

A Multi-center Study of a MicroRNA-based Assay for the Diagnosis of Pancreatic Ductal Adenocarcinoma in Fine Needle Aspirates

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SUMMARY

- The miRInform™ Pancreas LDT, interrogating expression of miR-130b, -135b, -148a, -196a, -375, -96 and -24, was developed and validated in accordance with CLIA and CAP regulations using 95 FFPE and 186 FNA pancreatic specimens, respectively.
- In conjunction with FNA cytology, the miRInform™ Pancreas LDT allows diagnosis of PDAC with 92.5% accuracy, as compared to 80.6% for FNA cytology alone.
- The miRInform™ Pancreas LDT enables resolution of “Indeterminate” cytology with an accuracy of 78.2%.

INTRODUCTION

Differential diagnosis between chronic pancreatitis (CP) and pancreatic ductal adenocarcinoma (PDAC) in patients with solid pancreatic masses often represents a clinical dilemma. Endoscopic ultrasound guided fine needle aspiration (EUS FNA) is widely used for obtaining a tissue diagnosis of masses suspected to be malignant. In high volume tertiary medical centers, EUS FNA has a reported sensitivity (Sens), specificity (Spec) and positive predictive value (PPV) approaching 100%. However, its negative predictive value (NPV) can be as low as 70%, resulting in up to 30% false negative results¹. This can stem from such confounding factors as co-existence of focal chronic pancreatitis mimicking pancreatic cancer and marked desmoplastic reaction creating peri-tumor fibrosis². Variations in the diagnostic yield of EUS FNA (i.e. non-diagnostic rate) can be another cause for concern in the process of obtaining a positive cytologic diagnosis of PDAC and deciding patient management³. The current diagnostic algorithm for pancreatic cancer provides opportunities for molecular biomarker tools, which could be used in conjunction with FNA cytopathologic evaluation to improve the accuracy of distinguishing between ambiguous benign conditions and pancreatic cancer.

Mature microRNAs (miRNA) are small 19-23 nt regulatory RNAs that control gene expression at the post-transcriptional level and whose mis-regulation has been linked to many human cancers, including pancreatic carcinomas. We previously identified a miRNA model consisting of miR-196a and miR-217 that distinguishes PDAC from chronic pancreatitis using frozen tissue and FNA specimens^{4,5}. We further established the excellent clinical performance of this model in accordance with CLIA and College of American Pathologists (CAP) guidelines using a blinded set of FFPE specimens, with the sensitivity and specificity of approximately 95%. Performance evaluation of this laboratory developed test (LDT) in resected pancreatic specimens was a key step to ensure success of the development and validation of a less-invasive miRNA test in pancreatic FNAs, the miRInform™ Pancreas LDT, for which final pathology is not always available and/or more difficult to obtain.

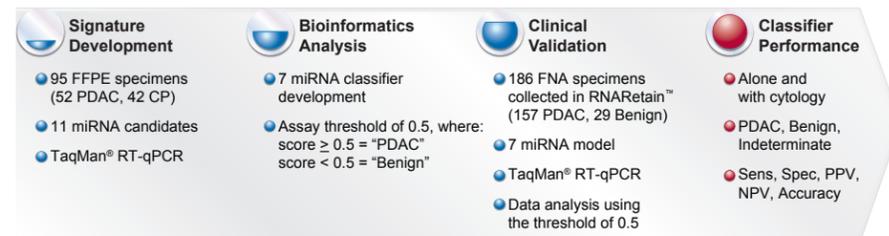


Figure 1: Flowchart describing the overview of development and validation of the miRInform™ Pancreas LDT. The development of the final model was performed on FFPE specimens using 11 miRNAs, pre-selected from previous studies based on their performance in differentiating PDAC and benign specimens^{4,5}. The clinical validation was carried out in FNA specimens preserved and shipped in RNARetain™, a RNA Stabilization Solution. Legend: FFPE – formalin fixed paraffin embedded, PDAC – pancreatic ductal adenocarcinoma, CP – chronic pancreatitis, RT-qPCR: real-time quantitative PCR, Sens – sensitivity, Spec – specificity, NPV – negative predictive value, PPV – positive predictive value.

