

## Circulating Thrombospondin-2 Enhances Prediction of Malignant Intraductal Papillary Mucinous Neoplasm

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### Abbreviations:

Intraductal papillary mucinous neoplasm (IPMN); High Grade Dysplasia (HGD); Thrombospondin-2 (THBS2); Body mass index (BMI); Area under the curve (AUC); Pancreatic Ductal Adenocarcinoma (PDAC); Pancreatic Stellate Cell (PSC)

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**ABSTRACT:**

**Background:** IPMNs are cystic pancreatic lesions with variable malignant potential.

Thrombospondin-2 (THBS2)—an endogenous, anti-angiogenic matrix glycoprotein—may modulate tumor progression. We hypothesized that circulating levels of THBS2 could aid in preoperative prediction of malignant IPMN.

**Methods:** Preoperative serum/plasma samples were procured from patients undergoing surgery. Circulating levels of THBS2 were measured (enzyme-linked immunosorbent assay) and compared to surgical pathology IPMN dysplastic grade.

**Results:** 164 patients underwent THBS2 testing (100 Low/Moderate-IPMN; 64 High-Grade/Invasive-IPMN). Circulating THBS2 (mean±SD) was greater in High-Grade/Invasive-IPMN than Low/Moderate-grade IPMN ( $26.6\pm 12.7\text{ng/mL}$  vs.  $20.4\pm 8.2\text{ng/mL}$ ;  $P<0.001$ ). THBS2 (AUC=0.65) out-performed CA19-9 (n=144; AUC=0.59) in predicting IPMN grade. The combination of THBS2, CA19-9, radiographic main-duct involvement, main-duct diameter, age, sex, and BMI (AUC 0.83; n=137) provided a good prediction model for IPMN grade.

**Conclusion:** Circulating THBS2 is correlated with IPMN dysplasia grade. THBS2 alone did not strongly predict IPMN grade but rather strengthened prediction models for High-Grade/Invasive IPMN when combined with other clinical/biomarker data.

**Keywords:**

Pancreatic cyst, Intraductal papillary mucinous neoplasm, IPMN, pancreatic cancer, Thrombospondin-2, biomarker

**INTRODUCTION:** Intraductal papillary mucinous neoplasms (IPMN), one of the few known premalignant lesions of the pancreas, may progress through grades of dysplasia to invasive cancer.[1] The most widely accepted management strategy for IPMN is outlined by the International Consensus Guidelines, which have proven to be limited by poor specificity for predicting high-risk lesions.[2, 3] The latest revision in 2016 is the first to introduce a blood biomarker, carbohydrate antigen (CA) 19-9 to the algorithm.[4]

CA19-9 has been shown to predict invasiveness of IPMN,[5] but must be used with caution. Benign biliary processes and other gastrointestinal malignancies have been associated with increased CA19-9 levels,[6] and approximately 5% of individuals lack the Lewis blood group antigen that allows for an increase in serum CA19-9 levels.[7] Thus, while CA19-9 may enhance our ability to risk-stratify patients with IPMN, these pitfalls, together with limitations of the International Consensus Guidelines largely based on clinical and radiological features, highlight the need for novel, complementary biomarkers.

Thrombospondin-2 (THBS2) is an extracellular matrix protein that modulates cell migration and angiogenesis,[8] two important features involved in tumor progression. Few have examined the role of THBS2 in pancreatic disease including pancreatic cancer. A recent study by Kim *et al.* revealed increasing levels of circulating THBS2 with increasing stage of pancreatic ductal adenocarcinoma (PDAC). Although 115 patients with IPMN were included in this study, they were treated as cases of benign pancreatic disease in data analysis and were not analyzed independently.[9]

As circulating THBS2 has shown promise in predicting progression of PDAC, we hypothesized that THBS2—particularly when combined with existing diagnostic modalities (radiographic findings, CA19-9)—may also enhance our ability to predict dysplastic grade of

IPMN preoperatively. With this knowledge, we may begin to build a panel of tumor biomarkers to complement our current management algorithm, and better select which patients require surgery for high-risk disease (high-grade (HGD)/Invasive-IPMN) while avoiding unnecessary surgical morbidity for patients with low-risk lesions (Low/Moderate-IPMN).

