# Using Tumor Marker Gene Variants to Improve the Diagnostic Accuracy of DUPAN-2 and Carbohydrate Antigen 19-9 for Pancreatic Cancer

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## ABSTRACT

**PURPOSE** Circulating carbohydrate antigen 19-9 (CA19-9) levels reflect FUT3 and FUT2 fucosyltransferase activity. Measuring the related glycan, DUPAN-2, can be useful in individuals unable to synthesize CA19-9. We hypothesized that similar to CA19-9, FUT functional groups determined by variants in *FUT3* and *FUT2* influence DUPAN-2 levels, and having tumor marker reference ranges for each functional group would improve diagnostic performance.

**MATERIALS AND METHODS** Using a training/validation study design, *FUT2*/*FUT3* genotypes were determined in 938 individuals from Johns Hopkins Hospital: 607 Cancer of the Pancreas Screening (CAPS) study subjects with unremarkable pancreata and 331 with pancreatic ductal adenocarcinoma (PDAC). Serum DUPAN-2 and CA19-9 levels were measured by immunoassay.

**RESULTS** In controls, three functional FUT groups were identified with significant differences in DUPAN-2 levels: FUT3-intact, FUT3-null/FUT2-intact, and FUT3-null/FUT2-null. DUPAN-2 training set diagnostic cutoffs for each FUT group yielded higher diagnostic sensitivity in the validation set for patients with stage I/II PDAC than uniform cutoffs (60.4% [95% CI, 50.2 to 70.0] *v* 39.8% [30.0 to 49.8]), at approximately 99% (96.7 to 99.6) specificity. Combining FUT/CA19-9 and FUT/DUPAN-2 tests yielded 78.4% (72.3 to 83.7) sensitivity for stage I/II PDAC, at 97.7% (95.3 to 99.1) specificity in the combined sets, with higher AUC (stage I/II: 0.960 *v* 0.935 for CA19-9 + DUPAN-2 without the FUT test; $P < .001$); for stage I PDAC, sensitivity was 62.0% (49.1 to 73.2; AUC, 0.919 *v* 0.883; $P = .03$). CA19-9 levels in FUT3-null/FUT2-null PDAC subjects were higher than in FUT3-null/FUT2-intact subjects (median/IQR; 24.9/57.4 *v* <1/2.3 U/mL; $P = .0044$). In a simulated CAPS cohort, AUC precision recall ($AUC_{PR}$) scores were 0.51 for CA19-9 alone, 0.64 for FUT/CA19-9, 0.73 for CA19-9/DUPAN-2, and 0.84 for FUT/CA19-9/DUPAN-2.

**CONCLUSION** Using a tumor marker gene test to individualize CA19-9 and DUPAN-2 reference ranges achieves high diagnostic performance for stage I/II pancreatic cancer.

ACCOMPANYING CONTENT

Data Supplement

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## INTRODUCTION

Pancreatic cancer is the third leading cause of cancer death in the United States, and although survival is improving (now approximately 12% at 5 years),[1] most patients present with advanced disease and have a poor outcome.[2] Survival is best for patients diagnosed with localized disease,[3] especially stage I pancreatic ductal adenocarcinoma (PDAC).[4] Early detection of PDAC, as shown in high-risk individuals who maintain pancreas surveillance, offers the best chance for cure.[5,6]

Blood-based early detection of pancreatic cancer remains elusive. Carbohydrate antigen 19-9 (CA19-9), also known as sialyl-Lewis$^a$ (sLe$^a$), outperforms candidate biomarkers of PDAC despite its limitations as a diagnostic test, one being that approximately 10% of the population do not synthesize CA19-9.[7] The CA19-9 precursor, sLe$^c$ (also known as DUPAN-2[8]), is converted to CA19-9 by fucosyltransferase 3 (FUT3),[9,10] and CA19-9 is converted to sLe$^b$ by fucosyltransferase 2 (FUT2[11]; Fig 1). DUPAN-2 was first reported as a pancreatic cancer antigen in 1982,[8] is commonly elevated

## CONTEXT

**Key Objective**

To evaluate whether using a FUT2/FUT3 tumor marker gene test to predict an individual's capacity to synthesize the circulating tumor markers carbohydrate antigen 19-9 (CA19-9) and DUPAN-2 can improve diagnostic performance for detecting cases with stage I/II pancreatic cancer versus controls with familial/genetic risk undergoing pancreatic surveillance.

**Knowledge Generated**

The tumor marker gene test can be used to personalizing the reference ranges of CA19-9 and DUPAN-2 according to an individual's FUT2/FUT3 variant status. Doing so improves the diagnostic performance of CA19-9/DUPAN-2 for stage I (AUC, 0.919) and stage I/II pancreatic cancer (AUC, 0.960), improving diagnostic sensitivity by approximately 12% at 98% specificity.

**Relevance *(E.M. O'Reilly)***

These data add to the body of evidence that blood-based biomarkers have utility in pancreas cancer, including providing options for patients who do not express CA 19-9. Nonetheless, further study is needed to support day-to-day integration into clinical care.*

*Relevance section written by *JCO* Associate Editor Eileen M. O'Reilly, MD.

in patients with pancreatic cancer,[12,13] and is used in Japan to monitor disease burden, particularly for patients without measurable CA19-9.[13-16] We have previously shown that *FUT2* and *FUT3* gene variants can be used to classify individuals with normal pancreata into one of four groups with respect to their CA19-9 reference range, and classifying patients in this manner improves the diagnostic performance of CA19-9.[17] FUT variants that affect CA19-9 synthesis and metabolism are expected to affect DUPAN-2 levels.

In this study, we evaluated whether assigning individuals to functional groups predicted by their *FUT2* and *FUT3* variants improves the diagnostic performance of DUPAN-2 for early-stage (stage I/II) pancreatic cancer, and whether diagnostic performance could be further improved by combining the FUT/CA19-9 and FUT/DUPAN-2 tests.

