

CLINICAL VERIFICATION OF THE PATHWORK® TOO TEST ON POORLY DIFFERENTIATED METASTASES

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ABSTRACT

Background: Tissue of origin assessment for poorly differentiated or undifferentiated metastatic tumors and cancers of unknown primary site (CUPS) may be enhanced through the use of microarray-based gene expression testing. One such assay, the Pathwork® Tissue of Origin (TOO) Test, uses expression signals from 1668 probe sets in a gene expression microarray, to quantify the likelihood that a tumor specimen is derived from 1 of 15 different tissues. Here, we evaluated the performance of this test on 20 cancer specimens obtained from biopsies or resections submitted to surgical pathology.

Materials and Methods: Residual snap-frozen tissue from these 20 specimens, corresponding to poorly differentiated or undifferentiated metastatic tumors, including 1 CUPS sample, were obtained under VCU IRB-approved protocols, and stored at -80°C until RNA extraction. The Pathwork TOO Test was performed on each case, and the TOO Test results were compared to the pathology report (PR) diagnoses. Agreement between PR diagnosis and TOO Test result was evaluated only when a diagnosis other than CUPS or a TOO result other than indeterminate (IND) was obtained, and was assessed by use of kappa statistics. Discordant cases were further evaluated by performing additional immunohistochemical (IHC) stains.

Results: From 19 samples with a diagnosis other than CUPS, 2 samples corresponding to tissue types not covered by the TOO Test, and 1 sample corresponding to an undifferentiated ovarian tumor, rendered IND results. Of the remaining 16 samples, 6 cases showed more than one possible tissue of origin in the PR diagnosis and the TOO Test result was in agreement with one of the differentials in 5 of them. Of the remaining 10 samples that had single PR diagnoses, the TOO results showed agreement in 7 of them. However, in 2 of the 4 overall discordant cases, additional IHC stains were supportive of the TOO Test result. Thus, very good agreement (14/16; $K = 0.855$) between the TOO Test result and the PR diagnosis and/or IHC results was obtained.

Conclusions: Our study suggests that the Pathwork TOO Test may be a useful ancillary tool for pathologists when classifying poorly differentiated or undifferentiated metastatic tumors.

BACKGROUND

The diagnosis of the primary site for malignancies arising in unexpected locations or with poorly differentiated morphologies is a challenge to pathologists and diagnosticians, and it is currently performed with immunohistochemistry (IHC) and advanced imaging testing. However, in some cases, the primary tumor is never identified. Improved diagnostic testing is needed to help in the choice of therapy for patients presenting such malignancies.

Recently, genome-wide gene expression microarrays have enabled the measurement of gene expression on a global scale to assess the tissue of origin of undifferentiated metastases from unknown primary sites.

Currently, the Pathwork® Tissue of Origin Test, an in vitro diagnostic test for evaluating the tissue of origin (TOO) in poorly differentiated or undifferentiated tumors, offers a microarray-based gene expression test that quantifies the similarity of tumor specimens to 15 tissues on the TOO Test panel by measuring over 1550 genes.

The tumor types included in the Pathwork TOO Test are: bladder, breast, colorectal, gastric, testicular germ cell, hepatocellular, kidney, non-small-cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid. The reproducibility of this test has already been evaluated.¹

In the study presented here, we performed the clinical verification of this test by assessing a cohort of undifferentiated and poorly differentiated tumor specimens, including 1 CUP, acquired at our institution. The Pathwork TOO Test results were compared to the initial pathology report. In case of disagreement, TOO Test results were also compared to IHC and imaging results, such as CT scan images and agreement with the complete diagnosis was assessed.