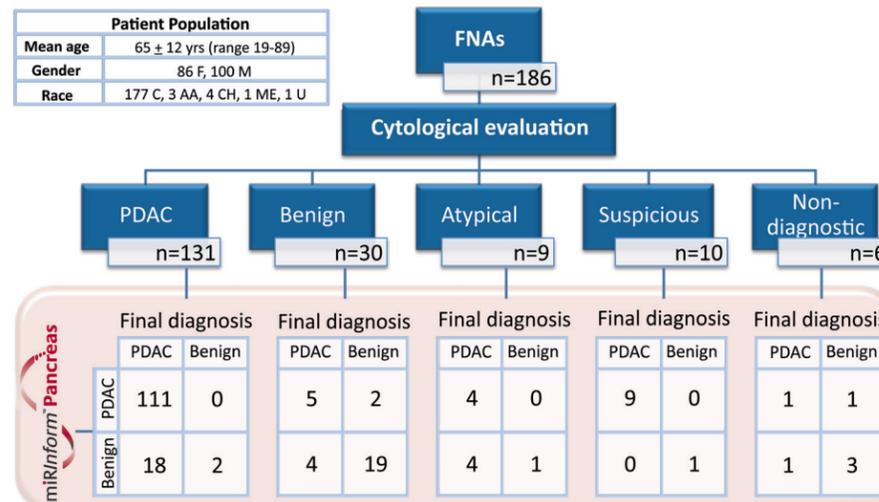
MATERIALS AND METHODS

FFPE specimens were collected according to a protocol approved by the ethics committee of the Ruhr-University Bochum (permission no. 3534-09 and 2392-04). Three to five 12µm FFPE tissue slices were extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE Tissues protocol (Ambion/Life Technologies). FNA specimens were collected according to the institutional IRB-approved protocol as a part of standard clinical care. Total RNA from FNA specimens preserved in RNARetain™ (Asuragen) was extracted with a modified miVana PARIS™ procedure and proprietary Asuragen protocols. Concentration and purity of RNA were measured using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc). RT-qPCR was performed using the TaqMan® 7900 system and TaqMan® miRNA Assays (miR-130b, -135b, -148a, -196a, -375, -96, -24, -210, -217, -155 and -223) (Applied Biosystems) according to in-house developed protocols using 30ng total RNA.

A panel of 95 FFPE samples was used to construct a single model to predict PDAC status on an independent FNA sample set. The final model has two key features: a set of biomarkers and a classification algorithm to summarize the expression values into a single score. The relative performance of several models were assessed by measuring Youden's index and area under the receiver characteristic curve based on replicated 5-fold cross-validation. The model selection was performed only on the FFPE sample set (training set).

For bioinformatics evaluation of FNA cytology and miRInform™ Pancreas performance, the Final diagnosis was reached by the participating institutions either via histopathological evaluation of the resected specimen or using EUS combined with cytopathological assessment of an FNA, and other clinical correlates (CEA, CA19-9 testing, etc.). For non-diagnostic findings on initial cytology, the EUS FNA procedure was repeated.

Patient Population, Study Design and Performance Evaluation Algorithm



*miRInform™ Pancreas consists of miR-130b, -135b, -148a, -196a, -375, -96 and miR-24

Figure 2: Patient demographic information and algorithm describing the approach to performance evaluation of the miRInform™ Pancreas LDT. According to cytology, FNAs were classified into PDAC, Benign, Atypical, Suspicious and Non-diagnostic. According to the Final diagnosis (defined in Materials and Methods), FNAs were categorized into PDAC and Benign (acute, chronic and autoimmune pancreatitis, inflammatory changes, normal, benign acinar cells). The miRInform™ Pancreas LDT classified specimens as PDAC (scores ≥ 0.5) and Benign (scores < 0.5). For bioinformatics analysis, an FNA was considered Benign when the Final diagnosis was Benign and when cytology as well as the miRInform™ Pancreas LDT were in agreement. The FNA was considered PDAC when the Final diagnosis was PDAC and either cytology or miRInform™ Pancreas LDT were in agreement. Legend: F – Female, M – Male, C – Caucasian, AA – African American, CH – Caucasian Hispanic, ME – Middle eastern, U – unknown.

Performance of FNA Cytology and miRInform™ Pancreas Alone

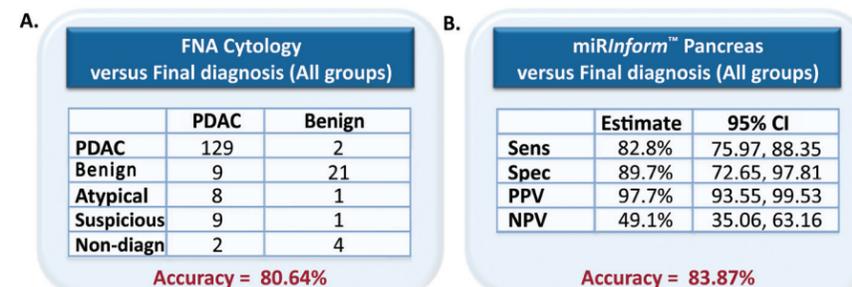
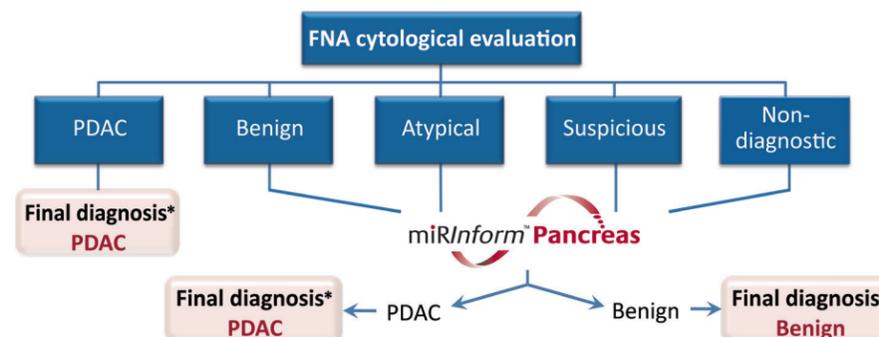


