	9.5	11.5	Pancreas, spleen, gallbladder
2	63	Unilateral	4	6	Pancreas, soft tissue
3	60	Unilateral	5.6	6.3	Pancreas, lymph nodes
4	60	Bilateral	15	6.3	Pancreas, spleen, gallbladder
5	78	Bilateral	3.5	4	Pancreas
6	78	Bilateral	3.5	2.2	Pancreas
7	80	Bilateral	7.7	7	Pancreas, lymph nodes, colon
8	62	Bilateral	21	17	Pancreas
9	71	Unilateral	9	9	None
10	68	Unilateral	19	19	None
11	63	Unilateral	23	22	None
12	62	Unilateral	34	15	None
13	64	Unilateral	34	15	None
14	64	Unilateral	34	15	None
15	36	Unilateral	12	11	None
16	72	Unilateral	4	11	Lymph nodes
17	46	Bilateral	12	13.5	Peritoneal noninvasive implants
18	74	Unilateral	19	19	None
19	26	Unilateral	26	26	None
20	65	Unilateral	35	18	None
21	60	Unilateral	16.8	16.8	None
22	39	Unilateral	18	18	None
23	39	Unilateral	18	18	None
24	67	Unilateral	29	29	None

## Table 2

Immunohistochemical Findings			
Case no.	CK7	CK20	CDX2
1	Strong	Strong	Negative
2	Negative	Strong	Negative
3	Negative	Strong	Strong
4	Negative	Strong	Strong
5	Strong	Strong	Strong
6	Negative	Strong	Strong
7	Strong	Moderate	Strong
8	Negative	Strong	Weak
9	Strong	Strong	Moderate
10	Strong	Strong	Strong
11	Strong	Strong	Weak
12	Strong	Strong	Moderate
13	Strong	Strong	Strong
14	Strong	Strong	Negative
15	Strong	Strong	Negative
16	Strong	Negative	Negative
17	Strong	Negative	Negative
18	Strong	Strong	Negative
19	Strong	Strong	Negative
20	Strong	Strong	Negative
21	Strong	Negative	Negative
22	Strong	Strong	Negative
23	Strong	Moderate	Negative
24	Strong	Moderate	Negative