## MATERIALS AND METHODS

### Study Population and Sample Collection

The study population included 607 controls who were prospectively enrolled in the Cancer of the Pancreas Screening (CAPS) studies between 2003 and 2022, almost all of whom met guideline criteria for pancreatic surveillance,[18] as well as 331 patients undergoing surgical resection for PDAC at Johns Hopkins Hospital between 2005 and 2022. Blood samples from PDAC cases were collected before pancreatic resection. None of the PDAC cases had received neoadjuvant therapy. Tumor staging was by surgical pathology. A patient flowchart is provided (Data Supplement, Fig S1, online only).

This study was approved by the Johns Hopkins Institutional Review Board and written informed consent was obtained from all study participants.

### FUT2/FUT3 Variant Sequencing

Variants (Data Supplement, Table S1) were sequenced as previously described.[19] Seven *FUT3* and two FUT2 variants were used to classify individuals. Four FUT2/FUT3 patient groups were characterized with respect to CA19-9 levels, three groups for DUPAN-2, and five groups for CA19-9/DUPAN-2 combined (Fig 1).

### CA19-9 and DUPAN-2 Measurement

Serum CA19-9 levels were measured in the Johns Hopkins Clinical Chemistry CLIA Reference Lab (laboratory-defined reference range in healthy individuals: <36 U/mL). Serum DUPAN-2 levels were measured by enzyme-linked immunosorbent assay (ELISA; Determiner DUPAN-2; Minaris Medical Co, Ltd, Tokyo, Japan).

### Statistical Analysis

FUT functional groups for DUPAN-2 and DUPAN-2 diagnostic cutoffs were determined using training set control data that were then evaluated in the validation set. The diagnostic performance of the combined CA19-9/DUPAN-2/FUT test was compared with that of CA19-9 and DUPAN-2 with/without the FUT test. Cutoffs for CA19-9 were set at the 99th percentile for each FUT group in our previous study where CA19-9 levels were measured in the Johns Hopkins Clinical Chemistry laboratory and FUT gene sequencing was

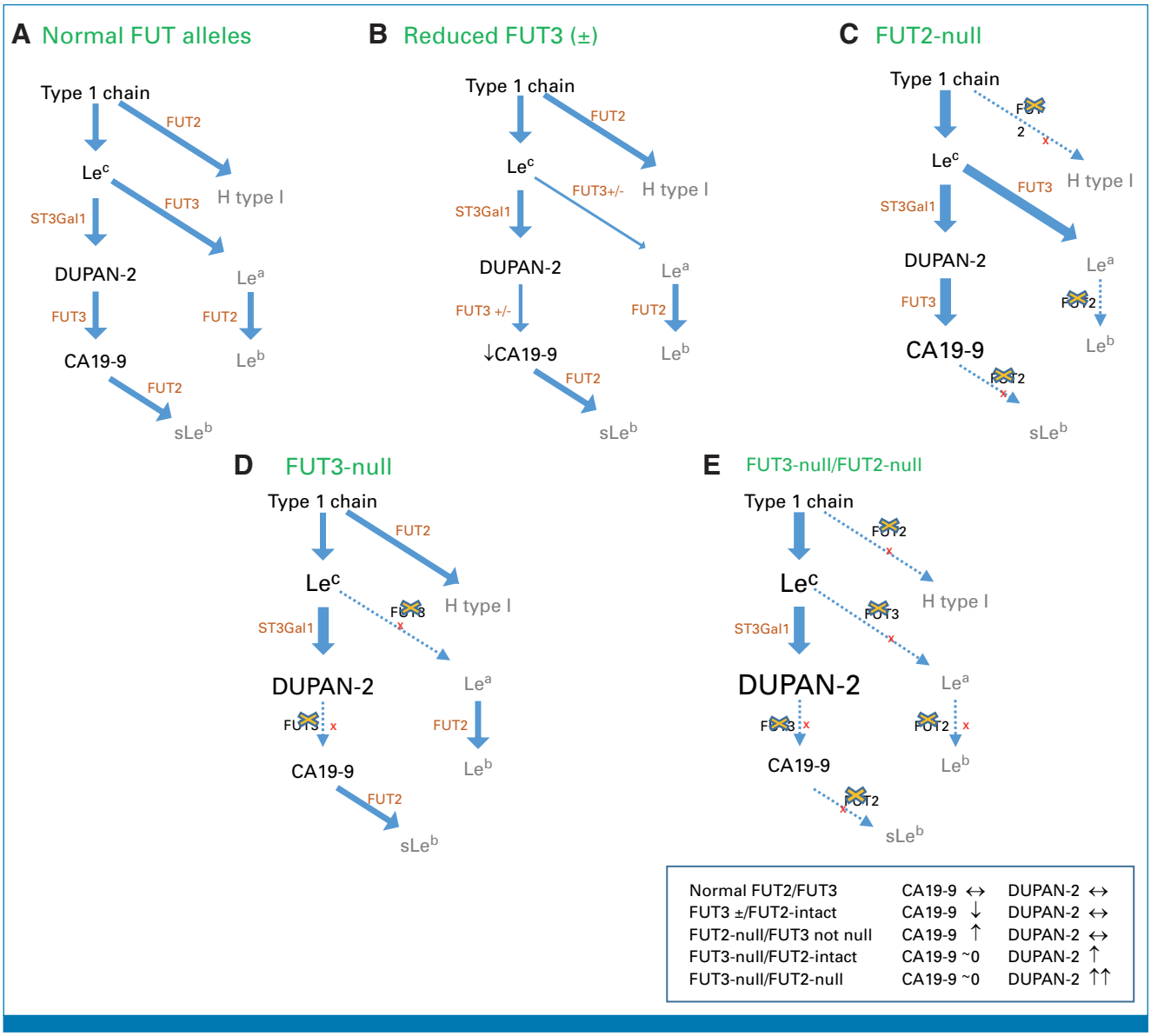


**FIG 1.** The effect of FUT functional groups on CA19-9 and DUPAN-2 synthesis. Synthetic pathway for Lewis antigens and related molecules in subjects with (A) normal FUT2 and FUT3 function (as predicted by the gene sequencing); (B) FUT3± and FUT2-intact; FUT3± indicates heterozygous for a null variant (lower CA19-9 levels), FUT2-intact meaning at least one functional FUT2 allele; (C) FUT2-null/FUT3 not null (higher CA19-9 levels); (D) FUT3-null/FUT2-intact (absent CA19-9/higher DUPAN-2 levels); (E) FUT3-null/FUT2-null (absent CA19-9 levels/highest DUPAN-2 levels). CA19-9, carbohydrate antigen 19-9.