**METHODS:** After obtaining informed consent, blood samples were prospectively gathered from patients undergoing care for IPMN or pancreatic cancer at Indiana University Health-University Hospital. Samples were collected at the time of preoperative endoscopic evaluation or surgery (May 2003 - February 2017). Blood samples were transported on ice and then processed to isolate plasma or serum. Aliquots were stored at -80°C. Samples were thawed on ice and tested for circulating levels of thrombospondin-2 (THBS2) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cat #DTSP20; R&D; Minneapolis, MN). Only first- or second-thaw samples were used; tests were performed on a subset of patients to determine that this number of freeze/thaw cycles or the use of plasma versus serum did not affect the results.

Diagnoses for all patients were confirmed by surgical pathology using the World Health Organization criteria for dysplastic grade. Clinical data were supplemented through review of the electronic medical records. Pancreatitis was a clinical diagnosis and included those with characteristic symptoms (epigastric abdominal pain radiating to the back) noted to be pancreatitis by the physician, or prior hospitalization for pancreatitis, with or without documented elevation in serum pancreatic enzymes. All data were gathered and recorded in compliance with the Indiana University Institutional Review Board guidelines.

Patients were grouped based on grade of dysplasia. Biomarker levels, demographic features, and clinical characteristics were compared between groups using Student's-t test or

analysis of variance (ANOVA) for continuous variables and Chi-square test for categorical variables. A p-value of <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was used to determine predictive accuracy of individual features and combinations.

**RESULTS:** A total of 164 patients underwent testing for THBS2 (100 Low/Moderate-IPMN, 38 HGD-IPMN, 26 Invasive-IPMN). Groups based on dysplasia (Low/Moderate, HGD/Invasive) were similar in age, sex, race, and clinical history of pancreatitis ( $p>0.05$ ). Those with HGD/Invasive-IPMN had slightly lower BMI (25.9 vs. 27.9;  $p=0.015$ ), larger main-duct diameter (8.6mm vs. 5.3mm;  $p<0.0001$ ), and more frequent main-duct involvement based on pre-operative imaging (78% vs. 36%;  $p<0.0001$ ).

Patients with Low/Moderate-IPMN had significantly lower levels of circulating THBS2 compared to patients with HGD/Invasive-IPMN (mean  $\pm$  standard deviation:  $20.4 \pm 8.2$ ng/mL vs.  $26.6 \pm 12.7$ ng/mL;  $p=0.0007$ ) (**Figure Ia**). When patients were sub-divided further (Low/Moderate-IPMN vs. HGD-IPMN vs. Invasive-IPMN), there was a step-wise increase in circulating THBS2 with progressive grade of dysplasia. Patients with Invasive-IPMN had the highest level of circulating THBS2 ( $30.4 \pm 12.7$ ng/mL) compared to those with HGD-IPMN ( $24.1 \pm 12.1$ ng/mL) and Low/Moderate-IPMN ( $20.4 \pm 8.2$ ng/mL) ( $p<0.0001$ ) (**Figure Ib**). On individual t-tests, circulating levels of THBS2 were significantly different between Invasive-IPMN vs. HGD-IPMN ( $p=0.0495$ ) and Low/Moderate-IPMN ( $p=0.0006$ ), but not between HDG-IPMN and Low/Moderate-IPMN ( $p=0.092$ ). When patients with conventional pancreatic adenocarcinoma (PDAC) were included, this trend of increased THBS2 levels and progressive dysplasia continued. Those with PDAC ( $n=30$ ) had greater levels of circulating THBS2 ( $37.4 \pm 19.1$ ng/mL) than all other groups, and mean levels varied significantly by dysplastic grade on

ANOVA ( $p < 0.0001$ ) (**Figure 1c**). Individual t-tests showed significant differences in the levels of THBS2 between patients with Low/Moderate-IPMN versus PDAC ( $p < 0.0001$ ), as well as patients with HGD-IPMN versus PDAC ( $p = 0.0016$ ). There was a similar but non-significant difference between those with Invasive-IPMN and PDAC ( $p = 0.104$ ).

On ROC curve analysis, THBS2 outperformed the traditionally used blood biomarker, CA19-9, in predicting HGD/Invasive-IPMN. Circulating CA19-9 levels did not differ significantly between patients with Low/Moderate-IPMN versus HGD/Invasive-IPMN ( $30.1 \pm 62.1$  U/mL vs.  $468.2 \pm 1,858.3$  U/mL;  $p = 0.075$ ), though there was a trend toward increasing levels of CA19-9 and increased IPMN grade. THBS2 alone provided an area under the ROC curve (AUC) of 0.65 compared to only 0.59 for CA19-9; together, these blood biomarkers revealed a predictive accuracy of 0.66. The addition of two clinical features—main-duct diameter and radiographic main-duct involvement—to a model including THBS2 and CA19-9 yielded an AUC of 0.81; this was strengthened with the addition of age, sex, and BMI to the model (AUC 0.83). Predictive accuracies of individual components and their combinations are outlined in **Table I**.