Figure 3: Classification results using FNA cytology (A) and the miRInform™ Pancreas LDT (B) for all 186 FNA specimens using Final diagnosis as a reference. The Final diagnosis was defined in Materials and Methods. The miRInform™ Pancreas LDT classified specimens using a pre-specified threshold of 0.5 (see Figure 1) as PDAC (scores ≥ 0.5) and Benign (scores < 0.5).

Integration of FNA Cytology and miRInform™ Pancreas LDT



*Determined by a physician using FNA cytology, miRInform™ Pancreas and other clinical information.

miRInform™ Pancreas LDT Improves Accuracy of FNA Cytology

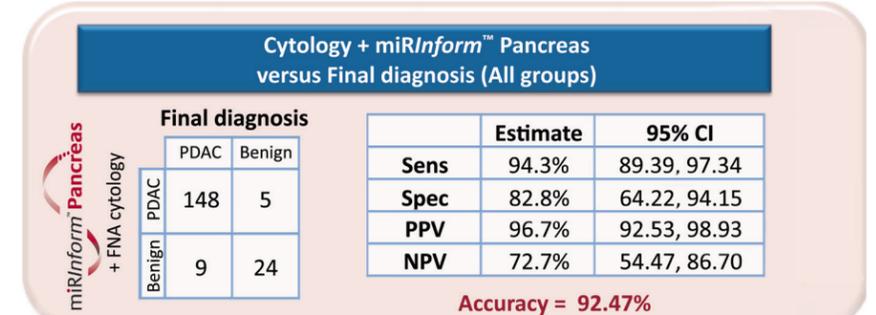


Figure 4: The performance and accuracy of miRInform™ Pancreas LDT in conjunction with FNA cytology using all 186 FNA specimens as compared to the Final diagnosis. Final diagnosis was defined as described in Materials and Methods. The miRInform™ Pancreas LDT classified specimens using a pre-specified threshold of 0.5 (see Figure 1) as PDAC (scores ≥ 0.5) and Benign (scores < 0.5).

miRInform™ Pancreas LDT Aids in Resolving Indeterminate FNA Cytology

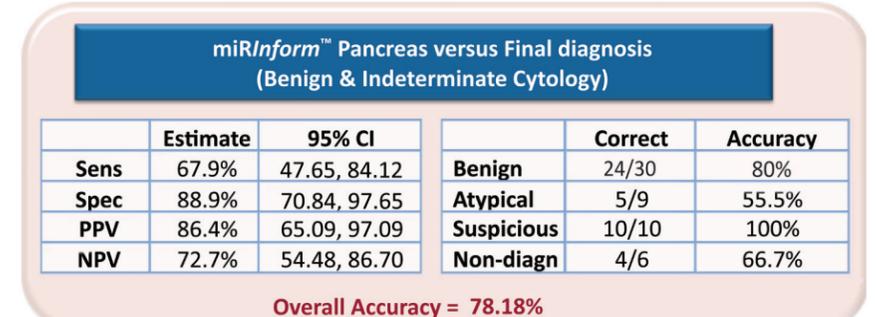


Figure 5: The performance and accuracy of miRInform™ Pancreas LDT as compared to the Final diagnosis for FNA specimens in the Benign and Indeterminate cytology groups. Final diagnosis was defined as described in Materials and Methods. The miRInform™ Pancreas LDT classified specimens using a pre-specified threshold of 0.5 (see Figure 1) as PDAC (scores ≥ 0.5) and Benign (scores < 0.5).

CONCLUSIONS

Using 95 FFPE pancreatic specimens and 186 FNA specimens preserved in RNARetain®, we developed and validated a seven miRNA model comprised of miR-130b, -135b, -148a, -196a, -375, -96 and -24 intended to aid in obtaining a differential diagnosis between PDAC and benign pancreatic diseases. This test, the miRInform™ Pancreas, was validated the Asuragen CLIA Laboratory in accordance with CLIA and CAP regulations. When used in conjunction with conventional FNA cytology, this test allows diagnosis of PDAC with 92.5% accuracy, as compared to 80.6% for FNA cytology alone. It also enables resolution of indeterminate FNA cytology specimens, including Atypical, Suspicious and Non-diagnostic, with an overall accuracy of approximately 78.2%.

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