performed in the Johns Hopkins Molecular Diagnostic laboratory (3 U/mL for FUT3-null, 34.9 U/mL for FUT3±/FUT2-intact, 41.8 U/mL for FUT3-wild-type/FUT2-intact, and 89.1 U/mL for FUT3-intact/FUT2-null).[20] In addition to comparing diagnostic sensitivity at approximately 99% specificity, AUCs were calculated for descriptive purposes. Logistic regression models estimated predicted probabilities of being a PDAC case. Three sets of models were estimated: (1) CA19-9 only, (2) DUPAN-2 only, and (3) CA19-9 and DUPAN-2 combined. For each, models with CA19-9 and/or DUPAN-2 were compared with models with and without functional FUT cutoffs and their interactions. For models where FUT cutoffs significantly improved discrimination, AUCs were calculated separately according to functional FUT cutoffs and combined across FUT cutoffs to estimate a weighted average AUC, using the inverse variance as weights.[21] Calibration curves corresponding to each AUC were constructed. AUC precision recall curves ($AUC_{PR}$)[22,23] were calculated for each test. We simulated how each test would perform when applied to a typical cohort of patients undergoing pancreas surveillance (incidence of PDAC 0.5% per year in the simulation). Additional methods are provided in the Data Supplement (Materials).

**TABLE 1.** Characteristics of Controls and PDAC Cases

| Characteristic | Training Set | | | Validation Set | | |
|---|---|---|---|---|---|---|
| | Controls (n = 304) | PDAC (n = 175) | P[a] | Controls (n = 303) | PDAC (n = 156) | P[a] |
| Age, years, mean (SD) | 59.6 (10.6) | 68.4 (9.8) | **<.0001** | 59.3 (11.6) | 68.0 (9.9) | **<.0001** |
| Sex, No. (%) | | | | | | |
| Male | 145 (47.7) | 90 (51.4) | .432 | 135 (44.6) | 74 (52.6) | .557 |
| Female | 159 (52.3) | 85 (48.6) | | 168 (55.4) | 82 (47.4) | |
| Race, No. (%) | | | | | | |
| Caucasian | 270 (88.8) | 155 (32.4) | .208 | 270 (89.1) | 130 (83.3) | .147 |
| African American | 22 (7.2) | 8 (4.6) | | 25 (8.3) | 22 (14.1) | |
| Others | 12 (4.0) | 12 (6.9) | | 8 (2.6) | 4 (2.6) | |
| BMI, mean (SD) | 27.3 (4.9) | 25.6 (4.7) | **.001** | 28.3 (6.0) | 25.8 (4.7) | **<.0001** |
| FUT-3, No. (%) | | | | | | |
| WT | 127 (41.8) | 79 (45.1) | .534 | 118 (38.9) | 65 (41.7) | .305 |
| Het | 128 (42.1) | 66 (37.7) | | 117 (38.6) | 65 (41.7) | |
| Null | 49 (16.1) | 30 (17.1) | | 68 (22.4) | 26 (16.7) | |
| FUT-2, No. (%) | | | | | | |
| WT | 61 (20.1) | 36 (23.5) | .610 | 87 (28.7) | 40 (29.2) | **.004** |
| Het | 144 (47.4) | 66 (43.1) | | 172 (56.8) | 60 (43.8) | |
| Null | 99 (32.6) | 51 (33.3) | | 44 (14.5) | 37 (27.0) | |
| Stage AJCC eighth ed, No. (%) | | | | | | |
| IA | | 12 (6.9) | | | 17 (10.9) | |
| IB | | 23 (13.1) | | | 19 (12.2) | |
| IIA | | 4 (2.2) | | | 1 (0.6) | |
| IIB | | 73 (41.7) | | | 64 (41.0) | |
| III | | 63 (36) | | | 30 (19.2) | |
| IV | | 0 (0) | | | 25 (16.0) | |

NOTE. Bold indicates statistically significant results at $P < .05$.
Abbreviations: AJCC eighth ed, American Joint Committee on Cancer, eighth edition (all staging by surgical pathology); Het, heterozygous for a null variant; PDAC, pancreatic ductal adenocarcinoma; SD, standard deviation; WT, wild-type or reference allele.
[a]*P* values for *t*-tests or Fisher's exact tests for differences between controls and PDAC cases.

## RESULTS

### DUPAN-2 Levels in Controls by FUT Variant Status

The demographics of the CAPS control population and the PDAC cases are presented in Table 1. Forty-one percent of CAPS controls had one or more (almost all subcentimeter) pancreatic cysts.

We first determined which FUT functional groups best predicted DUPAN-2 levels among controls. Because FUT3 converts DUPAN-2 to CA19-9,[9] FUT3-null controls should have higher DUPAN-2 levels than those with intact FUT3 (Fig 1), which is what we found ($P < .0001$ in both the training and validation sets; Fig 2A). FUT2-null subjects shunt more precursors to DUPAN-2 synthesis.[11] Therefore, we compared DUPAN-2 levels within the FUT3-null group according to FUT2 status: FUT2-null versus FUT2-intact subjects. Among FUT3-null subjects, FUT2-null subjects had significantly higher DUPAN-2 levels than FUT2-intact subjects ($P = .049$, training set; $P < .0001$, validation set; Fig 2; Data Supplement, Table S2 and Fig S2). The training set DUPAN-2 control skewness (12.4) and kurtosis scores (171.6) improved considerably when subjects were subgrouped into these functional groups (skewness, 1.8, kurtosis 3.5, for FUT3-intact; 3.1 and 10.2 for FUT3-null/FUT2-intact, and 2.5 and 6.3 for the FUT3-null/FUT2-null groups). The characteristics of controls by their FUT group is provided in the Data Supplement (Table S3).