**DISCUSSION:** Despite extensive clinical evaluation, predicting which IPMN will progress to invasive cancer is difficult. While the International Consensus Guidelines continue to improve,[4] they are heavily focused on clinical and radiographic features to guide management. In an effort to understand and manage pancreatic cysts at a more individual level, studies focused on tumor genetics, molecular profiling, and serum/cyst fluid biomarkers have appropriately gained favor. In the present study we examined whether circulating levels of THBS2 could predict HGD or Invasive IPMN. We report that THBS2 levels were significantly greater with progressive grades of IPMN dysplasia. Moreover, THBS2 out-performed the currently used

biomarker, CA19-9, on ROC curve analysis. Our findings suggest that a more practical use of THBS2 as a biomarker is in combination with other clinical features, as this more realistically approximates clinical decision-making. We showed that together with other factors commonly available in routine workup (demographics, radiographic features, CA19-9 level), THBS2 enhances prediction modeling for IPMN dysplasia. Ultimately, this will aid the clinician in deciding which patients with IPMN should continue to be surveilled and which should undergo surgical resection.

A recent study by Kim *et al.* revealed that THBS2 levels showed great promise in predicting progressive stages of PDAC. THBS2 alone provided an AUC of 0.875 for distinguishing between patients with PDAC and healthy controls. With a THBS2 level of 42 ng/mL as the cutoff point, they reported 52% sensitivity and 99% specificity for detecting PDAC. As in our study, they found that THBS2 levels were complementary to other clinical features in predicting PDAC: when combined with CA19-9 levels, sensitivity and specificity for PDAC reached 87% and 99%, respectively. The ability of THBS2 to distinguish IPMN (n=115) from conventional PDAC was also examined, with a reported AUC of 0.784. However, the grades of IPMN dysplasia were not considered, and IPMN were not examined as a separate entity.[9] As IPMN are pre-malignant lesions of the pancreas, with Invasive-IPMN resembling PDAC (arising from pancreatic intraepithelial neoplasia), it follows that a biomarker of PDAC such as THBS2 may also predict IPMN progression. However, compared to the study by Kim *et al.*, our results for Low/Moderate-IPMN compared to HGD/Invasive-IPMN were more modest. This is plausible, as Low/Moderate/HGD-IPMN are all neoplastic, *pre-malignant* lesions, and one would not expect to see such a stark contrast in THBS2 levels amongst these dysplastic grades when compared to the extremes of PDAC and healthy controls reported in the prior study.

The exact mechanisms linking circulating THBS2 and pancreatic disease, including PDAC and IPMN, are not well understood. Previous studies have suggested a link between elevated pancreatic enzymes, pancreatitis or inflammation and progressive grades of IPMN dysplasia.[10] Pancreatic stellate cells (PSC) are a type of mesenchymal cell that play a central role in the production of extracellular matrix after a pancreatic injury, as in pancreatitis. They have also been implicated as an important part of the pancreatic tumor environment.[11, 12] A study by Neuschwander-Tetri *et al.* demonstrated that THBS2 levels increased significantly in a mouse model of induced pancreatitis; PSCs were isolated, and found to be a potential source of this increase in THBS2.[11] Another study by Farrow *et al.* examined the role of PSCs in cancer invasion rather than pancreatitis or fibrogenesis. In this cell-culture study, PSCs expressing greater levels of THBS2 promoted migration of pancreatic cancer cells, whereas PSCs with low THBS2 expression attenuated pancreatic cancer cell invasion.[12] While this study by Farrow *et al.* focused on PDAC rather than IPMN, these studies considered together may suggest a connection between inflammation, pancreatic stellate cells, THBS2, and progressive dysplasia of pancreatic neoplasms, including PDAC and IPMN. Of note, there was no difference in the rate of reported pancreatitis between those with Low/Moderate-IPMN and HGD/Invasive-IPMN in our study.