MATERIALS AND METHODS

Figure 1. Specimen Workflow

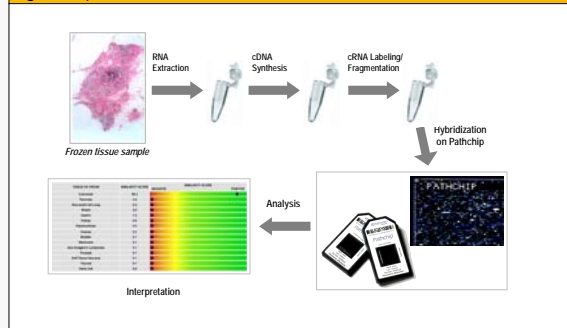


Table 1. List of Undifferentiated or Poorly Differentiated Cases

20 initial cases			3 new cases		
Alias	Biopsy Site	Diagnosis	Alias	Biopsy Site	Diagnosis
PATH1	Abdominal Wall	Uterus - Ovarian	PATH20	Brain	Breast
PATH2	Lymph Node	Uterus	PATH21	Peritoneal	Pancreas
PATH3	Brain	Lung - Breast	VSOT1	Lymph Node	Salivary Gland
PATH4	Colon	Lymphoma			
PATH5	Neck Mass	Lung - Others			
PATH6	Lymph Node	Retroperitoneal cystadenocarcinoma			
PATH8	Liver	Cholangiocarcinoma			
PATH9	Omentum	Colon			
PATH10	Uterus	Uterus - Cervix - Ovary			
PATH11	Brain	Lung			
PATH12	Thigh	Sarcoma			
PATH13	Omentum	Gastric			
PATH14	Pleura	Pancreas			
VSOT2	Neck Mass	Nasopharyngeal			
VSOT3	Axillary Mass	Lung			
VLN1	Lymph Node	CUP			
VLN6	Lymph Node	Breast - Lung - Endometrium - Cervix			
VNE2	Parotid	Salivary Gland, Lung, Skin			
VLOO3	Maxilla	Sinonasal			
VVO16	Ovary	Ovary			

RESULTS

Table 2. TOO Agreement with Original Diagnosis $K = 0.697$ [95% CI = 0.470 to 0.925]

Alias	Biopsy Site	Diagnosis	SS	TOO Result	Agreement with Diagnosis
PATH1	Abdominal Wall	Uterus - Ovarian	94.7	Ovarian	Yes
PATH2	Lymph Node	Uterus	59.9	Breast	No
PATH3	Brain	Lung - Breast	42.3	Colorectal	No
PATH4	Colon	Lymphoma	90.0	Lymphoma	Yes
PATH5	Neck Mass	Lung - Others	66.5	Lung	Yes
PATH6	Lymph Node	Retroperitoneal cystadenocarcinoma	36.6	Gastric	Yes
PATH8	Liver	Cholangiocarcinoma	<0	IND*	N.A.
PATH9	Omentum	Colon	89.4	Colorectal	Yes
PATH10	Uterus	Uterus - Cervix - Ovary	54.0	Ovarian	Yes
PATH11	Brain	Lung	44.2	Lung	Yes
PATH12	Thigh	Sarcoma	49.9	Soft Tissue Sarcoma	Yes
PATH13	Omentum	Gastric	38.4	Gastric	Yes
PATH14	Pleura	Pancreas	51.6	Kidney	No
VSOT2	Neck Mass	Nasopharyngeal	<0	IND	N.A.
VSOT3	Axillary Mass	Lung	83.0	Lung	Yes
VLN1	Lymph Node	CUP	44.2	Lung	N.A.
VLN6	Lymph Node	Breast - Lung - Endometrium - Cervix	91.4	Breast	Yes
VNE2	Parotid	Salivary Gland, Lung, Skin	56.2	Lung	Yes
VLOO3	Maxilla	Sinonasal	45.9	Melanoma	No
VVO16	Ovary	Ovary	<0	IND	N.A.
PATH20	Brain	Breast	98.3	Breast	Yes
PATH21	Peritoneal	Pancreas	34.1	Pancreas	Yes
VSOT1	Lymph Node	Salivary Gland	66.5	Lung	No

* Similarity Score = Indeterminate

Figure 2. IHC and CT Results on Disagreement Cases

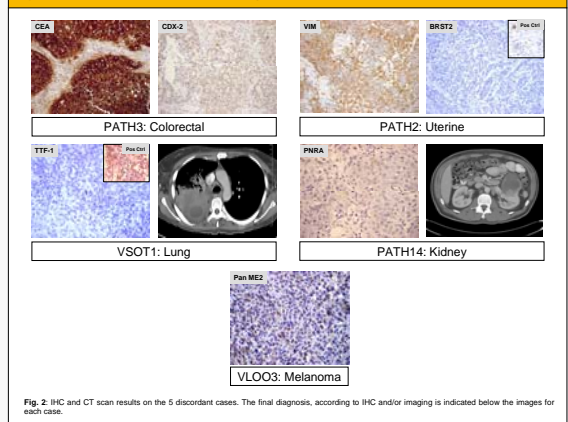


Fig. 2. IHC and CT scan results on the 5 discordant cases. The final diagnosis, according to IHC and/or imaging is indicated below the images for each case.