### The Diagnostic Performance of DUPAN-2 With FUT Test

DUPAN-2 levels in patients with PDAC also differed significantly according to FUT group (Data Supplement, Fig S3),

with little overlap with their corresponding FUT group–matched controls (Figs 2B and 2C). To evaluate the diagnostic performance of assigning FUT groups for DUPAN-2, we set approximately 99.5th percentile diagnostic cutoffs for each functional FUT subgroup in the training set controls (ie, 1,358.0 U/mL for FUT3-null/FUT2-null, 233.8 U/mL for FUT3-null/FUT2-intact, and 73.7 U/mL for FUT3-intact; Data Supplement, Table S2). We compared these DUPAN-2 cutoffs to uniform cutoffs, including those used clinically in Japan (>150 U/mL for screening, >400 U/mL for diagnosis),[12,24,25] and 238.8 U/mL, the uniform 99% specificity cutoff in our training set controls. DUPAN-2 had superior diagnostic accuracy when subjects were assigned functional FUT group cutoffs versus a uniform cutoff (>238.8 U/mL) for all subjects: 58.0% versus 41.1% sensitivity for stage I/II PDAC training set cases at approximately 99% diagnostic specificity (Data Supplement, Table S4).

### Validation Cohort

We applied the training set FUT group–stratified cutoffs to a validation set of 303 controls and 156 patients with PDAC, including 101 with stage I/II PDAC. Using the conventional uniform diagnostic DUPAN-2 cutoff used in Japan (<400 U/mL) yielded a diagnostic sensitivity of 28.7% for stage I/II PDAC, the uniform 238.8 U/mL cutoff yielded 39.8%, while the FUT group cutoffs yielded 60.4%, at 99.3%, 99.0%, and 98.7% specificity, respectively (Data Supplement, Table S4). DUPAN-2 diagnostic sensitivity increased with disease stage: 42.2%, 66.9%, 67.7%, and 72% for stages I, II, III, and IV, respectively (FUT group–based, both PDAC sets). DUPAN-2 cutoffs without the FUT test classification yielded an AUC of 0.872 for stage I/II PDAC in the validation set, while the FUT group cutoffs yielded an AUC of 0.904 (Data Supplement, Table S5). In the combined sets, DUPAN-2 with the FUT test yielded a significantly higher AUC than DUPAN-2 alone ($P < .001$; Table 2; Fig 3).

### The Diagnostic Performance of CA19-9 With DUPAN-2

The uniform CA19-9 cutoff (measured in the Johns Hopkins Clinical Chemistry laboratory [normal range <36 U/mL]) yielded poor diagnostic specificity compared with FUT group–based reference range cutoffs (Table 3). CA19-9 alone without the FUT test yielded an AUC of 0.827 and 0.855 for stage I/II PDAC in the training and validation sets, respectively (Data Supplement, Table S5), 0.839 overall (Table 3), significantly lower than those achieved with FUT grouping ($P < .001$; Fig 3). Applying the FUT functional group cutoffs to CA19-9 levels[19] yielded diagnostic sensitivities of 68.8% and 62.4%, respectively, at 98.7% specificity in the training and validation sets of stage I/II PDAC cases and controls (Table 3; Data Supplement, Table S6). In FUT3-null patients, CA19-9 performed poorly as expected, but DUPAN-2 yielded 76.7% and 71.4% sensitivity, at 98.0% and 97.1% specificity (at the FUT-defined cutoffs) in the training and validation sets, respectively (Data Supplement, Table S7), corresponding to an AUC of 0.983 and 0.938, respectively (Data Supplement, Table S8).

Combining CA19-9 and DUPAN-2 (either test-positive) and classifying subjects according to their FUT group (Fig 1) yielded 81.3% and 75.2% sensitivities, respectively, for stage I/II PDAC in the training and validation sets, at 98.0% and 97.4% specificity (Table 3; Data Supplement, Table S6), with an AUC of 0.960 for the whole cohort (Table 2). This combined test compares favorably to CA19-9 alone (uniform cutoff AUC 0.839 among all stage I/II PDAC cases) and to a combined CA19-9/DUPAN-2 test without FUT grouping (AUC, 0.935; $P < .001$; Table 2; Fig 3). Similar improvements in AUC were found when PDACs of all stages were included (Table 2; Data Supplement, Fig S4).

### CA19-9 Levels in FUT3-Null Subjects: FUT3-Null/FUT2-Null Versus FUT3-Null/FUT2-Intact

CA19-9 is elevated in a minority of FUT3-null PDAC cases,[26] perhaps reflecting FUT3 activity from high expression of variants with minimal enzymatic activity (Data Supplement, Table S7 and Fig S5). We suspected FUT2 status could explain why some FUT3-null patients with PDAC produce CA19-9, because FUT3-intact/FUT2-null controls have higher CA19-9 levels than FUT3-intact/FUT2-intact controls, as more precursors are shunted into CA19-9 synthesis. We compared CA19-9 levels among FUT3-null PDAC patients by FUT2 status. CA19-9 levels in PDAC subjects were higher in FUT3-null/FUT2-null subjects (median/IQR CA19-9; 24.9/57.4 U/mL) than in FUT3-null/FUT2-intact subjects (CA19-9; <1/2.3; $P = .0044$; Mann-Whitney). A significantly greater proportion of FUT3-null/FUT2-null individuals had CA19-9 levels above 3 U/mL (the diagnostic cutoff for FUT3-null controls[19]), 11 of 13, than FUT3-null/FUT2-intact individuals, nine of 43 ($P = .0001$).