The present study poses several limitations and opportunities. As a surgical series, this cohort does not well represent the entire IPMN population, as most patients with IPMN in the current era undergo surveillance rather than surgical resection. It is therefore unclear if our findings are generalizable to patients with known IPMN undergoing primary surveillance. Longitudinal collection of blood samples from the same patient over time, to observe the trends in THBS2 levels on an individual level, may be of benefit if a patient under surveillance



undergoes malignant transformation and eventually requires surgery. A steady increase in THBS2 levels over time, with eventual HGD/Invasive-IPMN on surgical pathology would further support our findings. Though our database overall is prospectively maintained, certain clinical features were retrospectively gathered. Finally, while THBS2 did enhance predictive capacity for IPMN dysplastic grade, it did not reach the levels of sensitivity and specificity to be used as a stand-alone test. Though THBS2 out-performed CA19-9 on predicting HGD/Invasive-IPMN, it alone was only a moderate predictor of IPMN grade. This observation is not surprising, and supports the need for ongoing research to build a panel of complementary biomarkers that may predict which IPMN truly require surgery for high-risk biology. To our knowledge, this is the first study to date examining the ability of THBS2 to predict dysplastic grade of IPMN.

Therefore, validation of our results is warranted in larger, prospective, multi-institutional studies.

**CONCLUSION:** Optimal management of IPMN relies on the clinician's ability to determine which lesions are high-risk (i.e. harbor HGD or Invasive disease). We examined the level of circulating THBS2 in 164 patients with IPMN, and found that THBS2 level increases with progressive grades of IPMN dysplasia. Though THBS2 considered alone was a modest predictor of IPMN dysplasia grade, the addition of THBS2 in models utilizing currently employed features (CA19-9 level, radiographic findings) enhanced the ability to predict HGD/Invasive IPMN. The use of THBS2 may assist in more appropriately recommending surgery for high-risk disease while avoiding unnecessary resection of low-risk lesions.

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**TABLE****Table I.** Predictive accuracy of selected biomarkers for IPMN malignancy (High Grade/Invasive IPMN vs. Low/Moderate IPMN)

Prediction Models	N	AUC	95% CI
Model 1: THBS2	164	0.65	0.56 – 0.73
Model 2: CA19-9	144	0.59	0.49 – 0.70
Model 3: THBS2, CA19-9	144	0.66	0.56 – 0.75
Model 4: Rad. MD involvement	164	0.71	0.64 – 0.78
Model 5: MPD diameter	155	0.74	0.66 – 0.82
Model 6: MPD diameter, Rad. MD involvement	155	0.78	0.71 – 0.85
Model 7: MPD diameter, Rad. MD involvement, age, sex, BMI	213	0.79	0.73 – 0.85
Model 8: MPD diameter, Rad. MD involvement, THBS2	189	0.79	0.73 – 0.86
Model 9: MPD diameter, Rad. MD involvement, CA19-9	189	0.79	0.73 – 0.86
Model 10: MPD diameter, Rad. MD involvement, THBS2, CA19-9	137	0.81	0.73 – 0.88
Model 11: MPD diameter, Rad. MD involvement, age, sex, BMI, THBS2	152	0.82	0.75 – 0.89
Model 12: MPD diameter, Rad. MD involvement, age, sex, BMI, CA19-9	189	0.82	0.76 – 0.88
Model 13: MPD diameter, Rad. MD involvement, age, sex, BMI, THBS2, CA19-9	137	0.83	0.76 – 0.90

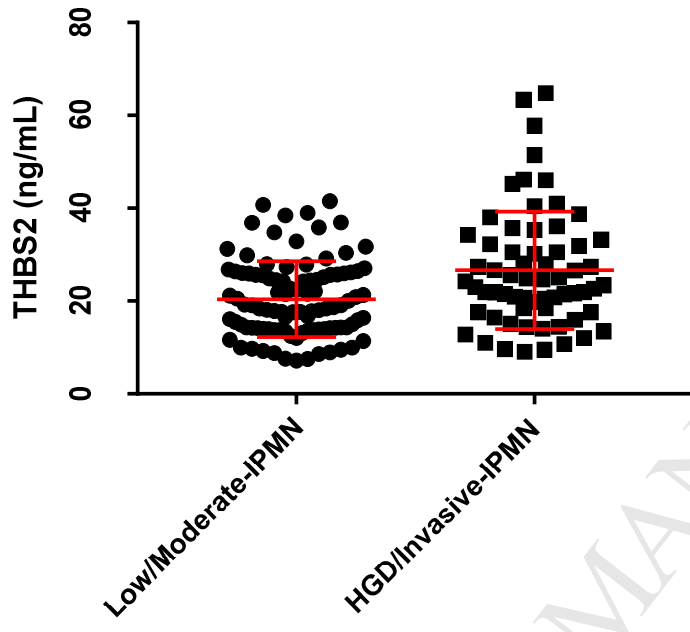
*AUC* Area Under the Receiver Operating Characteristic curve; *THBS2* Thrombospondin-2; *Rad. MD involvement* Radiographic main-duct involvement; *MPD* Main pancreatic duct diameter; *BMI* Body mass index