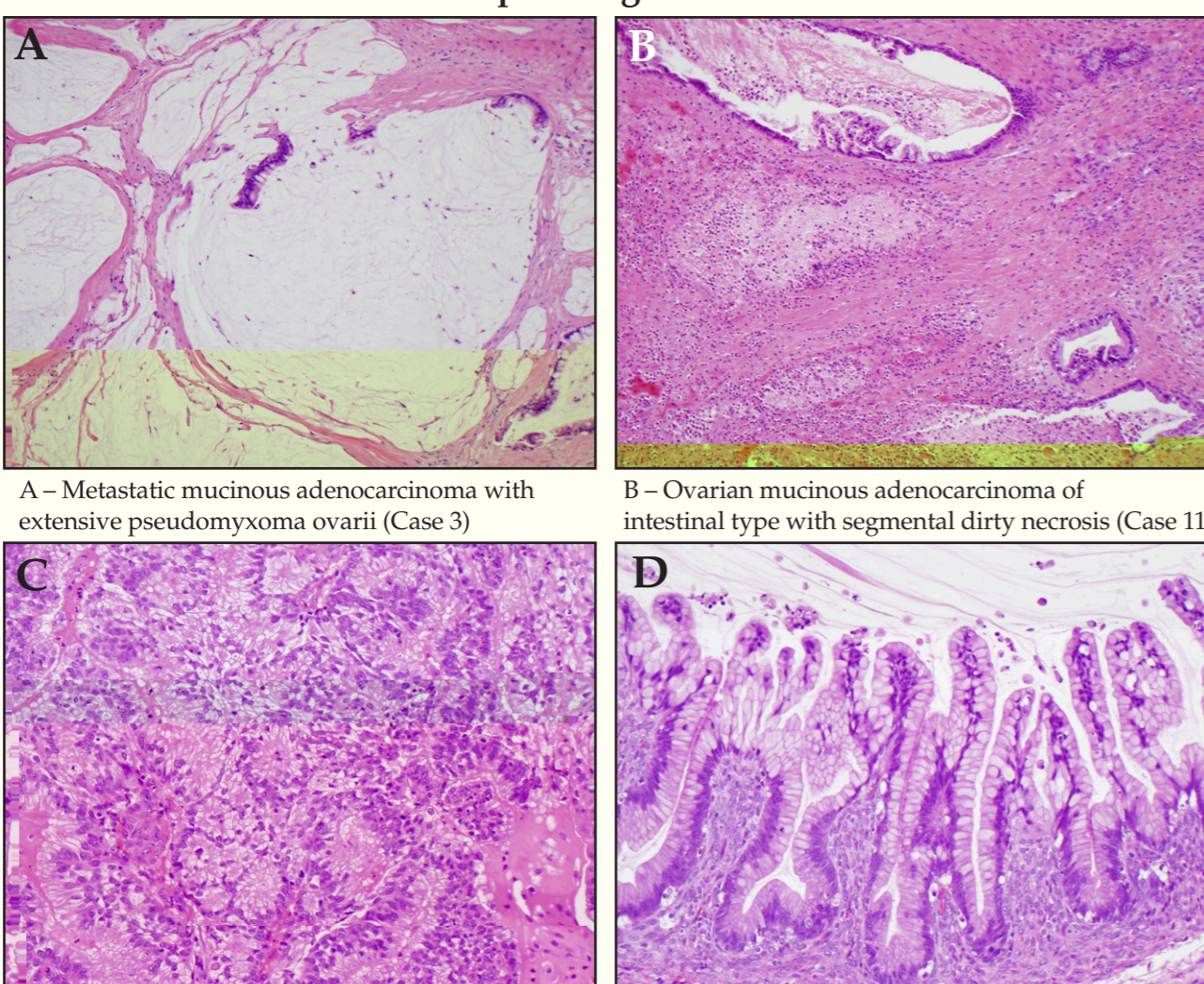
## Table 3

Case no.	Original diagnosis	Tumor grade	Revised Morphology	TOO result	Known origin for metastasis	Relapse or disease progression
1	Metastatic mucinous adenocarcinoma	1	GI	Colorectal	Appendix	Yes
2	Metastatic mucinous adenocarcinoma	2	GI	Indeterminate	Colon	No
3	Metastatic mucinous adenocarcinoma	2	GI	Colorectal	Colon	Yes
4	Metastatic mucinous adenocarcinoma	1	GI	Colorectal	Colon	No
5	Metastatic mucinous adenocarcinoma	1	GI	Pancreas	Appendix	No
6	Metastatic mucinous adenocarcinoma	2	GI	Ovary	Colon	Unknown
7	Metastatic goblet cell carcinoma	2	GI	Ovary	Appendix	No
8	Ovarian mucinous adenocarcinoma, intestinal type	1	Indeterminate	Pancreas	No	Yes
9	Ovarian mucinous adenocarcinoma, intestinal type	2	Indeterminate	Indeterminate	No	No
10	Ovarian mucinous adenocarcinoma, intestinal type	2	Indeterminate	Gastric	No	No
11	Ovarian mucinous adenocarcinoma, intestinal type	1	Indeterminate	Pancreas	No	Yes
12	Ovarian mucinous adenocarcinoma, intestinal type	2	Indeterminate	Pancreas	No	No
13	Ovarian mucinous adenocarcinoma, intestinal type	1	Indeterminate	Pancreas	No	Unknown
14	Ovarian mucinous adenocarcinoma, müllerian type	2	Ovary	Ovary	No	No
15	Ovarian mucinous adenocarcinoma, müllerian type	1*	Ovary	Ovary	No	No
16	Endometrioid adenocarcinoma with mucinous differentiation	2	Ovary	Ovary	No	No
17	Serous borderline tumor with focal mucinous differentiation	NA	Ovary	Ovary	No	No
18	MBT, müllerian type with intraperitoneal carcinoma and microinvasion	NA	Ovary	Ovary	No	Unknown
19	Mucinous borderline tumor, intestinal type with microinvasion	NA	Ovary	Pancreas	No	No
20	Mucinous borderline tumor, intestinal type	NA	Indeterminate	Pancreas	No	No
21	Mucinous borderline tumor, intestinal type	NA	Ovary	Pancreas	No	No
22	Mucinous borderline tumor, intestinal type	NA	Ovary	Pancreas	No	No
23	Mucinous borderline tumor, intestinal type	NA	Ovary	Indeterminate	No	No
24	Mucinous borderline tumor, intestinal type	NA	Ovary	Pancreas	No	Unknown

\* This case had an intratumoral nodule of anaplastic carcinoma

## Figure 1

### Histopathologic features



A – Metastatic mucinous adenocarcinoma with extensive pseudomyxoma ovarii (Case 3)

B – Ovarian mucinous adenocarcinoma of intestinal type with segmental dirty necrosis (Case 11)

C – Ovarian mucinous adenocarcinoma of Müllerian type (Case 14)

D – Mucinous Borderline tumor of intestinal type (Case 22)

## Conclusions

- All primary ovarian mucinous adenocarcinomas or mucinous borderline tumors of the müllerian type were recognized as ovarian tumors by the TOO test.
- The TOO test assigned a gastrointestinal origin to 57% of metastatic adenocarcinomas from appendiceal or colonic origin.
- All ovarian mucinous adenocarcinomas or borderline tumors of the intestinal type were interpreted by the TOO Test as pancreatic or gastric origin.
- These findings suggest that ovarian mucinous tumor of intestinal type share with upper digestive system tumors not only the histopathologic and immunohistochemical features, as previously shown, but also the gene expression profile.
- Considering the lack of reliable criteria to differentiate primary versus metastatic mucinous ovarian tumors and the great impact of this distinction, the TOO test may be of significant clinical utility.

## References

Dumur CI, Lyons-Weiler M, Scully C, et al.: Interlaboratory Performance of a Microarray-Based Gene Expression Test to Determine Tissue of Origin in Poorly Differentiated and Undifferentiated Cancer. J. Mol. Diagn., January 1, 2008; 10(1): 67 - 77.