### The Diagnostic Performance in Stage I PDAC

We performed a separate analysis of stage I PDAC cases. The diagnostic sensitivity of DUPAN-2 alone for stage I PDAC was superior using the functional FUT group diagnostic cutoffs (48.6% for the cutoffs in the training set and 36.1% in the validation set), compared with uniform cutoffs (25.7% and 16.7%, respectively; Data Supplement, Table S4). The corresponding AUCs are presented in the Data Supplement (Table S5; 95% CIs in the Data Supplement, Table S6, and overall results in Table 2).

Among all stage I cases, the combined FUT/CA19-9/DUPAN-2 test had an overall sensitivity of 62.0% versus 49.3% for CA19-9/DUPAN-2 without the gene test (Table 3), and the AUC was higher: 0.919 with the FUT test versus 0.883 without ($P = .032$; Table 2; Fig 3).

Stage I PDAC cases positive for both CA19-9 and DUPAN-2 had larger-sized tumors than those negative for both markers (excluding FUT3-negative subjects whose CA19-9 was uninformative; Data Supplement, Table S8).

To further compare differences in biomarker performance, we calculated areas under the precision-recall curve

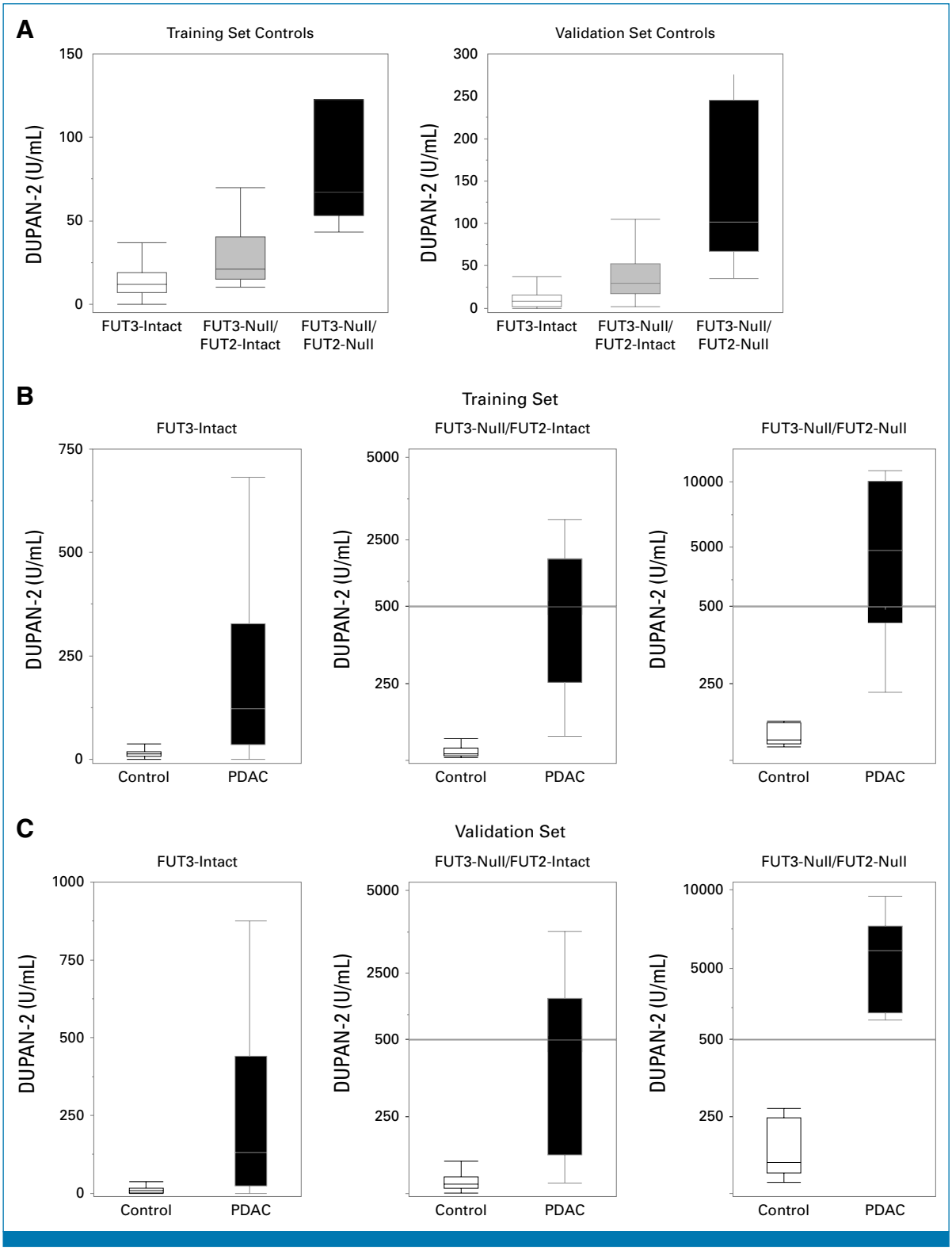


**FIG 2.** FUT functional group–stratified DUPAN-2 levels. (A) DUPAN-2 levels among controls classified into three functional FUT groups. In both the testing and validation set controls, DUPAN-2 levels differ significantly between each group. (B and C) Within each FUT group, DUPAN-2 levels are significantly higher among PDAC cases compared with controls ($P < .05$) in (B) the training set and (C) the validation set. The control box plots show the mean, and the 25th and the 75th percentile of values. Note that the *y*-axis scale is different for each group and the horizontal line at the 500 U/mL level indicates a change in the *y*-axis scale. PDAC, pancreatic ductal adenocarcinoma.