**FIGURE HEADINGS:****Figure 1**

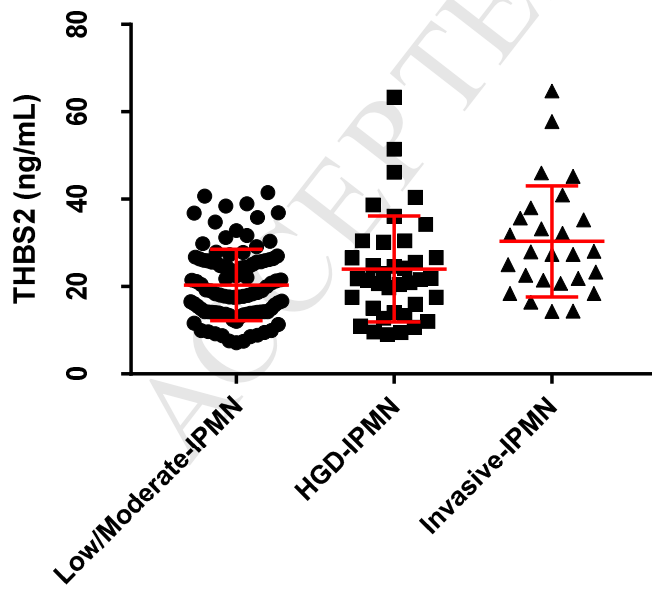
- a.** Scatterplot of Circulating Thrombospondin-2 Levels for Low/Moderate-IPMN versus HGD/Invasive-IPMN (mean and standard deviation bars shown in red)
- b.** Scatterplot of Circulating Thrombospondin-2 Levels for Low/Moderate-IPMN versus HGD-IPMN versus Invasive-IPMN (mean and standard deviation bars shown in red)
- c.** Scatterplot of Circulating Thrombospondin-2 Levels for Low/Moderate-IPMN versus HGD-IPMN versus Invasive-IPMN versus PDAC (mean and standard deviation bars shown in red)

Figure I

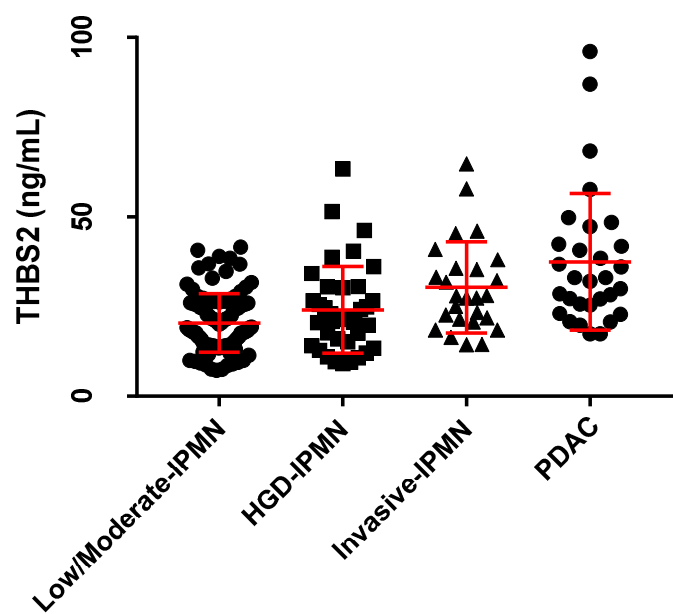
a.



b.



c.



**HIGHLIGHTS/SUMMARY:**

Optimal management of IPMN relies on the clinician's ability to determine which lesions are high-risk (i.e. harbor HGD or Invasive disease). Though THBS2 considered alone was a modest predictor of IPMN dysplasia grade, the addition of THBS2 in models utilizing currently employed features (CA19-9 level, radiographic findings) enhanced the ability to predict HGD/Invasive IPMN. The use of THBS2 may assist in more appropriately recommending surgery for high-risk disease while avoiding unnecessary resection of low-risk lesions.