Table 3. TOO Agreement with Complete Diagnosis $K = 0.939$ [95% CI = 0.822 to 1.055]

Alias	Biopsy Site	Diagnosis	SS	TOO Result	Agreement with Diagnosis / IHC / CT scan
PATH1	Abdominal Wall	Uterus - Ovarian	94.7	Ovarian	Yes
PATH2	Lymph Node	Uterus	59.9	Breast	No
PATH3	Brain	Lung - Breast	42.3	Colorectal	Yes
PATH4	Colon	Lymphoma	90.0	Lymphoma	Yes
PATH5	Neck Mass	Lung - Others	66.5	Lung	Yes
PATH6	Lymph Node	Retroperitoneal cystadenocarcinoma	36.6	Gastric	Yes
PATH8	Liver	Cholangiocarcinoma	<0	IND	N.A.
PATH9	Omentum	Colon	89.4	Colorectal	Yes
PATH10	Uterus	Uterus - Cervix - Ovary	54.0	Ovarian	Yes
PATH11	Brain	Lung	44.2	Lung	Yes
PATH12	Thigh	Sarcoma	49.9	Soft Tissue Sarcoma	Yes
PATH13	Omentum	Gastric	38.4	Gastric	Yes
PATH14	Pleura	Pancreas	51.6	Kidney	Yes
VSOT2	Neck Mass	Nasopharyngeal	<0	IND	N.A.
VSOT3	Axillary Mass	Lung	83.0	Lung	Yes
VLN1	Lymph Node	CUP	44.2	Lung	N.A.
VLN6	Lymph Node	Breast - Lung - Endometrium - Cervix	91.4	Breast	Yes
VNE2	Parotid	Salivary Gland, Lung, Skin	56.2	Lung	Yes
VLOO3	Maxilla	Sinonasal	45.9	Melanoma	Yes
VVO16	Ovary	Ovary	<0	IND	N.A.
PATH20	Brain	Breast	98.3	Breast	Yes
PATH21	Peritoneal	Pancreas	34.1	Pancreas	Yes
VSOT1	Lymph Node	Salivary Gland	66.5	Lung	Yes

CONCLUSIONS

The microarray-based Pathwork® TOO Test has been shown to be a robust and highly reproducible test to assess the tissue of origin of poorly differentiated and undifferentiated tumors. However, the clinical verification against complete histopathologic diagnosis, including immunohistochemistry (IHC), and imaging, including CT scanning, is yet to be evaluated.

In this study, we performed the clinical verification of the Pathwork TOO Test on 23 poorly differentiated and undifferentiated tumors, including 1 CUP specimen. The Pathwork TOO Test results were compared to the original diagnosis as stated on the Pathology Report. Selected cases were further correlated with ancillary IHC results and CT scanning.

Of the 23 cases, 4 cases could not be used in agreement analyses because they did not have a definitive diagnosis (1 CUP) or they rendered indeterminate (IND) results on the Pathwork TOO Test (3 cases with SS < 30). Two of these 3 cases corresponded to tissue types not covered by the Pathwork TOO Test.

The results of this study showed a 73.7% accuracy with respect to the original diagnosis on the 19 remaining cases, with a good agreement ($k = 0.697$). Four of the 5 cases that showed a disagreement between the original diagnosis and the Pathwork TOO Test, showed IHC and, when possible, CT scan imaging results that were in agreement with the Pathwork TOO Test. Thus, the overall accuracy of the test rose to 94.7%, with a very good agreement ($k = 0.939$) with the complete diagnosis.

Altogether, these results strongly suggest that the Pathwork TOO Test is an accurate assay that will become a useful ancillary tool to pathologists, and diagnosticians in general.

REFERENCES

1. Dumur CI, Lyons-Weiler M, Scillilli C, Garrett CT, Schrijver I, Holley TK, Rodriguez-Paris J, Pollack JR, Zehnder JL, Price M, Hagenkord JM, Rigi CT, Buturovic LJ, Anderson GG, Monzon FA. Interlaboratory performance of a microarray-based gene expression test to determine tissue of origin in poorly differentiated and undifferentiated cancers. J Mol Diagn. 2008 Jan;10(1):67-77.