**TABLE 2.** AUCs for Pancreatic Cancer Diagnosis of DUPAN-2 and CA19-9 Alone With and Without the Tumor Marker Gene Test

| | Stage I PDAC | | | Stage I/II PDAC | | | All PDAC Stages | | |
|---|---|---|---|---|---|---|---|---|---|
| Blood Test | AUC | 95% CI | *P* | AUC | 95% CI | *P* | AUC | 95% CI | *P* |
| CA19-9 uniform cutoffs | 0.820 | 0.803 to 0.869 | | 0.839 | 0.799 to 0.880 | | 0.836 | 0.803 to 0.869 | |
| CA19-9 functional FUT group cutoffs | 0.898 | 0.852 to 0.944 | | 0.927 | 0.904 to 0.949 | | 0.924 | 0.905 to 0.944 | |
| AUC difference | 0.078 | 0.024 to 0.133 | <.005 | 0.088 | 0.052 to 0.122 | <.001 | 0.088 | 0.058 to 0.118 | <.005 |
| Weighted AUC | 0.911 | 0.869 to 0.953 | | 0.948 | 0.928 to 0.968 | | 0.945 | 0.928 to 0.962 | |
| DUPAN-2 uniform cutoff | 0.793 | 0.727 to 0.860 | | 0.870 | 0.840 to 0.903 | | 0.879 | 0.854 to 0.905 | |
| DUPAN-2 functional FUT group cutoffs | 0.840 | 0.782 to 0.898 | | 0.899 | 0.871 to 0.927 | | 0.909 | 0.887 to 0.930 | |
| AUC difference | 0.047 | 0.017 to 0.076 | .002 | 0.028 | 0.011 to 0.044 | <.001 | 0.030 | 0.016 to 0.043 | <.001 |
| Weighted AUC | 0.861 | 0.806 to 0.916 | | 0.925 | 0.902 to 0.948 | | 0.931 | 0.912 to 0.950 | |
| CA19-9 and DUPAN-2 uniform cutoffs | 0.883 | 0.833 to 0.932 | | 0.935 | 0.913 to 0.957 | | 0.942 | 0.926 to 0.959 | |
| CA19-9 and DUPAN-2 functional FUT group cutoffs | 0.919 | 0.877 to 0.962 | | 0.960 | 0.942 to 0.977 | | 0.959 | 0.945 to 0.973 | |
| AUC difference | 0.036 | 0.003 to 0.07 | .032 | 0.025 | 0.011 to 0.038 | <.001 | 0.017 | 0.007 to 0.026 | <.001 |
| Weighted AUC | 0.919 | 0.880 to 0.958 | | 0.959 | 0.942 to 0.976 | | 0.959 | 0.945 to 0.973 | |

NOTE. For the combined set of training and validation PDAC cases versus controls.
Abbreviations: CA19-9, carbohydrate antigen 19-9; PDAC, pancreatic ductal adenocarcinoma.

($AUC_{PR}$),[23] precision referring to the positive predictive value, and recall a synonym for diagnostic sensitivity. An $AUC_{PR}$ curve uses the same data as an AUC, but its scores are a function of disease prevalence; as sensitivity increases, positive predictive value drops.[22] The Data Supplement (Figs S6-S8) shows $AUC_{PR}$ curves alongside their corresponding AUC for the stage I, stage I/II, and all PDAC cases and controls, respectively. Since cases constituted approximately one third of our study population, the $AUC_{PR}$ and AUC curve scores were similar, for example, an AUPR of 0.935 for FUT/CA19-9/DUPAN-2 (AUC, 0.960) versus 0.806 for CA19-9 alone (AUC, 0.84) (Data Supplement, Fig S7).

Next, we calculated the $AUC_{PR}$ scores one could expect if these tests were applied to a typical cohort undergoing CAPS surveillance with a pancreatic cancer prevalence of 1/200.[5] In this scenario where the pretest probability is low, the differences in $AUC_{PR}$ between the tests are more striking. For example, applying the stage I/II test biomarker test to 5,000 subjects in a simulated cohort yielded an $AUC_{PR}$ for CA19-9 alone of 0.51; for the FUT/CA19-9 test, it was 0.64, for the CA19-9/DUPAN-2 test, it was 0.73, and for the FUT/CA19-9/DUPAN-2 test, it was 0.84 (Data Supplement, Fig S9).

Beyond evaluating a test's discrimination using AUC, measures of calibration help determine test performance by considering a model's estimation of risk[27] and involve plotting observed versus predicted probability for each result (Data Supplement, Figs S10 and S11). See the Data Supplement (Materials) for additional results (Data Supplement, Tables S9-S12).

## DISCUSSION

We find that assigning individuals to the biomarker reference range that reflects their FUT functional group improves the diagnostic performance of CA19-9 and DUPAN-2. FUT group assignment improves DUPAN-2's diagnostic performance as much as it does for CA19-9, although the relevant FUT groups differ, reflecting the relative roles of FUT2 and FUT3 in the metabolism of each biomarker.

DUPAN-2 was identified as a pancreatic cancer marker approximately 40 years ago,[8,12,13] and several decades ago, investigators reported that DUPAN-2 can be particularly elevated in patients with pancreatic[14] [11] and colorectal cancer[11] who do not produce CA19-9. Because DUPAN-2's diagnostic performance as a standalone marker is somewhat inferior to that of CA19-9, it is not used clinically except in Japan, where it is used mainly in patients with undetectable CA19-9.[14,28] We find not only are DUPAN-2 levels significantly higher in control subjects lacking functional FUT3, they are higher still if they also lack functional FUT2. DUPAN-2 had the highest diagnostic performance in FUT3-null individuals, in whom CA19-9 performs poorly. The FUT test–stratified CA19-9/DUPAN-2 combination achieved high diagnostic performance with an AUC of 0.960 (diagnostic sensitivity of approximately 80% among stage I/II PDAC cases, approximately 12% point improvement over CA19-9/DUPAN-2 alone, at approximately 98% specificity). The performance of the FUT/CA19-9/DUPAN-2 test was impressive in patients with stage I PDAC (AUC of 0.919), with 62% of stage I PDACs detected (68.75% of stage I cases with PDACs of ≥1 cm; CA19-9 with FUT alone detected 52.1% of all stage I PDAC cases in this study; Table 3).

The $AUC_{PR}$ curves for the simulated CAPS cohort (Fig 3) illustrate how the tests might perform in clinical practice. If the FUT/CA19-9/DUPAN-2 test was applied to 5,000 high-risk subjects undergoing pancreatic surveillance where 0.50% would be expected to have a detectable pancreatic cancer[5], it would be predicted to yield 20 true positives, five

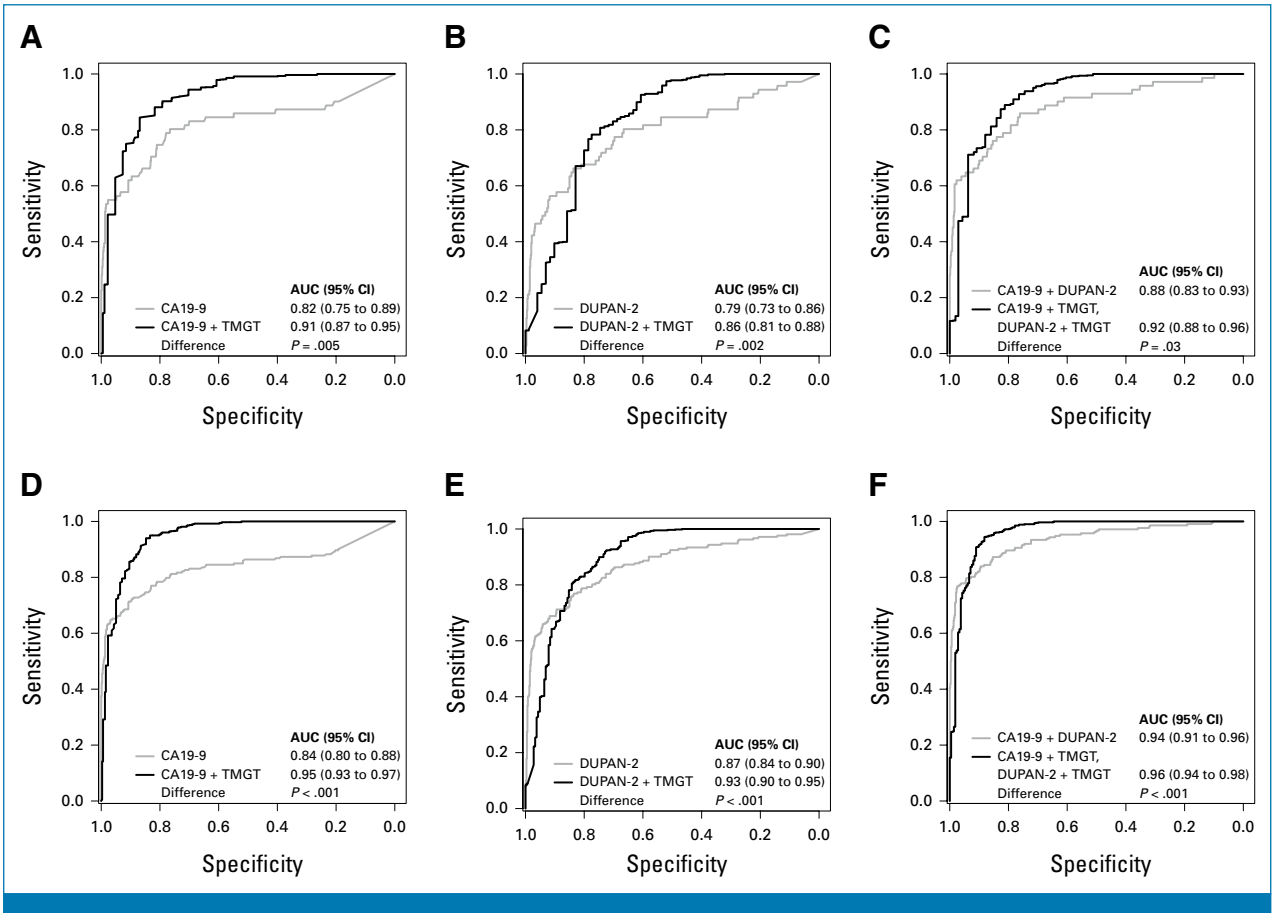


**FIG 3.** Diagnostic performance of CA19-9 and DUPAN-2, alone and with FUT group classification. Receiver operating characteristic curve analysis for stage I PDAC of CA19-9 alone versus CA19-9 by FUT group determined by the (A) FUT TMGT, (B) DUPAN-2 alone versus DUPAN-2 by FUT group, and (C) CA19-9 and DUPAN-2 combined (either test-positive) without FUT group, versus by FUT group. (D-F) Stage I/II PDAC versus controls. FUT group defined by FUT2/3 gene variants. CA19-9, carbohydrate antigen 19-9; PDAC, pancreatic ductal adenocarcinoma; TMGT, tumor marker gene test.

false negatives, 100 false positives, and 4,870 true negatives, and an additional small number (approximately 5) might have a positive test because of the presence of another cancer. The ratio of true positives to false positives and false negatives for the FUT/CA19-9/DUPAN-2 test reflected in the $AUC_{PR}$ score (0.84 *v* 0.51 for CA19-9 alone) is fairly typical of other promising cancer screening blood tests.[29] Evaluating the clinical utility of the FUT/CA19-9/DUPAN-2 test requires further study, and its ultimate utility will depend on the clinical context and having an optimal downstream testing strategy that reflects the diagnostic context, such as starting with repeating the positive test before deciding on additional downstream testing. For example, the diagnostic yield might be lower if the test were used primarily between annual imaging tests to detect interval cancers, but it could still have considerable value in this setting.

The high diagnostic accuracy of the FUT/CA19-9/DUPAN-2 test approaches the diagnostic performance required for further evaluation in clinical trials. Although it is tempting to consider the advantages of the blood test–first approach for pancreas surveillance, pancreatic imaging is superior for early detection in many respects. Although not directly compared in a clinical trial, EUS/MRI can detect small, subcentimeter pancreatic cancers missed by FUT/CA19-9/DUPAN-2[5] and remains the preferred modality for pancreas surveillance. One potential study design to further evaluate a FUT/CA19-9/DUPAN-2 blood test would be one that compares it with diagnostic imaging (ie, where the blood test is performed at the same time as EUS or MRI for pancreatic surveillance). Subsequent studies could evaluate the blood test as a standalone test that complemented imaging-based surveillance (such as at the 6-month mark between annual EUS or MRI).

An ideal pancreatic cancer early detection test requires high diagnostic sensitivity for stage I PDAC while maintaining high specificity. Evaluating biomarkers for stage I PDAC detection is challenging; only approximately 5%-10% of PDAC cases are currently diagnosed at stage I, and with the expanded use of neoadjuvant therapy, pretreatment staging often misses nodal involvement evident by surgical pathology.[4]

**TABLE 3.** CA19-9 and DUPAN-2 for PDAC Diagnosis With and Without the Tumor Marker Gene Test

| | Training Set | | | | Validation Set | | | | Combined Sets | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stage I (n = 35) | Stage I/II (n = 112) | All Stage (n = 175) | | Stage I (n = 36) | Stage I/II (n = 101) | All Stage (n = 156) | | Stage I (n = 71) | Stage I/II (n = 213) | All Stage (n = 331) | |
| Blood Test | | Sensitivity[a] | | Specificity[a] | | Sensitivity[a] | | Specificity[a] | | Sensitivity[a] | | Specificity[a] |
| CA19-9 conventional cutoff (>36 U/mL) | 57.1 | 72.3 | 71.4 | 90.8 | 58.3 | 62.4 | 64.1 | 95.0 | 57.7 | 67.6 | 68.0 | 92.9 |
| CA19-9 uniform high-specificity cutoff (>67.2 U/mL) | 37.1 | 55.4 | 57.7 | 98.7 | 41.7 | 45.5 | 50.6 | 99.3 | 39.4 | 50.7 | 54.4 | 99.0 |
| CA19-9 FUT group cutoffs | 54.3 | 68.8 | 69.1 | 98.7 | 50.0 | 62.4 | 66.0 | 98.7 | 52.1 | 65.7 | 67.7 | 98.7 |
| CA19-9 + DUPAN-2 (uniform conventional cutoffs) | 68.6 | 85.7 | 85.1 | 89.4 | 63.9 | 78.2 | 80.1 | 93.1 | 66.2 | 82.2 | 82.8 | 91.3 |
| CA19-9 + DUPAN-2 uniform high-specificity cutoffs | 51.4 | 70.5 | 72.6 | 98.0 | 47.2 | 61.4 | 67.3 | 98.3 | 49.3 | 66.2 | 70.1 | 98.2 |
| CA19-9 + DUPAN-2 FUT group cutoffs (either positive) | 68.6 | 81.3 | 82.3 | 98.0 | 55.6 | 75.2 | 78.8 | 97.4 | 62.0 | 78.4 | 80.7 | 97.7 |
| CA19-9 + DUPAN-2 FUT cutoffs (double positive) | 34.3 | 45.5 | 49.1 | 100.0 | 30.6 | 46.5 | 49.4 | 100.0 | 32.4 | 46.0 | 49.2 | 100.0 |

NOTE. Conventional cutoff, DUPAN-2 >150 U/mL and CA19-9 >36 U/mL; combined uniform cutoff for 99% specificity, DUPAN-2 >238.8 U/mL and CA19-9 >67.2 U/mL.
Abbreviations: CA19-9, carbohydrate antigen 19-9; PDAC, pancreatic ductal adenocarcinoma.
[a]95% CIs are presented in the Data Supplement (Table S5).

For these reasons, most biomarker studies report the diagnostic performance for stage I/II PDAC rather than stage I PDAC alone.

CA19-9 and DUPAN-2 have been extensively studied for decades, primarily as prognostic markers for patients with pancreatic cancer.[15,30,31] These studies indicate that both serum CA19-9 and DUPAN-2 levels reflect the tumor burden. Indeed, we found that the diagnostic sensitivity of DUPAN-2 increased with tumor stage.

The FUT gene test is a one-time test that can be performed in a molecular diagnostic laboratory. DUPAN-2 can be measured by a simple ELISA assay; although DUPAN-2 is only approved for use in Japan, it is not currently approved as a test for clinical use in the United States nor available. CA19-9 and DUPAN-2 are not pancreatic cancer–specific tests. A diagnostic algorithm for a positive test would require consideration of other cancers that might elevate CA19-9 and DUPAN-2.[32,33] Localizing the source of a positive cancer screening blood test is a challenge for all multicancer detection tests.[34,35]

The prevalence of FUT variants varies across populations, although the overall prevalence of FUT3-null and FUT2-null subjects appears to be similar across multiple populations.[36] Approximately 10% of the American population is FUT3-null,[37] and approximately 20% or more are FUT2-null (NCBI dbSNP database). There is evidence that FUT2-null alleles are under positive selection.[38] One finding that requires additional study but may have clinical implications is the observation that FUT3/FUT2-double-null individuals are overrepresented among individuals of African descent.

Our CA19/DUPAN-2/FUT test has the potential to improve the assessment of prognosis and response to PDAC therapies, particularly in patients who cannot synthesize CA19-9, but determining this will require future studies.

This study has some limitations. Ours was a retrospective, single-institution study, and prospective validation of the FUT group tumor marker diagnostic cutoffs is needed.

In conclusion, using a FUT variant gene test to assign individuals to the tumor marker reference range that corresponds to their FUT functional group significantly improves the diagnostic accuracy of CA19-9 and DUPAN-2 blood tests for early-stage pancreatic cancer.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Using Tumor Marker Gene Variants to Improve the Diagnostic Accuracy of DUPAN-2 and Carbohydrate Antigen 19-9 for Pancreatic Cancer**

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